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Ministry of Agriculture and Food Industry of MOLDOVA
National Audit Centre of Alcoholic Products
Agricultural Technical Institute MECAGRO of A.S. M.
Scientific-Practical Institute of Horticulture and Food
Technologies of A.S. M

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- 2. Food Technology**
- 3. Chemistry and Microbiology of Food**
- 4. Economics and Management in the Food Industry**

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Section II

Food Technology



ESTABLISHMENT OF CHEMICAL COMPOUNDS RESPONSIBLE FOR ODORANT AREAS OF THREE WINES FROM LOCAL GRAPE VARIETIES FROM MOLDOVA

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Abstract. Three wines from local grape varieties from Republic of Moldova were submitted to both sensory and gas chromatography – olfactometry analyses (GC-O). Through descriptive analysis, a set of aroma attributes has been described. In order to identify these odor active compounds, the wines were evaluated using qualitative detection frequency analysis (n=7). The panelists generated in total 697 descriptions distributed in 126 odorant areas (OAs), but only 565 (81 %) distributed in 45 OAs were validated as being representative. According to coincidence of gas chromatographic retention data and on the similarity of odor with standards were identified the chemical compounds responsible for odorant areas.

Key words: gas chromatography – olfactometry, detection frequency analysis, odorant area.

Introduction

Wine, which is produced by fermentation of fresh grapes or must, is one of the most complex alcoholic beverages, and its aroma substances are responsible for much of this complexity. Wine flavor can be classified into three groups: varietal, fermentative and wine ageing aroma. Describing the aroma of wines is not a simple task for researchers, because more than 800 aroma compounds such as alcohols, esters organic acids, aldehydes, ethers, ketones and terpenes, etc., have been identified in them, with a wide concentration range varying between hundreds of mg/L to the µg/L or ng/L levels, and their combinations form the character of wine and differentiates one wine from another [1].

Chromatography is a method used to decompose complex mixtures of chemicals into their constituents. In essence, the method entails the forced transfer of chemical components along an adsorptive or dissolvent material, which usually is packed in a column or which constitutes the inner lining of a column.

When odorous chemicals elude from a capillary column, their presence may be detected by instruments like flame ionization detectors (FID) or by mass spectrometry (MS). Due to large differences in detection thresholds between odorants, the capacity of chemicals to invoke odor sensations at a given concentration level varies strongly. Therefore, relative quantities of the components in the mixture are poor indicators of their relative contributions to the mixture's aroma. A better estimate of each component's contribution to the aroma may be obtained by sensory evaluation of the separated constituents. Thus, by replacing the FID with a sufficiently large panel of subjects that sniff the effluents of the gas chromatograph in an effort to detect and characterize the odor-active chemicals.

The gas chromatography-olfactometry (GC-O) is an analytical method that combines the gas chromatography and sensory evaluation, using the human nose to assess odor components. The human nose has an odor detection limit of about 10^{-19} moles, making GC-O an extremely valuable and sensitive tool for odor detection.

After injection, the content of the sample is separated by the chromatographic column. Before leaving the column, the effluent is divided into two parts: the smallest is directed to the instrumental detector, usually a flame ionization detector (FID); the largest part is directed to a smelling device (sniffing port) placed at the evaluator's nose height. Therefore, this method provides simultaneously two signals: the chromatogram of the extract and the recording of odor events perceived by assessors [2].

The odorant areas frequency is correlated to the concentration logarithm of the compound responsible for stimulus. This relationship is based on the hypothesis that, for a certain compound, the perception threshold has a Gaussian distribution. Each assessor must perceive the beginning and the end of the flavor and describe it. The individual aromagrams are summed, yielding the global aromagram where frequency of detection is represented in dependence on time or retention index. The olfactometric indices can be used for ranking odorant areas in function of their olfactory impact [3].

The odorant areas obtained via GC-O are characterized by three parameters: olfactometric index, average linear retention index (LRI) or LRI interval and flavor descriptors. All this information is used later in the identification of compounds.

Material and method

For analysis were used wines made from Moldavian local grape varieties: Startovyi, Hibernat and Muscat of Ialoveni (harvest 2010) produced at the Practical Scientific Institute of Horticulture and Food Technology from Chişinău.

In order to extract aromatic compounds was used the dichloromethane extraction, based on the method proposed by Moio [4].

The olfactometric analysis was performed on 3 extracts by 7 assessors selected in advance and informed that they will analyze three white wines, but no other detail has been specified. The extracts were analyzed by the participants in a different and balanced sequence. Total length of a session was 45 minutes. After injection of the solution into chromatograph column, in order to avoid inhalation of the solvent, the assessor was asked to wait 5 minutes before approaching the nose to the sniffing port.

Gas chromatograph Hewlett-Packard 5890 was equipped with split/splitless injector and DB-1701 capillary column. Simultaneous processing of both signals was performed using EZchrom Elite (Agilent Technologies) and AcquiSniff® (© INRA).

Linear retention indices (LRI) of chromatographic peaks and odorant events were calculated using a daily injection of a solution of 13 n-alkanes (from C₇ to C₁₉), analyzed under the same chromatographic conditions as the extracts.

The results of each individual data processing were presented in Excel tables where the LRI peak, the assessor codes, the extract codes and their respective descriptors were indicated. Therefore, 21 tables with olfactometric data were obtained (3 wines x 7 assessors), that subsequently were submitted to mathematical processing. Mathematical processing of olfactometric data was performed using Matlab® (The Mathwork Inc.), which implements an iterative mathematical function to get a table that contains the number of detections for each tandem wine/odorant area.

Results and discussions

Initially the wines were submitted to sensory analysis sessions (Table 1). Though considerable dispersion of responses, it was achieved conclusive data. The intensity of wine aroma was appreciated with values within a range from 62.5 to 75 pts out of 100.

Table 1. Descriptors set out by tasters during the sensory evaluation

The wine	Types of aromas			
	Floral	Fruity	Vegetal	Spicy
Startovyi	Honey	Pear, apple, lemon	Freshly cut hay	Pepper, coconut
Hibernal	Basil, thyme	Pomelo, grapefruit	Herbaceous	Laurel leaves, paprika
Muscat of Ialoveni	Muscat intense, acacia flower	Citrus, pineapple	Celery	Nutmeg

The olfactometric study, using frequency detection method, generated 21 individual aromagrams. The number of odorant events related to each wine is situated between 228 (Muscat of Ialoveni) and 238 (Hibernal), meaning that for three wines, seven assessors had spotted 697 events. The assessors, with some exceptions, have described each event with only one term, the report terms / events being nearly 1.1.

In order to process data obtained by using Matlab® software, it was previously set an eliminatory threshold. This corresponds to the value of first quartile of distribution, i.e., to consider an odorant area as representative it must contain at least 5 odor events. Of the totality of 697 odor events, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area. Consequently, the areas with the number of events lower than the eliminatory threshold have been removed. Consequently, the areas with the number of events lower than the eliminatory threshold have been removed (Fig. 1).

The results obtained by GC-O analysis were summarized in Table 2.

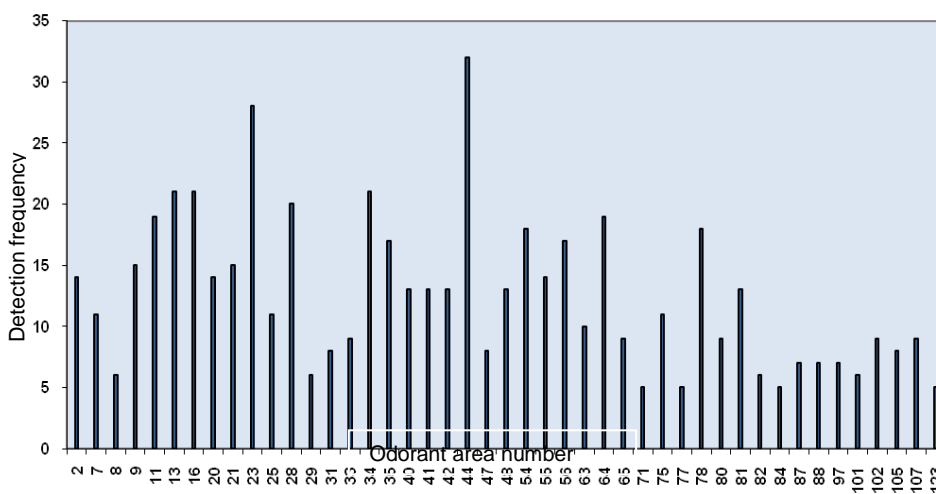
**Fig. 1.** Global aromagram of studied wines

Table 2. Characteristic of representative odorant areas for studied wines

Area number ¹	LRI ²	Detection frequency	Odorant area description	Chemical compounds responsible for odorant areas ³
2	695	14	Yoghurt, cream, butter	1,1-dietoxiethane
7	766	11	Fruity, solvent	ethyl acetate
8	770	6	Vinegar, pungent	acetic acid
9	778	15	Fruity, brandy	ethyl propanoate
11	816	19	Strawberries, pineapple	ethyl 2-methylpropanoate
13	845	21	Cocoa, chocolate, yeasty	3-methylbutan-1-ol
16	862	21	Tutti frutti, strawberries,	ethyl butyrate
20	906	14	Fruity, kiwi, pineapple	ethyl 2-metilbutanoate
21	912	15	Fruit candy, linden, verbena	ethyl 3-metilbutanoate
23	938*	28	Peanuts, roasted, banana, pear	2-metilfuran-3-thiol / isobutyl acetate / isoamyl acetate
25	957	11	Cheese	butanoic acid
28	1009	20	Cheese, rancid	3-methylbutanoic acid
29	1014	6	Apple, cheese	2-methylbutanoic acid
31	1027	8	Woody, fresh herbs, lime	<i>alpha</i> -pinene
33	1053	9	Cooked potatoes, gnocchi	3-methylthiopropanal
34	1060	21	Fruit candy, apple, citrus	ethyl hexanoate
35	1074	17	Black currant buds	4-mercapto-4-methylpentan-2-one
40	1149	13	Flowers	methyl octanoate
41	1154	13	Sulfurous, plastic	hexanoic acid
42	1174	13	Fruity, balsamic	ethylfuran-2-carboxylate
44	1194*	32	Lily of the valley, lavender, citrus, marshmallows	2-phenylacetaldehyde / linalool
47	1235	8	Caramel, chocolate	guaiacol
48	1240	13	Cotton candy, caramel	furaneol
54	1284	18	Honey, rose, lilac	2-phenylethanol
55	1292	14	Flowers	<i>alpha</i> -terpineol
56	1305	17	Caramel, cotton candy	homofuraneol
63	1350	10	Cheese, smoky, dusty	octanoic acid
64	1357	19	Spicy, curry, fennel	sotolon
65	1371	9	Bergamot, citrus	3-sulfanylhhexyl acetate
71	1432	5	Licorice	dehydro-ar-ionene (TDN)
75	1473	11	Floral, herbaceous	ethyl 3-phenylpropanoate
77	1489	5	Chemical, pharmaceutical	4-vinylphenol
78	1494	18	Balsamic, clove, curry	4-vinylguaiacol
80	1508	9	Polyfloral honey	<i>beta</i> -damascenone
81	1512	13	Prune, floral, smoky	phenylacetic acid
82	1518	6	Clove	eugenol
84	1529	5	Spicy	methyleugenol
87	1545	7	Mineral	2,6-dimethoxyphenol
88	1550	7	Floral, herbaceous	ethyl dihydrocinnamate
97	1619	7	Fruity, punch	ethyl cinnamate

Continue Table 2

Area number ¹	LRI ²	Detection frequency	Odorant area description	Chemical compounds responsible for odorant areas ³
101	1644	6	Sulfurous, fermented	decanoic acid
102	1662	9	Vanilla	vanillin
105	1728	8	Mulled wine, balsamic	methyl vanillate
107	1748	9	Coconut	<i>delta</i> -decalactone
123	1909	5	Fruity, candy	tyrosol

1 – Odorant areas that contain at least 5 events per area;

2 – Average LRI in DB-1701 capillary column (30 m x 0.32 mm x 1 µm).

3 – Identification based on coincidence of gas chromatographic retention data and on the similarity of odor with standards [5], [6].

* – co-eluted chemical compounds

This table contains the number attributed to the detected odorant areas (OA), the linear retention indices (LRI), the identity of the compounds and the main odor descriptors of the wines. According to the presented data, OA no. 44 (linalool and/or 2-phenylacetaldehyde) and OA no. 23 (2-methylfuran-3-thiol and/or isobutyl acetate / isoamyl acetate) have the highest average of detection frequency, probably due to co-elution of several chemical compounds. The OA no.13 (3-methylbutan-1-ol), OA no.16 (ethyl butyrate) and OA no.34 (ethyl hexanoate) were also highly detected probably due to their low perception threshold or their high concentration.

Conclusions

Olfactometry analysis (GC-O) allows the selection of odorant compounds using human analyzer, sequentially combining gas chromatography (instrumental analysis) and sensory perception (subjective analysis), thus being a very precious technique for detection of compounds with higher detection threshold than their concentration in wine, and thereby solving some problems in the aroma analysis.

The study presented here has shown that the wines from local grapes from Republic of Moldova (Startovyi, Hibernat and Muscat of Ialoveni) possess a large amount of odorants detectable by olfactometric studies.

The central method of this research was the olfactometry analysis by using the detection frequency method to generate 21 individual aromagrams, which were later summed into a global aromagram for all three wines.

According to mathematical processing of experimental data using Matlab® software, it was established that out of 697 odor events spread in 123 odorant areas, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area.

In spite of some limitations, The GC-O approach used in the study arises as a valid tool for determining the existence of intense odorants of wine.

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THE ANTIOXIDANT PROPERTIES DYNAMIC OF AGED RED WINES

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Abstract: This article presents the results of experiments that established the influence of the storage method of Cabernet Sauvignon and Merlot red wines on the phenolic complex and chromatic indices. The wines were kept in the bottle, moldavian and french oak shavings and oak barrel for a period of 275 days.

Key words: reduction capacity, chromatic indices, phenolic complex, red wine.

Introduction

Maturation is an important phase in the production of quality wines. Color stability and organoleptic quality indexes are improved by aging red wines. Color changes during aging of red wines is due anthocyanins which are incorporated into a more stable complex forming polymeric pigments in various reactions of condensation. Traditionally, wine is aging in oak barrels, but now the attention of researchers are on the alternative maturation with oak sources, such as chips, shavings and oak extracts.

Maturation wine in bottle is widely used in the process of maturation, as in oak casks, but more wine during aging [4].

Aging process refers only to wines from varieties of high quality red and white who had a good evolution during maturation.

Duration of aging red wines in bottles ranging from 8 to 20 years and even more in some varieties, especially in years with good harvests which were prepared and subject to strict technological processes. Duration of aging wines in bottles is optional for commercial purposes, for 6 to 12 months, depending on the type of wine and variety.

Materials and Methods

The intention of research were to kept Cabernet Sauvignon and Merlot red wines in: bottle, on the moldavian and french oak shavings [1,2] that each dose of 1 g/L, and in the barrel for a period of 270 days. The amount of administered shavings (1 g/L) was established in preventive investigations at the Department of Oenology, TUM.

Cabernet-Sauvignon and Merlot wines were produced in the Trifești wine center, Valul lui Traian geographical area, Burlacu wine realm (Vierul-Vin Winery), the harvest of 2011.

The research was performed in the science laboratory of Enology Department. For determine the basic physical-chemical and specific indices were used methods for analysis corresponding to current standards and recommended methods of OIVV. Dynamic analysis was carried out 7 days.

Results and Discussions

The wine samples studied were characterized by physical-chemical and organoleptic qualities shown in Table 1 and 2.

Table 1. Physical-chemical indices of Cabernet Sauvignon and Merlot wines

Wine	Alcohol, % vol.	Sugar, g/L	Titration acidity, g/L	Volatile acidity, g/L	SO ₂ free/total, mg/L
Cabernet Sauvignon	13,8	0,9	6,0	0,36	50/90
Merlot	12,2	1,5	5,7	0,4	26/68

Table 2. Organoleptic qualities of researched wines

Wine	Clarity	Color	Flavor	Taste
Cabernet Sauvignon	Clear, no sediment and foreign inclusions	Intense ruby	Shades of green peppers, saffian, red fruit (plums, cherries, currants)	Full body.
Merlot	Clear, no sediment and foreign inclusions	Dark ruby	Shades of red fruits and berries (plums, raspberries)	Full, soft and harmonious.

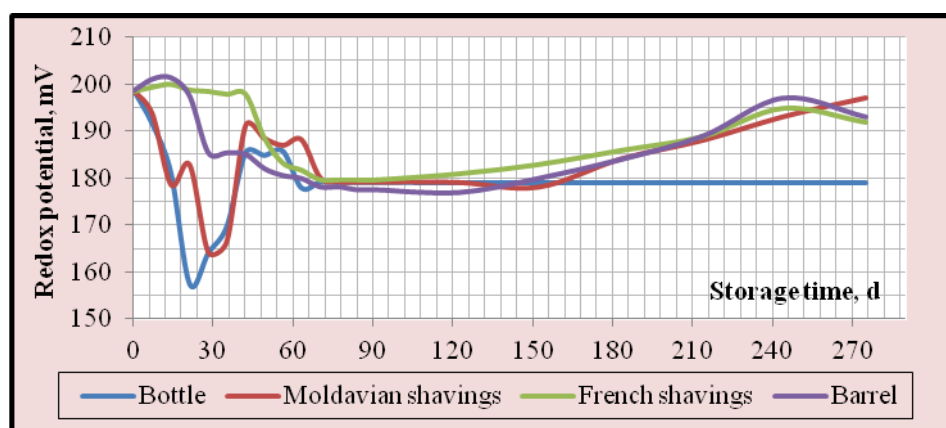
The analysis of Cabernet Sauvignon and Merlot red wines are characterized by physical-chemical indices falling within allowable values of normative documents. Cabernet Sauvignon and Merlot wines during implementation research were characterized by physical-chemical and microbiological stability, organoleptic changes were not improper. Specific and chromatic indices of investigated red wines are shown in table 3. Specific indices and chromatic values for Cabernet Sauvignon and Merlot wines are characteristic for wines obtained from the South of Moldova region, which is characterized by a sum of active temperatures (annual quota $3200 \div 3400$ °C), which ensures full ripening of the grapes and emphasize the value of wine area.

Evolution of redox potential during storage Cabernet Sauvignon and Merlot red wines aged in bottles, moldavian and french shaving and oak barrel is shown in Figure 1 (a and b). The initial value of redox potential in Cabernet Sauvignon wine was 198 mV. After the first week of storage, it increases with 5.28% compared to the control sample, for all modes wine storage. Since the second week of storage redox potential values gradually decrease until the end research environment maintained at 23.58% in the bottle, 7.78 and 16.54 % for samples maintained with french and moldovian shavings, and about 9.99% for wine kept in oak barrel, figure 1 (a).

Table 3. Specific and chromatic indices of Cabernet Sauvignon și Merlot wines

Wine	Total phenols, catechine mg/L	Total anthocyanins, mg/L	Monomeric/polymeric anthocyanins, mg/L	IPT	Hue, u. a.	Tint, u. a.	Total antioxidant capacity, mM Trolox	E _h , mV
Cabernet Sauvignon	3951	494	146/348	58,8	2,57	0,49	14,91	198
Merlot	2534	391	89/302	42,6	1,37	0,52	13,62	201

A similar situation is recorded at Merlot wine samples. Initial Eh value record an increase to about 6.74 % for wine maintained in bottle, with 1.42 % and respectively 7.80 % for samples maintained on moldovian and frenches shaving and wine kept in oak barrel is characterized by higher values by about 5.32 %, Figure 1 (b). Later, during the 77 days there is an almost continuous decrease of Eh-value with approximately 11% for all samples of wines. Since, redox potential expresses the oxidation or reduction level of wine, the initial increase in the Eh value with 5.28 % for Cabernet Sauvignon wine and 5.32 % for Merlot wine may be subject to achievement intense oxidation processes due to oxygen mass solvate in wine made by following last aerations before pouring in the bottle / barrel and oxygen that enters by pores of oak staves, as well as the interaction of oxygen with reducing compounds in wine (phenolic substances). Well-onset reduction can be conditioned by the presence of SO₂ concentration of 100 mg/L administered in order to preserve wine. Subsequent stability of redox potential during storage is determined by the environment in which the wine tight and small changes that occur as doses of oxygen ingress due to opening containers.



a)

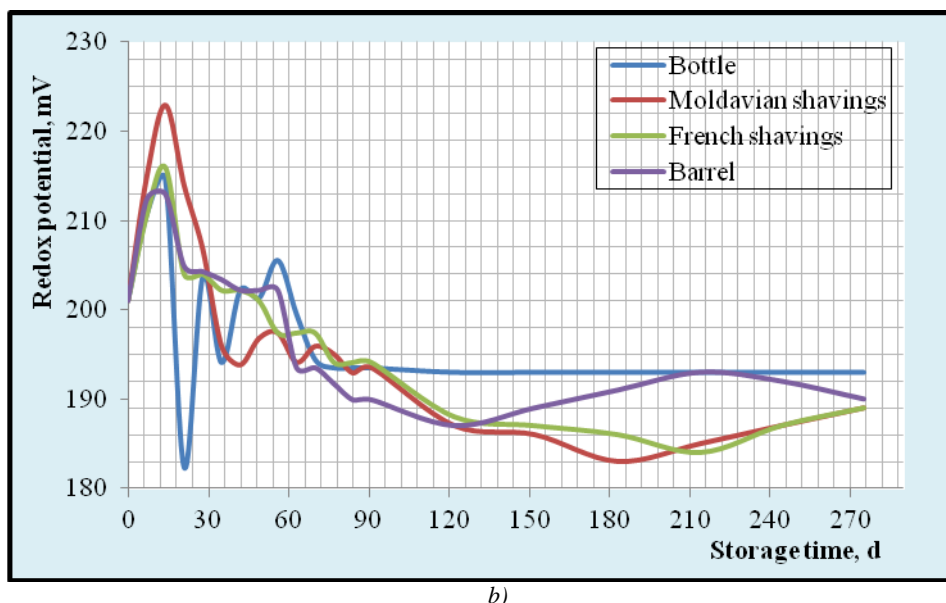


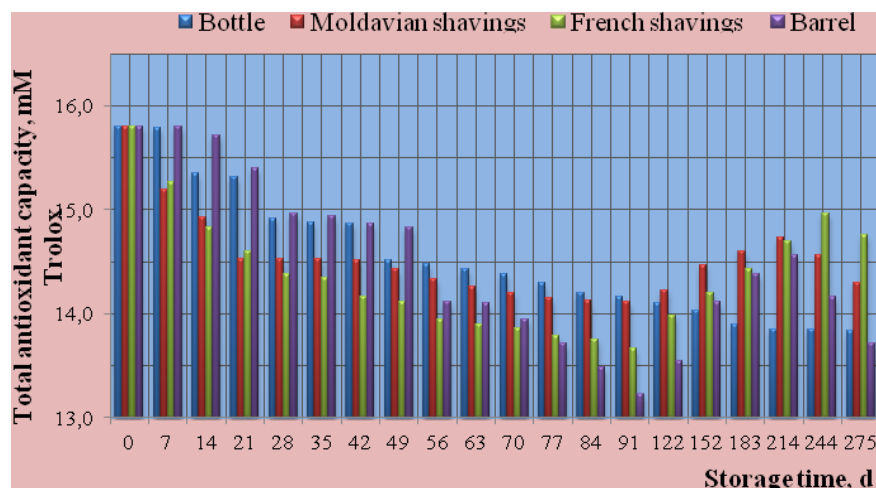
Fig. 1 The changing of redox potential values in Cabernet Sauvignon (a) and Merlot (b) wines
Note: d – days.

Evolution of total antioxidant capacity (TAC) during storage of Cabernet Sauvignon and Merlot red wines kept in bottles, moldavian and french shavings and barrel is reflected in Figure 1 (a and b). Initial value of total antioxidant capacity, or as it is called antioxidant power of wine is the 14,91 mM Trolox [7] for Cabernet Sauvignon wine. Subsequently, for wine maintained in barrel, for the first week, although insignificant, but an increase in antioxidant capacity by 0,19 % due to possible extraction of phenolic compounds from oak wood, but then diminishes over time by about 8,12 %.

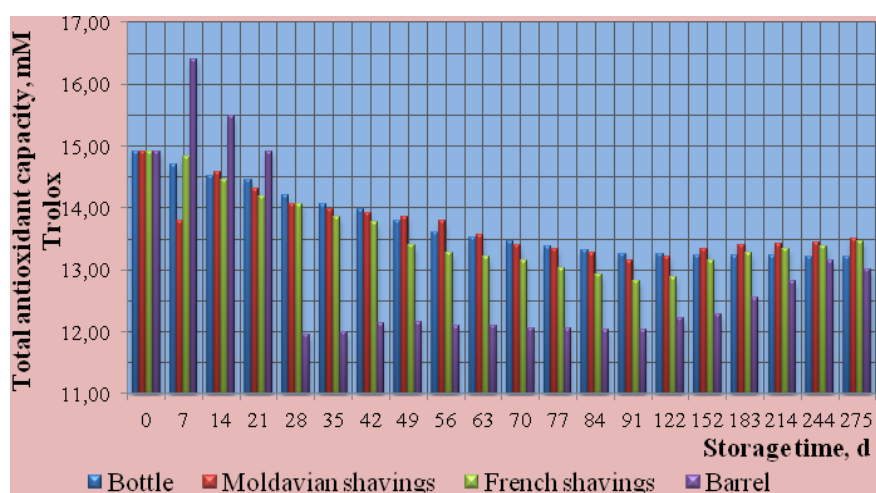
A decrease in total antioxidant capacity of Cabernet Sauvignon wine is registered to keep the wine in bottle by 9,6 %, on moldovian and french shavings 8,08 % and respectively 6,32 %. Merlot wine also recorded throughout the research one decrease in antioxidant capacity on average 8,70 % for wine sample remained in the bottle, 7,62 % and 6,98 % on moldovian and french shavings. It is worth mentioning that if wine sample maintained in barrel are an increase of 10,06 % in the first week, 3,91 % and 0,08 % in the second and third week and later to drop by an average by 19,07 % over the 70 days.

The reducing of antioxidant power during investigations, 180 days, is due to physical-chemical reactions (polymerization, condensation, oxidation) in which phenolic substances from both the raw material and the oak. An evident decrease of total antioxidant capacity is observed when Cabernet Sauvignon and Merlot wines are maintaining in barrel, figure 2, due to the porous structure of oak wood which facilitates penetration of oxygen [1, 2, 5, and 6].

Cabernet Sauvignon wine compared to Merlot wine has a higher antioxidant capacity by about 1,5 times, this would be a consequence of higher phenolic substances technology stocks for Cabernet Sauvignon.



a)



b)

Fig. 2. Evolution of total antioxidant capacity during storage of Cabernet Sauvignon (a) și Merlot (b) wines

Note: d – days.

Conclusions

On based research about how to preserve Cabernet Sauvignon and Merlot red wines kept in bottle, on the moldovian and french shavings, and oak barrel, we can conclude the following:

- The dynamic of redox potential values during wine storage reflect obvious fluctuations while the period of 40 days for the wines stored in barrels and kept on shavings. Subsequent stability of redox potential value is determined by the formation of a reductive environment, especially for samples stored in the bottle.

- The antioxidant activity of phenolic substances gives wines a subsequent reduction potential that contributes to their stability over time. Most value of total capacity is 1167.4 mM Trolox in Cabernet Sauvignon wines, samples maintained in the bottle and moldovian shavings.

The time of storage influence the stability of specific indices in red wines, registering obvious changes for a period since 30 to 40 days.

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ISOFLAVONES DISTRIBUTION IN THE PROCESS OF SOY PROTEIN RECOVERY

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Abstract

Soy bean contain soy isoflavones which have an effect on different metabolic disorders in human. In this study we have analysed distribution of soy isoflavone between different product under recovery of soy protein. Using mass-spectroscopy and NMR analysis we have detected main isomers in soy bean daidzin and genistin. It was shown that isoflavone fraction is extracted to [alcohol](#) solution in processing of protein concentrate.

Having analysed products of protein isolate processing we detected that isoflavones were extracted to alkaline solution during protein extraction. Only about 17 % of isoflavones have been remaining in protein isolate. Main part of them was detected in whey water after protein precipitation. And it is possible to elicit them according to standart procedure using extraction by ethylacetate and precipitation by chloroform. Taking into account our results we are proposing to use the by-products of soy protein processing for isoflavone concentrate producing.

Keywords: soy isoflavones, protein concentrate, protein isolate

Introduction

Isoflavones are the phenolic substances that are detected only in some plant family. The highest content of isoflavones have been found in soy bean. The content of these substances differs in different soy lines, depends on weather conditions, maturity of seed and so on. (Britz, Schomburg & Kenworthy, 2011). It was shown that these substances had weakly estrogenic and anticarcinogenic effect on human (Lu, Anderson, Grady & Nagamani, 1996), they could be used for prevention cardiovascular and another metabolic disorders (Wagner et al, 1997, Nestel et al, 1997).

A number of studies have been done to investigate isoflavone content in different soy products and soy protein supplements. Having done research on isoflavone concentration in soybean samples on a C18 reverse-phase column using a two-step gradient solvent system, Wu & Muir (2008) showed that producing soybean hydrolysate led to a nearly 40% loss of isoflavones compared with the original soybean flour. Barnes, Kirk & Coward (1994) showed that high temperature treatment (higher than 80 °C) resulted in changes in isoflavone isomers content and toasted flour and protein isolate contained only moderate amounts of each isomer. Yerramsetty, Mathias, Bunzel & Ismail (2011) detected interconversions of malonylgenistin and its isomer in buffered and soymilk systems. Significant interconversions of isoflavone isomers was also observed in thermally treated soymilk at pH 7 and pH 9 (Nufer, Ismail & Hayes, 2009).

Genovese & Lajolo (2010) reported that production of soy isolates leads to an increase in the percentage of free aglycones. Coward, Barnes, Setchell & Barnes (1993) have shown decreased isoflavone content in such soy protein products from American and Asian diets as soy sauce, alcogol-extracted protein concentrates and protein isolates. Low isoflavone content in alcogol-extracted protein concentrates and protein isolates had demonstrated also by Wang & Murphy (1994). Lin, Krishnan & Chunyang (2006) have

shown effect of precipitating and washing temperatures on retention of isoflavones and saponins during processing of soy protein isolate. The purpose of our study was to investigate the soy isoflavones distribution between different by-products and final products during soy protein processing.

Materials and methods

Isoflavones extraction. Isoflavones were extracted from defatted soy meal using [ethyl alcohol](#) solution (70 %, v/v) in the same extractor during some 4 hour. Than solvent was evaporated on the rotor evaporator and residue was treated by hot water. Received solution was filtrated through paper filter to remove insoluble substances. Filtrate was evaporated again and residues were extracted three time by ethylacetate. Obtained extracts were combined and dried by sodium sulfate. Isoflavones were precipitated by chloroform and filtrated.

Isoflavones analysis. Isoflavones composition was analysed by column chromatography on silicagel with system solvents [ethyl alcohol](#):water. Eluate was analysed photometrically. Mass-spectroscopy was used for identification molecular mass of isoflavone isomers.

Isoflavones identification. ^1H nuclear magnetic resonance (NMR) spectra were measured on a Bruker Avance DRX300 spectrometer and were used for isoflavones identification. Chemical shifts obtained NMR spectra were compared with standarts (Pouchert, 1983).

Obtaining of protein concentrates. Defatted soy seed was used for protein concentrate obtaining according to next procedure. Defatted soy seed was mixed with [ethyl alcohol](#) solution (70 %, v/v) in relation 1:10 and exposed during 40-50 min with stirring under 45-50 °C. After this insoluble residue was precipitated by centrifugation. The supernatant (extract of soluble substances) was analysed on isoflavone isomer content. Pellet was dried to 8-10 % fluidity and protein and isoflavone content was analysed.

Obtaining of protein isolate. Protein was extracted from defatted soy seed by alkaline solution (pH 8.5-9.5) under constant stirring and temperature 50-55 °C during 40-50 min, relation cake:solution was 1:10. After this insoluble residue was precipitated by centrifugation. The supernatant (protein extract) was used for isoelectric protein precipitation at pH 3.8-4.5. After protein coagulation pellet was separated by centrifugation (3 000 x g). Protein pellet was collected and dried to 6-8 % fluidity. Protein and isoflavone isomer content was analysed in protein product and in supernatant (whey water).

Results and discussion

After extraction of isoflavones from soy bean we have detected that total content of isoflavones was 0.39 %, calculated on the dry substances of soy meal. Obtained mixture of isoflavones from defatted soy seed was studied on the isoflavone composition. The three individual substances were detected on chromatogram in a ratio 1:1:0,03. The holding time of these components was very close – 1.38, 1.41 and 1.53 min.

Mass-spectroscopy analysis gave possibility to estimate molecular mass of detected isomers. They were 417,4 and 433,4. Such molecular masses correspond to daidzin and genistin respectively. Daidzin is a glukoside of isoflavone daidzein and genistin correspondingly - of genisteine. We could not determine the third component due to its very insignificant content.

For identification of detected isomers we have analysed ^1H NMR spectra of isoflavone mixture from soy seed. We have analysed aromatic part of spectrum for this purpose (Fig.1). And it was confirmed the presence of daidzin and genistin in isoflavone extract, their ratio was about 1:1.

We used defatted soy meal for processing of protein concentrate. We had proposed that isoflavone substances could be extracted from soy meal simultaneously with other soluble substances to [ethyl alcohol](#) solution. That is why we had used [ethyl alcohol](#) extract obtained under protein concentrate producing to recover isoflavones. In this case we followed the same procedure as for isoflavone extraction from defatted soy meal. We have obtained 0.39 % (from the initial mass of dry substances meal) output of isoflavone concentrate, that means that almost whole isoflavone fraction is extracted to [ethyl alcohol](#) extract in processing of protein concentrate. Mass-spectroscopy and ^1H NMR analysis have confirmed the presence of two isomers of isoflavones - daidzin and genistin in ratio about 1:1 (Fig.2).

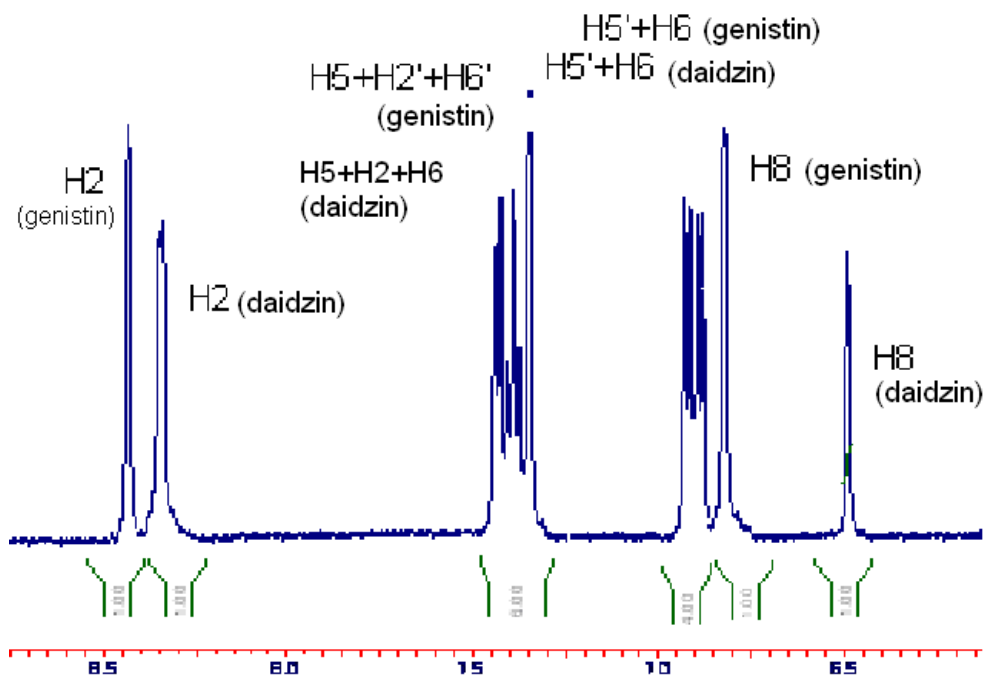


Fig. 1. ^1H Nuclear magnetic resonance (NMR) spectrum of isoflavone extract from soy bean.

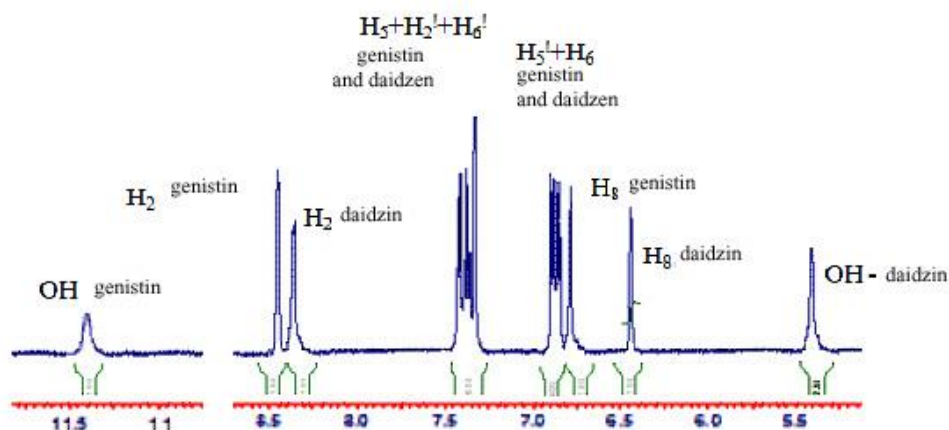


Fig. 2. ^1H Nuclear magnetic resonance (NMR) spectrum of isoflavone extracted from alcohol solution obtained in protein concentrate processing.

We have studied also isoflavone content in different by-product during protein isolate processing and in protein isolate. To obtain protein isolate protein were extracted by alkaline solution and then isoelectrically precipitated.

Firstly we have determined isoflavone content in whey water after protein precipitation. For this purpose whey water was evaporated on the [rotary evaporator](#) and residue was extracted three time by ethylacetate and obtained extracts were combined. In other respects the procedure was the same as described earlier. We have analysed the isoflavone content and composition (Table 1). About 87% of whole isoflavone fraction of soy bean is containing in whey water. We have detected three isoflavone isomers– daidzin, genistin and malonildaizn. Their ratio was about 1:1:0.33 respectively.

Analysis of insoluble residue after protein extraction indicated only trace content of isoflavone in this by-product (Table 1). Thus almost whole isoflavone fraction is extracted to alkaline solution together with protein. These data are a little surprising because it is known that these substances are not soluble in water solution. We suppose that this result may be due to isoflavones are bound with protein in soy bean and that why these substances are extracted simultaneously.

Our results have demonstrated insignificant isoflavone content in protein isolate. It was about 17 % (on the protein isolate mass). These our results do not agree with the data obtained by Jun Lin et al. which have shown that isoflavone content in protein isolate was significantly higher including whole range of investigated temperature [Yerramsetty V.et al., 2011].

Table 1. Isoflavone isomers extracted from different products of soy bean processing. Mass-spectroscopy and NMR data.

Product	Molecular mass of isoflavone isomer	Isoflavone isomer	Ratio	Common output, % of soy bean mass
Soy bean	417,0	daidzin	1	0.39
	433,6	genistin	1	
Ethyl alcohol extract from defatted soy meal	417,0	daidzin	48	0.39
	433,6	genistin	42	
Protein concentrate		Not detected		
Insoluble residue after protein extraction	417,0	daidzin	45	0.01
	433,6	genistin	43	
	503.2	malonildaizin	9	
Whey water after protein precipitation	417,0	daidzin	39	0.26
	433,6	genistin	43	
	503.2	malonildaizin	14	
Protein isolate	417,0	daidzin	46	0.05
	433,6	genistin	43	
	503.2	malonildaizin	7	

Conclusions

Our results showed the presence of three main isoflavone isomers in soy beans and in [by-product](#) of their processing – daidzin, genistin and malonildaizin. During soy processing these substances are extracted to alcohol and water solution. Only about 17 % of initial content isoflavones is containing in protein isolate. We did not detect them in protein concentrate. To retain the main part of soy isoflavones it is possible to extract them from [by-product](#) of protein processing. It is obviously that isoflavone concentrate will have higher medicinal effect than soy protein.

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STUDY ON REDUCING THE CALCIUM CONCENTRATION IN WHITE WINES

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Abstract: The article presents results of research on the comparative study of the reduction of calcium content of white dry wine material treated Aligote, harvest in 2008 by chemical methods. Data are presented for carrying out the process at different temperatures and the process. Reduction was performed with use of calcium preparations "Decalcic" and "Crystal-ex".

Proved that temperature of 18-22°C reduction of calcium content about 25 mg/dm³ occurs within 12 days of preparation "Decalcic" and the "Crystal-ex" - within 30 days. To decrease the calcium content of 50 mg/dm³ at the same temperature better use of preparation "Crystal-ex".

Spending process at a temperature of minus 3° C allows reducing the duration of treatment up to 2-3 days.

Keywords: chemical reduction, preparations "Decalcic" and "Crystal-ex", temperature, process.

With prolongation of the warranty period the wine storage need to a more thorough study of their composition in order to achieve long-term stability against physical and chemical haze.

Often wines that passed the complete cycle of technological treatments on the conclusion of the laboratory and determined to be stable to all kinds of haze, do not maintain the warranty period - they appear fine crystalline precipitate is not always accompanied by the opalescence. After filtration and subsequent testing the wines are resistant to all kinds of haze. Most susceptible to the formation of such sediments are young white wines.

The reason for the formation of precipitation in bottled wines are often to be found in excessive amounts of calcium and its compounds with organic acids of wine.

According to the literature it is known that the real danger of calcium haze exists for mass content of calcium in wine 80 - 100 mg/dm³ [1,2,3], according to other sources - more than 40 mg/dm³ [4, 5].

According to C. Țîrdea [6] calcium causes physical - chemical haze of wine, forming in the cold season (winter) crystals of calcium tartrate, and in warm weather (summer) - crystals of the calcium salt of malic acid. In wines with a pH over 3.3 its possible the formation of the precipitate of calcium tartrate, while at a pH of less than 3.2 the occurrence of these sediments is less likely. [7]

The deposition rate of calcium tartrate for cold treatment is very low, the balance can be achieved in a few months. Consequently, the cold treatment is ineffective against the opacities associated with the precipitation of calcium tartrate and stabilization of these wines can only be achieved by removing excess calcium from wine.

Widespread in Italy has received decalcification wines with racemic tartaric acid (DL - tartaric acid), [8]. Also known to remove excess calcium from the wine using the drug "Decalcic" (oxalate Amon 1 - aq), [9].

The purpose of this study was a comparative evaluation of removing excess calcium from the wine by chemical means using drugs "Decalcic» and «Cristal-ex» (DL - tartaric acid).

Materials and methods.

We studied processed natural dry white wine material Aligote 2008 harvest, which is defined as vulnerable to a clouding of the crystalline calcium to calcium tartrate saturation temperature on the device "IziKristaTest" (firm ERBSLÖH). Mass concentration of calcium was determined permanganometric method [10], other indicators - under existing procedures.

For the deposition of excess calcium used the drug firms "ERBSLÖH" – "Cristal-Ex" and the drug "Decalcic". Reducing the mass concentration of calcium was performed on 25mg/dm³ 50mg/dm³ and at different temperatures and duration of treatments.

Results and discussion.

The studies found that the natural dry white processed wine material Aligoté contains calcium 92 mg/dm³, the pH is 3.28, the concentration of total soluble solids - 871 mg/dm³ and conductivity - 1727 µS / cm.

It should be noted that the treatment of drug "Decalcic" We recommend a wine stock for clarification by settling for a period of not less than 2 days, and the drug «Cristal-Ex" - not less than 14 days.

The results of research on removing excess calcium from dry wine material processed Aligote preparations "Decalcic » and «Cristal-Ex" at different temperatures and duration of treatment are given in the table. The data show that at lower calcium content rate of 25 dm³ at 18 - 22 ° C removed 22 mg/dm³ to 12 days using the drug "Decalcic" and 24 mg/dm³ to 30 days preparation «Cristal-Ex". This results in a reduction to the value of pH 3.15 and 3.13, the concentration of total soluble solids to 779 and 692 mg/dm³ and electrical conductivity to 1547 and 1380 µS / cm, respectively.

In case the amount of calcium the rate of 50 mg/dm³ in temperature 18-22 ° C for 30 days actually removed 36 mg/dm³ drug "Decalcic" and 50mg/dm³, mean estimated amount of calcium preparation «Cristal-Ex":

From the data table shows that the treatment of a temperature of minus 3 ° C helps to reduce the duration of the process of the removal of calcium up to 2-4 days. Thus, with a decrease in calcium content for 25 mg/dm³ in the cold the desired effect is achieved by the preparation "Decalcic" for 2 days (23 mg/dm³ calcium is removed), and the drug «Cristal-Ex" - up to 3 days (removed 26 mg/dm³ calcium).

With an estimated reduction of calcium 50 mg/dm³ for 3 days at a temperature of minus 3° C removed 52 mg/dm³ drug «Cristal-Ex", while the drug "Decalcic" is removed only 36mg/dm³ to 4 days.

Thus, during the treatment of the cold to remove calcium at 25 mg/dm³, both used the drug can reach it in 2 - 3 days, and when deleting large amounts of calcium (50 mg/dm³) more effective is the drug «Cristal-Ex". The process of decalcification in the cold is accompanied by a natural decrease of the pH, total soluble solids and conductivity.

Table The results of research on removing excess calcium from dry white processed wine material Aligote

Treatment variant	Estimated reduction in the content of the calcium mg/dm ³	Processing time, day	The mass content of calcium after treatment mg/dm ³	Actual reduction in calcium content, mg/dm ³	Conductivity, $\mu\text{S}/\text{cm}$	Concentration of total soluble substance, mg/dm ³	pH
Temperature 18 – 22 °C							
Decalcic	25	2	83	9	1870	935	3,21
	25	6	81	11	1826	913	3,20
	25	12	70	22	1547	779	3,15
	50	4	76	16	1682	832	3,20
	50	12	60	32	1269	615	3,16
	50	16	58	34	1245	598	3,16
	50	30	56	36	1239	582	3,16
Cristal-ex	25	14	77	15	1656	829	3,15
	25	22	73	19	1516	758	3,13
	25	30	68	24	1380	692	3,13
	50	14	78	14	1814	907	3,15
	50	22	54	38	1573	774	3,13
	50	30	42	50	1341	642	3,10
Temperature minus 3 °C							
Decalcic	25	1	82	10	1826	914	3,20
	25	2	69	23	1529	767	3,15
	50	1	66	26	1452	726	3,20
	50	2	61	31	1344	684	3,20
	50	4	56	36	1311	656	3,15
Cristal-ex	25	1	88	4	1723	861	3,15
	25	2	78	14	1542	792	3,13
	25	3	66	26	1452	724	3,10
	50	1	74	18	1631	736	3,14
	50	2	55	37	1450	689	3,13
	50	3	40	52	1280	642	3,10

Conclusions

1. When removing excess calcium from dry white processed wine material Aligote for 25 mg/dm³ at 18 - 22 °C is better to use the drug "Decalcic" that allows you to reach this goal for 12 days (the drug «Cristal-Ex" - for 30 days).
2. When removing large amounts of calcium (50 mg/dm³) for the same duration of the process (30 days) is more efficient to use the drug «Cristal-Ex."
3. Duration of the process to remove the excess calcium at minus 3 °C can reduce the processing time of up to 2 - 3 days, and when you remove the excess calcium from the calculation (50 mg/dm³) rational use of the drug «Cristal-Ex."

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VALORISATION OF LOCAL RED VARIETIES IN DIVERSIFYING WINE ASSORTMENT

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Abstract: The present range of moldovian black grain grape varieties is composed mostly of french classics, as well as clones of these varieties. On global wine market several countries with great diligence promote wines from local varieties. In order to diversify assortment of red wines research were subjected wines made from indigenous varieties of 11 grape year 2011 under micro winemaking according to classical technology. The wines were determined physicochemical indices covered, as well as some complementary indicators (phenolic substances, anthocyanins, glycerol, 2,3-butylenglicol, the main non-volatile organic acids, etc.), note organoleptic based on preliminary results obtained were shown perspective to produce local varieties of dry red wines.

Keywords: local varieties, assortment, phenolic substances, anthocyanins, glycerol.

Lately more and more countries highlights the basic assortment some varieties, so-called "national".

Currently moldovan assortment of grape varieties with black bean consists mostly of french classics as well as from clones of these varieties, such as Cabernet Sauvignon, Merlot, Pinot Noir.

It is difficult to create from existing assortment national symbol moldovan wine.

In the current tough competition on the world wine market several countries with great diligence promote wines from local varieties. An example Rkațiteli varieties and Saperavi Georgia, Bulgaria - Mavrud, Rubin and Melnik, Romania - Fetească Neagră and Tămâioasă românească, Azerbaijan - Ширваншах, Баян ширей. [1,2,3].

Stress climate change in recent years have led to the need to attract attention clones domestic varieties that are adapted to local conditions and ensures quality wines [4].

On the range of Moldova to remember that not too far from the historically consisted of local varieties Rară Neagră, Plăvaie, Galbenă, Zghihară, Bătută neagră, Fetească Neagră, Țița caprei, Păsărească, Cabasmă and others.

Many local varieties such as Rară Neagră, Fetească Neagră, Busuioaca de Bohotin etc. had a mark on the history of the country and created typicity of wine vineyards.

To note that at the time ampelographic collection of PSIHFT comprises 41 varieties, „ancestors”, including 10 with black grain.

We currently some local red varieties, which are invaluable, but, unfortunately, they have not undergone thorough research regarding the potential of phenolic substances and coloring, grape processing technology efficiency.

The purpose of this research is to study the physico-chemical composition of red wines made from indigenous varieties for compliance with the requirements of normative documents submitted for red wines.

Materials and methods.

Research have undergone 10 experimental wines made from local red varieties, 2 varieties Codrinschi wines produced from grapes harvested in the Central region (Stăuceni) and South (Pleseni) wine season of 2011 in section Microvinificație Practical Scientific Institute of Horticulture and Food Technologies. Served as a witness Merlot wine. Samples of experimental wines were produced according to traditional technology: harvesting grapes → un bunch-crushing grapes → sulphite at a dose of 30 to 50 mg/dm³ → inoculate active dry yeast → fermentation maceration-pulp at a temperature of 25 to 28 ° C → pressing with liquid phase separation → postfermentation → drawing wine from wine yeast sediment → sulphite free sulfur dioxide content 15 -20 mg/dm³ → wine preservation and care.

Physical and chemical indexes were determined according to standard methods and OIV methods in laboratory Enologie și VDO and laboratory Verificarea calității. Organoleptic assessment was performed in experimental wines tasting Commission within PSIHFT [5].

Results and discussion.

The results of physico-chemical investigations mainly dry red wines made from indigenous varieties of the crop of 2011 are presented in Table 1. The data in Table 1 it can be seen that the wines subject research is characterized by an alcohol concentration range from 12.0 up to 14.9% vol, except for only wines made from varieties Bătuta Neagră (9.5% vol) Negru de Akkerman (9.7% vol). With a higher degree alcohol wines are distinguished from varieties Codrinschi Pleseni (14.9% vol), Feteasca Neagră (14.4% vol) and Tămîioasa de Bohotin (14.1% vol).

Sugar content, despite the high alcohol level does not exceed 2.1 g/dm³ and under the maximum allowable dry wines.

Titrateable acidity reaches values between 6.1 and 9.4 g/dm³, the lowest value recorded into wine variety Seină and the highest values in wines from varieties Kopceak (9.2 g/dm³) and Negru de Caușeni (9.4g/dm³). PH values are 3.20 to 3.62.

Volatile acidity in red wines is investigated within the 0.20 to 0.40 g/dm³, denoting compliance technology to produce experimental wines.

Fairly wide limits vary glycerol content - from 7.4 up to 9.5 g/dm³ except Bătuta Neagră and Negru de Akkerman, characterized by a low glycerol content of only 4.6 and 6 , 5 g/dm³ properly.

Most wines glycerol contained in Codrinschi (Pleseni) - 9.5 g/dm³ and Tămîioasă de Bohotin - 9.3 g/dm³.

In Ciorcuță neagră, Fetească Neagră, Rară Neagră, Codrinschi (Stăuceni) wines, glycerol contained in an amount of 8.6 to 8.8 g/dm³ and is close to the witness Merlot wine (8.9 g/dm³).

Table 1 Physicochemical indices mainly in dry red wines made from indigenous varieties, crop of 2011

Nr. d/o	Denumirea vinului (soiului de struguri)	Alcohol,% vol.	Sugar, g/dm ³	Titrate acidity, g/dm ³	Volatile acidity, g/dm ³	pH	Glycerol, g/dm ³	2,3 – buthilen-glycol, mg/dm ³	unreducible dry extract, g/dm ³	organoleptic note, point
1	Bătută Neagră	9,5	0,8	7,4	0,26	3,54	4,6	215	17,2	7,71
2	Brează	12,0	1,0	6,4	0,26	3,20	7,4	478	20,1	7,80
3	Ciorcuța Neagră	13,0	1,4	7,0	0,26	3,45	8,6	441	22,1	7,88
4	Fetească Neagră	14,4	1,7	7,6	0,26	3,62	8,8	520	24,6	7,94
5	Rară Neagră	13,5	1,8	7,7	0,33	3,45	8,7	848	25,0	7,80
6	Kopceak	13,8	1,6	9,2	0,26	3,37	8,2	511	25,8	7,92
7	Negru de Akkerman	9,7	0,8	7,3	0,20	3,23	6,5	319	19,5	7,81
8	Negru de Căușeni	13,7	1,3	9,4	0,26	3,14	7,8	576	27,4	7,96
9	Seină	12,7	1,4	6,1	0,40	3,59	7,9	454	21,3	7,84
10	Tămîioasă de Bohotin	14,1	1,6	7,7	0,26	3,46	9,3	634	25,5	8,09
11	Codrinschi (Stăuceni)	13,0	1,5	7,3	0,26	3,44	8,6	428	24,0	7,91
12	Codrinschi (Pleşeni)	14,9	2,1	7,5	0,26	3,43	9,5	633	27,1	8,02
13	Merlot (martor)	12,8	1,0	6,5	0,26	3,47	8,9	511	23,2	7,98

2,3 - Buthilenglycol ranging from 398 up to 848 m g/dm³, registering lower values only vinuril Bătută Neagră (215 mg/dm³), which has the lowest content in glycerol.

Unreducible extract reaches record 22.1 and 27.5 g/dm³ except varietal wines Bătută Neagră, Negru de Akkerman and Seină, where this indicator is only 17.2, 20.3 and 21.3 g/dm³ corresponding, which is not enough for a young red wine.

In Black wines Fetească Neagră, Rară Neagră, Kopceak, Negru de Căușeni, Busuioacă de Bohotin, Codrinschi Pleșeni unreducible dry extract exceeds 24.6 g/dm³.

Assessing organoleptic tasting samples from the Commission PSIHFT demonstrated that wine is healthy for their production have complied with the technological regimes. The highest organoleptic note 8.09 points (minimum grade being 7.80 points) was awarded the wine variety Tămîioasă de Bohotin (Busuioacă de Bohotin), which has a pale red to pink, an intense aroma with notes of basil, rose petals, fresh taste, with the same notes.

The lowest note organoleptic 7.71 points obtained Bătută Neagră wine variety, characterized by a dark pink, simple neutral flavor, taste simple, clean, acid, astringency typical red wines.

Brează varietal wines and Negru de Akkerman are characterized by open red to pink, clean aroma with floral and fruit nuances, but the taste is simple, aqueous (7.80 points). Sample of Ciorcuța Neagră variety (7.88 points) has a red, clean flavor, rich, full flavor, harmonious.

After physico-chemical composition closer to witness the variety Merlot wine stands Fetească Neagră, who stands with a dark ruby red color, aroma with notes of fruit and flowers, taste soft, full and was appreciated by 7.94 points. With a bright red color, with shades of solan flavor, slightly, fresh wine characterized Rară Neagră (7.80 points). Sample obtained from the variety Negru de Căușeni (7.96 points) has a dark red color, clean fruit flavor combined with slightly vegetal tones, full flavor, mining, acid, astringent phenolic potentially high. Codrinschi variety wine produced in the South was able to taste a touch higher (8.02 points) compared with that obtained in the Central region (7.91 points), because it highlights the rich aroma with notes of fruit, harmonious taste, mining, noble tannins. With a 7.92 grade point taste was appreciated Kopceak wine variety, characterized by a dark red, clean flavor, made with fruit tones, fresh taste fresh. Evidence - witness Merlot has a deep red color, rich aroma with notes of red fruit, taste extractive soft harmonious taste and obtained a grade of 7.98 points.

In red wines undergo major investigations were determined non-volatile organic acids, and the results of investigations are presented in Table 2.

The results show that the investigated red wines lactic acid shall not exceed 0.2 g/dm³ except wine variety Seină, which contains 1.0 g/dm³ lactic acid.

Succinic acid varies from 0.9 up to 1.2 g/dm³ and is within the limits described in the literature. Malic acid content is observed within the 1.3 to 2.8 g/dm³. Containing higher in malic acid (2.3 to 2.8 g/dm³) stands Kopceak varietal wines, Codrinschi (Stăuceni), Fetească Neagră and Brează. With lower values of malic acid (1.3 to 1.8 g/dm³) are distinguished varietal wines Bătuță Neagră, Merlot, Codrinschi (Pleseni), Seină, Ciorcuță Neagră and Negru de Akkerman.

On tartaric acid can be seen that the values of wider varies from 1.5 to 3.4 g/dm³ except wine Negru de Căușeni where this index is 4.1 g/dm³. It should be noted that the wines Bătuță Neagra, Negru de Akkerman, Negru de Căușeni and Codrinschi (Pleseni)

Table 2 Main organic organic acids content of red wines made from indigenous varieties, crop of 2011

Nr. d/o	Wine name	Organic acids, g/dm ³				
		Lactic	Succinic	Malic	Tartric	Citric
1	Bătuță neagră	0,1	1,0	1,3	3,4	0,1
2	Brează	0,2	1,0	2,4	2,1	0,2
3	Ciorcuță neagră	0,1	1,2	1,8	2,3	0,2
4	Fetească neagră	0,2	1,1	2,8	2,6	0,2
5	Rară neagră	0,2	0,9	2,1	1,7	0,3
6	Kopceak	0,1	1,1	2,3	2,8	0,2
7	Negru de Akkerman	0,2	0,9	1,7	3,4	0,2
8	Negru de Căușeni	0,1	1,0	2,1	4,1	0,3
9	Seină	1,0	1,1	1,7	2,4	0,3
10	Tămîioasă de Bohotin	0,2	1,1	2,2	1,4	0,3
11	Codrinschi (Stăuceni)	0,2	1,0	2,3	2,9	0,3
12	Codrinschi (Pleşeni)	0,2	1,2	1,4	3,1	0,3
13	Merlot (martor)	0,1	0,9	1,3	1,5	0,3

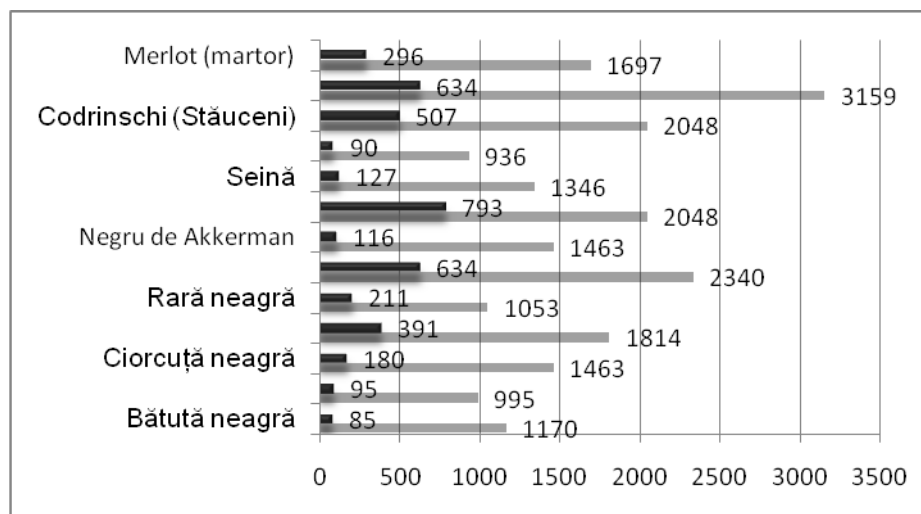


Fig. 1 phenolic substances and anthocyanin content of red wines from indigenous varieties, crop of 2011

■ - anthocyanins, mg/dm³

■ - Phenolic substances, mg/dm³

content tartaric acid is 2 times higher than malic acid content, and wines Tămîioasă de Bohotin, Rară Neagră, Brează and Feteasca Neagră tartaric acid content is less than malic acid content.

Containing almost equal tartaric and malic acids witness stands Merlot wine (1.5 and 1.3 g/dm³ respectively).

Citric acid in wines under study does not exceed 0.3 g/dm³.

Research in phenolic and anthocyanin content substances has shown that red wines from indigenous varieties are distinguished with different content in these compounds shown in Figure 1. It should be noted that the wine from Codrinschi Pleșeni is characterized by the highest potential of phenolic substances - 3159 mg/dm³, followed by varieties Kopceak (2340 mg/dm³) and Codrinschi from the Central region and Negru de Causeni (2048 mg/dm³). The highest anthocyanin content of the varieties mentioned above are recorded in wine variety Negru de Causeni - 793 mg/dm³. Feteasca Neagră wine variety has potential biological environment phenolic compounds - 1814 mg/dm³ and 391 mg/dm³ anthocyanin phenolic substances, hovering closer to wine - Merlot witness - in 1697 and 296mg/dm³.

Conclusions

1. Investigated phenolic substances in wine anthocyanins varies very broad:

- phenolic substances - from 936 up to 3159 mg/dm³;
- anthocyanins - from 90 up to 793 mg/dm³

With a high content in phenolic substances in red wine anthocyanins differ native varieties: • Codrinschi (Pleseni); • Negru de Caușeni; • Kopceak; • Codrinschi (Stăuceni); • Fetească Neagră; Rară Neagră and Busuioacă de Bohotin have a more modest phenolic compounds.

2. Wines Fetească Neagră, Codrinschi, South, Negru de Căușeni, Rară Neagră are subject to investigations of physical and chemical composition that meets the requirements of normative documents submitted to this category of wines and can be recommended for production for widening assortment of moldovan wines.

3. Investigated the varieties currently can not be recommended for the production of red wines varieties: Bătută Neagră, Brează, Seină and Negru de Akkerman.

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NUT BEER AS A FUNCTIONAL DRINK

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Abstract: Existing developments of beer enrichment by unconventional vegetable raw materials have been reviewed to improve the physicochemical, organoleptic, antioxidant properties of the beer. An additional vegetable raw material for the manufacturing of the original beer has been suggested and the chemical analysis of the extract has been conducted.

Keywords: original beer, herbal alternative, walnut, new sorts of the beer.

In recent years, despite the difficult economic conditions in Ukraine a very dynamic range of food industry has been developing, including production of beer and soft drinks. Nowadays the popularity of high-quality, expensive and middle class beer is growing. In order to maintain market position of their brands, manufacturers seek for the development new marketing and creative ways, including the introduction of special assortments of beer, such as the original.

The usage of beer alternative raw materials in the production of different types has long been known. Beers with different additives have their flavor and aroma, created often with the help of unconventional raw materials: ready soft drinks, fruit and herbs and spices that also make beers good for health.

Currently, as a non-traditional additives such various herbal products as honey, mineral salts, synthetic flavorings and other ingredients are being used. Beer with additives takes specific organoleptic and physico-chemical parameters and additives also affect its nutritional value [1]. Used in the beer manufacture innovative supplements correspond to different objectives in their application. Some of them perform technological functions, possessing bactericidal properties, contributing to the illumination beer wort (juniper, ginger, yarrow, nuts, etc.). [2]. The others form its organoleptic and physico-chemical, sometimes pharmacological properties, mitigating effects of alcohol on the body, increase the nutritional value of beer (food processing fruits, berries, honey, citruspeel, herbs, roots, etc.). [3].

Additives with technological purposes, are being used due to the presence of these carbohydrates, poly phenolic compounds which are similar in composition to hop, tannins, nitrogen and minerals. Additives are used in pharmacological purpose to create drinks that have a pronounced effect on the prevention of a disease (with anticarcinogen, hepatoprotective, antioxidant properties). Extracts of many plants which are used as additives often exhibit antioxidant effect [4].

The third group on purpose additives provides the formation setorganoleptic properties of beer. It can be made with the source of aromatic and flavoring substances (essential oils, essences, tinctures, extracts of aromatic raw materials) or products containing aromatic and flavor components (fermented base, soft drinks, wine, brandy, etc.) [5].

Recently, brewing very often use non-traditional vegetable raw materials. Promising plant material is black and green tea. Because the tea list is very rich in antioxidants, there is the possibility of using tea leaf extracts to enhance antioxidant properties of beer, as well as physical and chemical stability of the finished drink. Special attention should be devoted to green tea, which has more phenolic compounds than black.

Worthy of note is a development of national scientists who developed a special technology using ginger as conventional bio-active materials. Most of the technology is estimated to expand the range of beer, but the chemical composition of ginger indicates that in its application beverage enriched with compounds that provide the body antioxidant, immune-modulating properties required in terms of environmental pollution.

It is worth mentioning the development of Belgian scientists company Ajinomoto Natural Specialities, which is produced from oak wood (*Quercus* species) and leaf sumac tree (*Sumac*) purified and dried extract that positively affects the colloidal stability and taste of beer, precipitated metals with halotanin-protein complexes. These extracts tannins halo is only natural, recommended to the use of stabilizing materials [6].

In recent years, relevance of the creation of alcohol with drug infusions aromatic plants has increased: lemon balm, lavender, mint, sage etc. These extracts not only improve the organoleptic properties of products, but also enrich its biologically active substances (BAS), which makes it useful for human health.

An interesting perspective plant that grows in Ukraine is a walnut, which in its composition has all the substances that contribute to the stability of beer.

Walnut (*Juglans regia* L.) – a powerful rozkydyste family tree nut (*Juglans*) to 25-30 m tall. On older trunks the bark light gray, with cracks on the young - smooth. Leaves alternate, petiolate, odd. Leaflets elliptical or elongated, dark green above and light round bottom. Flowers unisexual, male, female - top, single are collected by 2-3. Fruit - false drupe. Flowers should be collected in May and June, fruits ripen in September.

Using walnut extract in brewing would provide stability, healing properties of the drink, and would provide antioxidant properties.

Leaves skin and amniotic walnuts contain high amounts of biologically active substances: substance that is easily oxidized in yuhlon, flavonoids (quercetin 3-arabinozid, 3-arabinozid kaempferol), aldehydes, essential oils, alkaloids, vitamins C, PP, carotene, fenolkarbon acid tannins, coumarins, flavonoids, anthocyanins, quin ones and hydrocarbons.

For example, extract amniotic pelts walnut in itself contains the necessary body substances. For example, vitamin C is required for normal connective tissue as well as for healing scars, quinine provides an tiarrhythmic effect, reduces the excitability of the heart muscle and also has a weak effect, ellagic acid according to some sources has such properties; yuhlon has antibacterial, antiseptic, antiparasitic, antioxidant, immunomodulatory, antitumor, restorative effect, regulates metabolism, has a moderate sugar decreasing effect, fungicidal (antifungal), choleric, salts, carotene is a precursor of vitamin A (retinol) and is a powerful antioxidant.

Infusion of amniotic pelts and Walnut leaves are used as an external means wound healing in the form of lotions, as a general agent, rinsing with inflammatory processes of the mouth and throat. Fresh crushed leaves of the walnut are applied to wounds to speed their healing. Immature nuts or nut partitions are used for the production of beverages, such as the production of tinctures, balsams and liqueurs [6].

Thus, balanced by amniotic peel walnut which can not only increase the nutritional value of the drink, but also provide optimal conditions for the life of yeast, together with the products of their exchange form quality organoleptic and physico-chemical properties of finished beverage. Making herbal beer additionally enriches with biologically active substances, organic acids, bioflavonoids, vitamins. Based on these data on the usefulness of the substances that make up the amniotic pelts walnut, it must be used in the production of special beers supplements in the form of water-alcohol extract.

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OPTIMISATION OF FATTY-ACID COMPOSITION OF VEGETABLE OILS

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Abstract: We researched the possible way to optimise vegetable oils in terms of number and ratio of essential fatty acids.

Such oil may be obtained through blending two or more vegetable oil with well-known fatty acids in proportions determined by the theory that has been empirically demonstrated.

Keywords: essential fatty acids, optimal ratio

Oil and fat are ubiquitous in food-stuff and represent one of the most important energy sources for humans. Vegetable and animal fats differ in types of fatty acids; those acid types are classified in saturated and unsaturated fats. Saturated fats are globally used for providing energy; an overrated use of them – namely taking more fat than needed by one's organism – leads to an increased level of low-density cholesterol and lipoprotein cholesterol in bloodstream, and results in cardiovascular diseases.

Amongst monounsaturated fatty acids the most common is oleic acid. Vegetable oils, rich in oleic acid, allow for a reduction of low-density lipoprotein in bloodstream; amongst technological advantages, one has to name a slower oxidation process.

Polyunsaturated acids have a particular significance in human food-stuff. These acids are irreplaceable and essential for they are not synthesized by the organism – they can only be ingested from food.

Polyunsaturated acids are divided into two groups: ω -3 and ω -6. The α -linolenic, eicosapentaenoic, and docosapentaenoic acids belong to the first group; while the linoleic acid, γ -linolenic, and arachidonic acids belong to the second. Polyunsaturated fatty acids are the building block of cell membranes and are used to ensure a steady development and adaptation of human organism in face of 'adverse' events from the environment. The lack of essential fatty acids is lacking stops the organism growth and may lead to serious diseases.

A minimum amount of polyunsaturated fatty acids is needed in food but one has to bear in mind an accurate ratio. With regard to the FAO/WHO recommendations the optimal ratio is ω -6: ω -3, from 5:1 to 10:1 [1]. If those fatty acids are not consumed according to the optimal ratio there is a high chance they will not be well assimilated by the organism resulting in a deficit in prostaglandins (hormone-like molecules that are crucial to regulating various processes in the organism).

Ω -6 fatty acids are present in almost all kind of oils. The main source of ω -3 fatty acids are mainly fatty fish and some sea-foods. As for vegetable oils ω -3 are present in linseed, soy-bean and crucifers-seed oils. Sunflower oil, which is the most consumed in the Republic of Moldova, does not contain any of such fatty acids.

To this point, it is to be noted that no natural oils present the fatty acid ω -6 and ω -3 ratio. The only oils that display this characteristic are synthetic (using selection or genetic

modifications of oilseeds). The surest way to creating oils with the right ratio of ω -6 and ω -3 polyunsaturated fatty acids is through blending vegetable oils according to the technique described hereafter: such oil may then be used for everyday consumption or be further processed. [2]

Our research's aim was to create a blended vegetable oil on the base of sunflower oil presenting the right ratio.

For optimising the quantity of fatty acids in sunflower oil through blending it is necessary to use other oils containing α -linolenic acid. Firstly, it is a flax oil in which according to data [3] depending on types and region of origin, flax may contain from 59.6% to 34.1% of α -linolenic acid. Besides, rapeseed oil may contain also quite high proportion of it (from 5.0% to 14.0%) and also soy-bean oil [4].

These oils (chosen for creating blended oils), were first researched into for containing fatty acids. Afterwards, some oil blends were calculated using Pearce, in accordance to which oil blends were created with a definite amount of fatty acids.

The very high proportion of fatty acids was determined via a method based upon turning fatty acid triglycerides into their methyl ethers and also upon a gas-chromatographic analysis of those former ethers.

During our research we used refined sunflower, rapeseed, and soy-bean oils, as well as non-refined flax oil. The fatty acid content of these oils is presented in table 1.

Table 1. Fatty acid composition of vegetable oils

Oil type	Proportion of each acid type in relation with triglyceride amount in oil, %						
	C _{14:0}	C _{16:0}	C _{16:1}	C _{18:0}	C _{18:1}	C _{18:2} ω -6	C _{18:3} ω -3
Sunflower	0.15	7.4	0.20	3.00	37.75	53.00	none
Flax	0.09	7.22	traces	1.58	23.40	19.20	48.5
Soy-bean	0.06	13.16	-	1.99	23.64	53.39	7.75
Rapeseed	0.10	5.19	traces	0.84	62.87	20.00	11.00

In compliance with obtained data we elaborated receipts that allows for an essential fatty acids content and we prepared compositions of vegetable oil blends presenting the optimal ratio between ω -3 and ω -6 fatty acids within the following boundaries: 1:5-10.

We determined factual content of fatty acids in obtained compositions and then we compared it to the estimated values that are presented in table 2.

These data demonstrate that the receipt estimation method we used allows for obtaining vegetable oil compositions containing the optimal ration of essential fatty acids.

Such oil may be obtained through blending two or more vegetable oil with well-known fatty acids in proportions determined by the theory that has been empirically demonstrated.

Table 2. Compositions of vegetable oils presenting the optimal ratio of ω -3 and ω -6 fatty acids

№	Composition content		Proportion of fatty acids in compositions			
	Oil type	Contain oil, %	Estimation		Fact	
			ω -3	ω -6	ω -3	ω -6
1	Sunflower Flaxseed	89 11	15.34	49.28	6.2	51.4
	ω -3: ω -6 ratio		1:9.2		1:8.3	
2	Sunflower Flaxseed	80 20	9.70	46.24	9.8	51.4
	ω -3: ω -6 ratio		1:4.8		1:5.2	
3	Sunflower Rapeseed	62,5 37,5	4.12	40.62	3.7	39.9
	ω -3: ω -6 ratio		1:9.9		1:10.8	
4	Sunflower Soy-bean	35 65	5.04	53.26	4.70	52.3
	ω -3: ω -6 ratio		1:10.6		1:11.1	
5	Sunflower Soy-bean, Rapeseed	48 36 16	4.46	44.6	4.35	46.2
	ω -3: ω -6 ratio		1:10		1:10.6	

Therefore, there is a possibility to create a vegetable oil of mass consumption, for example on the basis of sunflower oil with improved consumer properties.

Vegetable oil blend with optimal ratio of fatty acids ω -3 and ω -6 is a “healthy food product” whose utilization in adult and child nutrition can positively influence their health.

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A STUDY ON OXIDATIVE STABILITY OF VEGETABLE OIL BLENDS WITH OPTIMAL RATIO OF POLYUNSATURATED FATTY ACIDS

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Abstract: The characteristic of optimal fatty acid composition of vegetable oils is their balance by the ratio of polyunsaturated (essential) fatty acids, primarily linoleic and α -linolenic, referring respectively to the groups of ω -6 and ω -3. In accordance with the recommendations of physicians, the optimum ratio of ω -6 and ω -3 fatty acids is 5-10:1. There were elaborated recipes on the basis of which the vegetable oil blends with a specified ratio of ω -6 and ω -3 fatty acids were made from sunflower and corn oils with the addition of soy, rapeseed, linseed and grape oils with regard to the actual content of these acids. There was investigated the oxidation stability of the initial vegetable oils and their blends during storage in different conditions.

Key words: polyunsaturated fatty acids, optimal ratio

The characteristic of optimal fatty acid composition of vegetable oils is their balance by the ratio of polyunsaturated fatty acids, primarily linoleic and α -linolenic, referring respectively to the groups of ω -6 and ω -3. These acids are essential as they are not synthesized in the body and so must come from foods.

Polyunsaturated fatty acids are the building blocks of cell membranes and ensure the normal development and adaptation of the human organism to adverse environmental factors.

In the absence of essential fatty acid the growth of the organism stops, and some severe diseases may experience. Not only to the total essential fatty acids content is important in the human diet, but also their relationship. In accordance with the recommendations of [1], the optimum ratio of ω -6: ω -3 is from 5:1 to 10:1.

If these acids are not in the optimal ratio, they are poorly absorbed by the body and there is no full metabolism of prostaglandins - hormone-like molecules that are very important for the regulation of body processes.

Nearly all vegetable oils contain fatty acids of ω -6 group. The main source of ω -3 fatty acids are fatty fish and some seafood. As for the oils, the presence of ω -3 fatty acids is characteristic for linseed, soybean oil and also for oil from the seeds of crucifers. Sunflower oil, which is the most-used in the Republic of Moldova, does not contain such acids.

One of the areas of creating fat-based foods with balanced ratio of ω -6 and ω -3 fatty acids is obtaining vegetable oil blends and their subsequent use directly for food products manufacturing, and also as a fat basis for emulsion products (mayonnaise, sauces, etc.) [2].

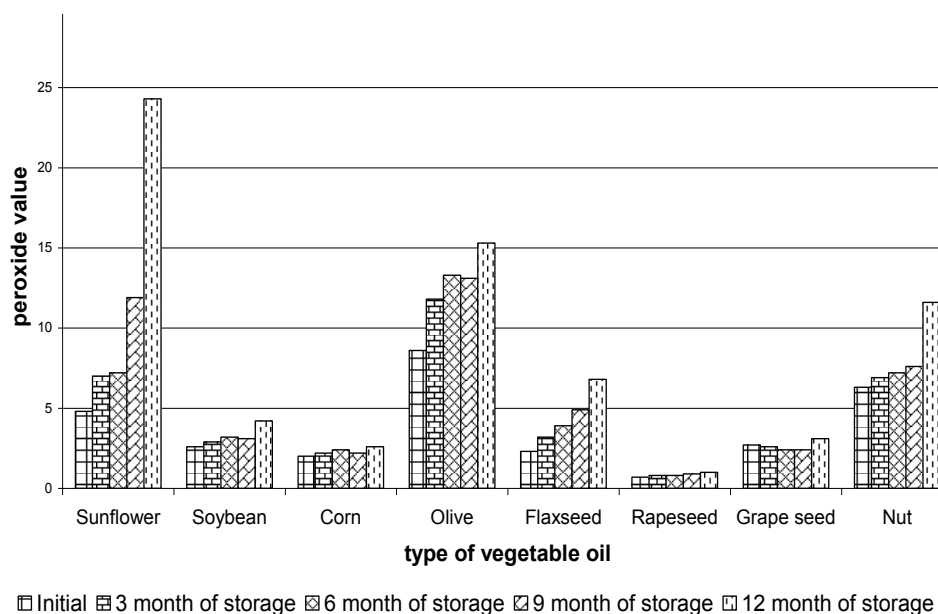
In drawing up such a vegetable oil blends the recipe quantity of each of the used oils can be calculated by dint of criss-cross rule on the basis of the specific content of ω -6 and ω -3 fatty acids.

With regard to the actual content of linoleic and α -linolenic acids vegetable oil blends were calculated and made from sunflower and corn oils with addition of soybean,

rapeseed, linseed and grape seed oils, with the required ratio of ω -6 and ω -3 fatty acids (Table 1).

In aim to study the oxidative stability of oil blends there was investigated the change of peroxide and acid values in initial oils and created oil blends during storage in the refrigerator and in the non-regulated temperature conditions - at room temperature.

The data regarding the changes of the main indicator of oxidative damage - peroxide value during refrigerator storage of oils are shown in Fig. 1. Analysis of the data shows that some vegetable oils are stable when stored under these conditions, peroxide value after one year of storage does not exceed the storage limit values – 10 milliequivalents of active oxygen/kg oil for refined oils and 15 milliequivalents – for cold-pressed oils (olive oil). Exception – is refined sunflower oil in which after one year of storage peroxide value is 2.4 times higher than the maximum allowable.



□ Initial ▨ 3 month of storage ▩ 6 month of storage ▤ 9 month of storage ▨ 12 month of storage

Fig. 1 Changes in peroxide value of vegetable oils during refrigerator storage

A great majority of the studied blends of vegetable oils are stable when stored – Fig. 2.

Exception – Blend 6 (sunflower, olive and canola), in which peroxide value after 9 months of storage increased by 8 times compared to the initial. Blends of sunflower and linseed oils also are not stable.

It is known that the oxidation stability of vegetable oils is due to the reactivity of the oxidizing agent (fatty-acid composition), to the presence of the inhibitor (tocopherols and other antioxidants) and to the oxidation initiators (peroxides, secondary oxidation products, transition metals, etc.). The latter compounds lead to an increase in the rate of oxidation [3].

Table 1. Vegetable oil blends with optimal ratio of polyunsaturated fatty acids ω -6 and ω -3

№ oil blend	Composition		Ratio between ω -3 and ω -6
	Vegetable oils	Proportion, %	
1	Sunflower Flaxseed	89 11	1:9,2
2	Sunflower Flaxseed	80 20	1:4,8
3	Sunflower Rapeseed	62,5 37,5	1:9,9
4	Sunflower Soybean	35 65	1:10,9
5	Sunflower Rapeseed Soybean	48 16 36	1:10
6	Sunflower Olive Rapeseed	59 6 35	1:10
7	Sunflower Grape seed Rapeseed	50 15 35	1:10,1
8	Corn Rapeseed	69 31	1:9,6
9	Corn Flaxseed	83,3 16,7	1:4,8
10	Corn Soybean	40 60	1:10,5

Content of tocopherols in vegetable oils resistant to oxidation - soybean, corn and canola oils is respectively mg/kg: 600-3370, 330-3720, 430-2680. In sunflower oil the content of tocopherols is below – 509-741 mg / kg [4].

In the olive oil the tocopherols content is at sunflower oil's level – 414-663 mg/kg, but it contains 2-3 times more oleic acid, which reduces the rate of oxidation [5].

In grape oil the tocopherols content is also small - 240-410 mg/kg, but this oil is the most resistant oil to oxidation. It can be explained by the fact that from the total tocopherols content 55-77% relates to tocotrienols - the strongest natural antioxidants, which are 40-60 times stronger than tocopherol [6]. So, both grape oil and its blends are stable.

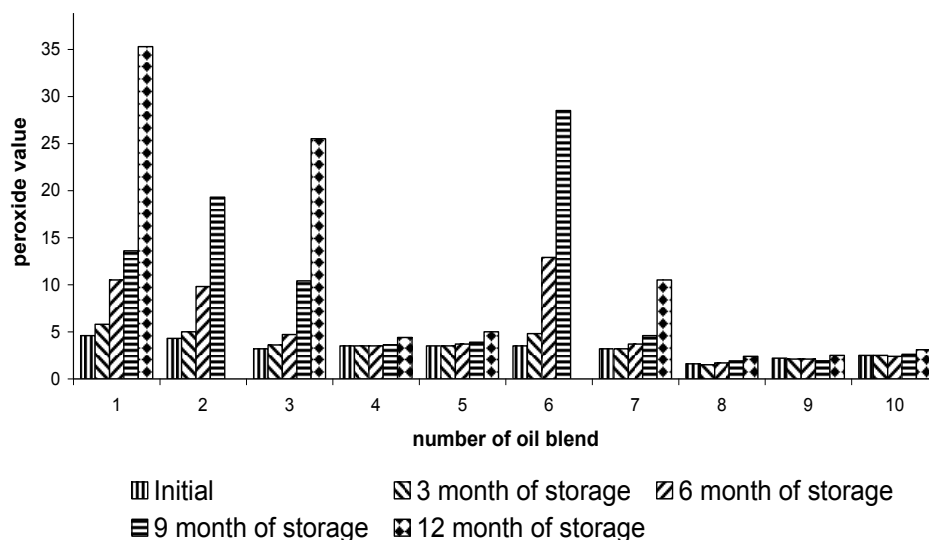


Fig. 2 Changes in peroxide value of vegetable oil blends during refrigerator storage

High stability is characteristic for vegetable oil blends based on corn oil, but not only with addition of soybean or canola oil, but also with linseed oil. This can be explained by the presence of tocotrienols in corn oil whose content can reach up to 700 mg/kg [4].

In another series of experiments there was investigated the oxidative stability of several oil blends stored at room temperature (Table 2). The obtained data suggest the high rate of oxidation of the oil blends based on sunflower oil. Blends based on corn oil are short-term stable; however, the duration of storage before reaching the limit peroxide value does not exceed 3 months.

Table 2. Changes in peroxide value of vegetable oil blends (during room-temperature storage)

Composition of oil blend	Peroxide value						
	Initial	During storage, weeks					
		2	4	6	8	10	12
Sunflower Flaxseed	6,8	8,0	8,9	11,6	13,5	14,7	15,6
Sunflower Rapeseed	3,9	4,5	4,7	5,8	8,3	13,7	18,8
Corn Flaxseed	3,0	3,3	3,7	4,2	5,6	8,0	14,4
Corn Soybean	3,0	3,4	3,5	3,7	4,0	5,3	8,9

In all studies, the objects are determined as acid value at the beginning and at the end of storage. This index practically has not changed during the studied methods and duration of storage. The acid values in all oil blends ranged between 0.2-0.5 mg KOH/g oil, which didn't exceed the limit value for refined oil – 0.6 mg KOH/g oil.

The results of the research can be used to justify the methods and shelf life of the vegetable oils blends with optimal ratio of polyunsaturated fatty acids.

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FUNCTIONAL BREAD WITH IODINE

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Abstract: A research has been conducted concerning the fortification of bread with iodine by using iodinated salt and iodine compounds in industrial conditions. Experimental samples of bread were manufactured. Physical-chemical, microbiological and sensory parameters were examined, as well as the iodine preservation rates.

The developed technology of iodinated bread was patented and was awarded the silver medal at the International Exhibition “Infinvent-2009” and also with the diploma of Great Mention “Invenția anului-2011”.

The collected data were used as a basis for the development of technology and industrial manufacturing of bread with guaranteed iodine content.

Key-words: iodine, technology, bread, industrial production

Introduction

The deficit of iodine is one of the most frequent alimentary diseases. According to the data of the World Health Organization, around 2 billion people on the Earth suffer from various degrees of iodine deficiency. The iodine insufficiency is caused by the low content of this microelement in potable water and foodstuffs conditioned by the particularities of geochemical composition of soils and by the alimentary patterns of population.

The natural environment of the Republic of Moldova is characterized by low values of iodine content: 4,5-5,3 mg/kg in the soil, 40 mcg/l in water and 0,03-0,22 mg/kg in vegetable products (recalculated to dry substance) - all these being a favoring factor for thyroid gland diseases. The standard food allowance of the country's population composed of traditional nutrients and ready dishes of sufficient caloric contents can not fully satisfy the need for all vitamins and minerals. Small quantities of iodine may be found in natural products (non-frozen sea products – sea wand, sea fish, shrimps, squid). Additional iodine consumption is assured by iodination of cooking salt and its use in the food industry [1], as well as by inclusion of iodine compounds into foodstuffs. This approach to the solution of the problem is well acknowledged in the entire world and is recommended by competent international organizations.

For the overwhelming majority of the country's population bread is a traditional foodstuff of mass consumption. Bread and bakery products occupy an important place in the people's food allowance and in this manner the enrichment of these foodstuffs with iodine is considered to be as the most rational perspective allowing to cover the widest social strata.

Aim of research

Elaboration of a technology of production of bread enriched with iodine. Investigation of the possibility of using iodinated salt and other iodine compounds permitted by the health protection authorities of the Republic of Moldova for the creation of a functional product with guaranteed iodine content.

Research methods

Technological research methods

For the conduction of works related to the enrichment of bread and bakery products with iodine a systemic approach was used to include: selection of iodine compounds, determination of their dosage; determination of compatibility of iodine compounds with the product components; simplicity and convenience of usage; study of the impact of iodine compounds on the bread quality, dough properties; preservation of iodine during the technological production process and during the storage of finite products, as well as the economic aspects of enrichment of bakery products with iodine [1].

The enrichment of bread with iodine was effected based on the following rule: every 200 – 250 g of consumed bread shall contain 75 mcg of bound iodine (for adults aged 12 and above), that corresponds to a half daily requirement for iodine. As possible iodine compounds for enrichment were considered: iodinated cooking salt (with KJO_3 supplement) – as per GOST 13830 and the following iodine compounds: potassium iodide (KI) – as per GOST 4232, potassium iodate (KJO_3) – as per GOST 4202, iodis-concentrate – ТУ У 14326060.003 (НПК «Йодис» Ukraine), iodine-casein – ТУ 9229-001-48363077-02 (НПП «Медбиофарм», Russia). The quantity of iodine containing supplement for the production of bread was determined based on the content of iodine in the compound itself minus the losses assumed during the production and storage of product.

The experimental samples of bread with use of iodinated salt and iodinated compounds based on the elaborated recipes and the reference samples of products were produced in industrial conditions on the bakery enterprise «ODIUS». The samples of finite products were packed into shrinkable film. Quality parameters were determined after baking as well as during 96 hours from production.

Physical-chemical and microbiological research methods

The physical-chemical parameters of bread and bakery products were determined in accordance with the following standardized methods: crumb humidity – as per GOST 21094; crumb acidity – as per GOST 5670; crumb porosity – as per GOST 5669; mass fraction of total iodine – as per IM 06.10/15.386 and GOST 13685; aerobic and elective anaerobe mesophilic organisms – as per GOST 10444.15; moulds – as per GOST 10444.12; potato disease of bread – as per SM 173;

Sensory evaluation

The sensory evaluation of finite products was performed by the Institute's Tasting Commission using a scale of 5 points.

Results and discussions

The content of iodine in the salt imported into the Republic of Moldova is at the level of 25 mgI/kg...35 mgI/kg of salt. Taking into consideration the salt consumption in the bread production prescribed by the respective recipes, the use of iodinated salt would provide a content of iodine in 200 g of bread at a level not exceeding 53% of the half-daily iodine requirement of an adult person. For this reason, in order to provide for a guaranteed half-daily requirement of iodine we have conducted a research of the possibility of combined use of iodinated salt and iodine supplements. The results of the said research are set out in the Tables.

The analysis of obtained data stipulates that the use of iodine preparations doesn't decrease the rated parameters of bread, including humidity, acidity and porosity of crumb compared to the reference alternative (see Table 1). Moreover, the use of potassium iodate improves considerably the properties of dough and gluten: thinning and smearing are reduced, a number of indicators is improved – specific volume, formation stability and porosity. In addition, the use of potassium iodide prevents the development of potato disease and moulds. During storage none of the samples displayed any signs of potato disease (table 2), that fully corresponds to the data provided in the literature [3].

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A high rate of iodine preservation was determined both in the bread production process with use of iodine supplements and during storage (70 to 95%). The content of iodine after three days of storage changes insignificantly compared to the initial values. The most evident loss of iodine was observed in the bread prepared with addition of bran where the crumb acidity was the highest (0,42% recalculated to lactic acid). This fact conforms to the data of iodine preservation in model systems where it was determined that the highest losses of iodine are observed in the system «iodine-casein - 1% acetic acid». The cooking salt exerts a protective action on the iodine preservation, while the organic acids tend to reduce it [4].

The use of iodine supplements increases the bread production costs somehow: use of potassium iodide or potassium iodate – by 1 bani, iodine-casein - by 4,5 bani, iodis-concentrate – by 41 bani.

Table 1. Results of microbiological analyses of bread performed during storage at temperatures 20-25°C, packed

Bread description/ sample number	Iodine preparation	Parameters							
		After baking			96 hours after baking			Iodine preservation n rate, % of input	
		crumb humidity, %	crumb acidity, deg	crumb porosity, %	crumb humidity, %	crumb acidity, deg	crumb porosity, %		
Top grade wheat flour bread - SM 173 / 1	-	33,0-46,0	2,5-3,5	min .65	33,0-46,0	2,5-3,5	Min. 65	-	
Top grade wheat flour bread, control / 2	-	41,1	3,3	74,8	40,4	3,2	74,4	-	
Top grade wheat flour bread / 3	KI	40,2	2,0	75,0	39,9	1,9	74,8	78	
Top grade wheat flour bread / 4	KIO ₃	40,8	2,3	76,2	40,0	2,2	76,0	80	
Top grade wheat flour bread / 5	KIO ₃	40,0	1,8	77,1	37,6	1,9	76,3	96	
Top grade wheat flour and bran bread / 6	KIO ₃	40,5	4,8	75,2	39,8	4,5	77,6	70	
Top grade wheat flour bread / 7	Iodineasein	41,2	2,0	73,4	39,9	1,8	72,0	95	
Top grade wheat flour bread / 8	Iodis- concentrate	40,9	1,8	72,9	39,9	1,8	71,8	96	
Wheat and rye flour bread- GOST 28807/ 9	-	41,0-53,0	5,5-12,0	min.46	41,0-53,0	5,5-12,0	Min. 46	-	
Rye and wheat flour bread experimental sample / 10	KI	41,1	6,0	66,7	41,0	6,0	66,4	91	
Rye and wheat flour bread experimental sample / 11	KIO ₃	41,1	5,5	67,4	41,0	5,5	65,8	94	

Table 2. Results of microbiological analyses of bread performed during storage at temperatures 20-25°C, packed.

Product	Indicators	Norm by SANPiN 2.3.2.1078-01	Seeding value during storage				
			Background	24 hours	48 hours	72 hours	96 hours
Sample 3	Coliforms, mesophilic, anaerobe and opportunistic anaerobe germs, KOE/cm ³	No more than 1x10 ³	-	-	-	-	-
	Coliform group germs / 1g	Not allowed	not determined	not determined	not determined	not determined	not determined
	Moulds, KOE/ cm ³	No more than 50	< 1x10	< 1x10	< 1x10	< 1x10	< 1x10
	Potato disease	Not allowed	not determined	not determined	not determined	not determined	not determined
Sample 6	Coliforms, mesophilic, anaerobe and opportunistic anaerobe germs, KOE/cm ³	No more than 1x10 ³	-	5,2x10 ¹	5,0x10 ¹	4,4x10 ¹	4,5x10 ¹
	Coliform group germs / 1g	Not allowed	not determined	not determined	not determined	not determined	not determined
	Moulds, KOE/ cm ³	No more than 50	< 1x10	< 1x10	< 1x10	< 1x10	< 1x10
	Potato disease	Not allowed	not determined	not determined	not determined	not determined	not determined
Sample 10	Coliforms, mesophilic, anaerobe and opportunistic anaerobe germs, KOE/cm ³	No more than 1x10 ³	8,2x10 ¹	8,0x10 ¹	7,5x10 ¹	6,9x10 ¹	6,2x10 ¹
	Coliform group germs / 1g	Not allowed	not determined	not determined	not determined	not determined	not determined
	Moulds, KOE/ cm ³	No more than 50	< 1x10	< 1x10	< 1x10	< 1x10	< 1x10
	Potato disease	Not allowed	not determined	not determined	not determined	not determined	not determined

The developed technology of iodinated bread was patented and was awarded the silver medal at the International Exhibition “Infinvent-2009” and also with the diploma of Great Mention “Invenția anului-2011”.

Conclusions

The obtained results allow us to make a conclusion that the use of iodine preparations doesn't alter the organoleptic quality parameters of the bread enriched with iodine compared to the reference samples, fully confirming the compatibility of iodine supplements with the components of dough and bread specified by the applicable recipes and the opportunity of their use in the industrial production of bread. The data obtained in the result of the performed research may be used as basis for the organization of industrial production of bread with a guaranteed iodine content in the Republic of Moldova.

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MODERN EXPERIENCE OF THE USE OF IODINATED SALT IN THE PRODUCTION OF CANNED VEGETABLES

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Abstract: In this work the results of researches on the utilization of the iodated salt at the production in industrial conditions of the tinned food „Red natural beans”, „Whine natural beans”, „Canned green peas”, „Canned sweet corn”, „Pickled cucumbers”, „Salted tomatoes” and „Salted cucumbers” are presented. There have been settled the indices of their quality (exterior factors, physico-chemical factors, microbiological factors, factors of safety) in comparison with the control samples of the same production that was made using the common salt. The obtained results are to be used in the decision taking on the general use of the iodated salt in the canned goods industry of the Republic of Moldova.

Key words: iodine salt, tinned food, industrial production

Introduction

Water and soil in the Republic of Moldova are known for their low iodine content. The low content of iodine in foodstuffs is not sufficient for covering the people's need for iodine. The country's population, as a rule, consumes foodstuffs produced on their individual farms or foodstuffs produced from local raw materials containing insufficient quantities of iodine. Given all above mentioned, it is urgently required to enrich the foodstuffs with iodine in order to reduce the iodine deficiency and the incidence of diseases caused by it. The Government of the Republic of Moldova has initiated a series of activities aimed to liquidate the iodine deficiency. In the year 2011 was adopted the second National Program of Iodine Deficiency Liquidation until the year 2015 [1].

Regardless of the fact that in many countries (Switzerland, Germany, Poland, Hungary, Slovakia, Denmark and others.) the cooking salt is subject to compulsory iodination [2, 3, 4], the specialists of production enterprises and the consumers of the Republic of Moldova are critical to the use of iodinated salt for the production of foodstuffs and namely canned foodstuffs due to the fact that iodine may alter the color of canned vegetables, soak the tissues of vegetables in the pickled or salted finite products, especially tomatoes, cucumbers, cabbage, paprika, gourds and others.

In order to obtain the necessary arguments to recommend the enterprises of canning industry and the consumers to use iodinated salt, the Scientific-Practical Institute of Horticulture and Food Technologies of the Republic of Moldova has conducted tests of canned products within the shelf life periods provided for in the applicable standards.

The aim of this research is determination of the possibility of using iodinated salt for the production of canned vegetables by the enterprises of the Republic of Moldova.

Canned vegetables (canned sweet corn, natural red beans, natural white beans, canned green peas, marinated cucumbers, salted cucumbers, salted tomatoes) produced with the use of iodinated salt – experimental lots. Products produced in accordance with the prevailing normative documents were used as reference samples.

There were selected the following places for the research activities: Practical Scientific Institute of Horticulture and Food Technologies of the Republic of Moldova;

enterprises of the food canning industry of the Republic of Moldova (S.A. „Cosnita Canning Factory”; S.A. „Alfa-Nistru” Soroca; F.C. „Mercon” Mereni village, LLC „Gutarom” Budesti village, S.A. „Natur-Bravo” Cupcini village); Center of Standardization and Determination of Quality of Canned Foodstuffs.

Methods and materials

The activities in the view of determining the possibility of using iodinated salt in the production of canned vegetables included: analysis of the assortment of canned vegetables produced in the Republic of Moldova with use of salt; determination and selection of the most vulnerable (from the producers' point of view) assortment of canned foods; production of canned vegetables in industrial conditions with use of iodinated salt – experimental samples and reference samples based on traditional methodology; determination of criteria and quality characteristics of canned foods to be analyzed during the production and storage; determination of the periodicity of quality assessments.

The storage of canned products was arranged for by and at the respective producers at the prescribed temperature and relative humidity of air.

Physical-chemical, microbiological and organoleptic properties considered upon the determination of quality of the canned vegetables.

The research described above was performed in accordance with the standardized norms and methodologies accepted in the scientific practice. In accordance with the respective standards were determined the external appearance of packaging, the tightness of packaging and metallic cans, net weight, mass fractions; pH, optical density of covering liquid at $\lambda = 540$ nm, mass fractions of cooking salt, titrable acids, mineral and vegetal impurities, iodine content in the cooking salt and in the final product, dry soluble substances, foreign bodies, mercury, arsenic, copper, cadmium, zinc, lead and tin. Besides all these, microbiological analyses have been performed to determine the number of germs from the group of intestinal Coliforms (E Coli count).

The basic criterion for the determination of the possibility of using iodinated salt for the production of canned foods in the case of conformity with all the standardized characteristics has been the organoleptical one: external appearance, taste, smell, consistence measured by a system of 5 points - all these were determined during the open sessions of the Institute's Tasting Commission with the participation of interested organizations (representatives of producers, of the Ministry of Health, of the Ministry of Agriculture and Food Industry, of the Service for Standardization and Metrology, of the National Institute for Standardization and Metrology, as well as of the Ministry of Economy and Commerce, UNICEF, mass-media, etc.).

Results

Both the tested and the reference samples were produced of the same raw materials, following the same technological scheme, with observation of all requirements to the preparation and processing of raw materials, sterilization and storage. The content of salt and other ingredients was identical both in the experimental and the reference samples.

The mass fraction of iodine in the iodinated salt used for the production of canned vegetables „Sweet corn” was 32,0 mg/kg; „Red natural beans” – 34,4 mg/kg; „White natural beans” – 32,8 mg/kg; „Salted tomatoes” – 31,7 mg/kg; „Canned green peas” - 35,7 mg/kg; „Picked cucumbers” – 37,0 mg/kg; „Salted cucumbers” – 31,2 mg/kg.

The analysis of quality characteristics was performed at the canning factory and after one year from the date of production, on quarterly basis (for salted products – after six months of storage).

The comparative analysis of physical-chemical standardized properties of the experimental and reference samples, including the safety aspects of the cans investigated during their normal shelf life has demonstrated full compliance with the provisions of normative documentation. The optical density of the solutions covering the top of the can contents diluted to 20 times is approximately at the same level both for the experimental and reference samples.

The results of organoleptic evaluation obtained by tasting of samples of canned products during their storage period are set out in the Table 2.

The results of organoleptic evaluation confirm the absence of any differences between the samples produced with iodinated salt and usual salt.

The members of the tasting commission and the invitees have mentioned that the use of iodinated salt did not affect the organoleptic properties of the products and were unable to detect that iodinated salt was used for the production of any particular product.

The iodine preservation rates in the canned products were measured during the entire storage period of the considered products. The results set out in the Table 3 imply that around 44-58% of the initial quantity of iodine is preserved in the products.

Conclusions

The preliminary results have demonstrated that pursuant to the analyses of physical-chemical and microbiological characteristics, as well as based on the results of safety tests conducted during the storage period no noticeable difference has been established between the products prepared with iodinated salt and the ones prepared with usual salt.

The analysis of organoleptic characteristics has demonstrated that the iodinated salt doesn't affect the consistence, the color, taste and smell of the considered canned products during storage.

The research of the quality of experimental and reference samples will be continued for the entire unexpired guaranteed shelf life of the considered products.

Table 1. Some physical-chemical characteristics of cans

Parameters	Normal value as set out in normative documents	Values			
		Initial lot value		After storage during one year	
		experimental	control	experimental	control
1	2	3	4	5	6
„Canned sweet corn”, S.A. „Canning Factory of Cosnita” (in metallic cans of 425 ml)					
Mass fraction of iodine, mg/kg	no standard value	0,58	0,13	0,30	-
Mass fraction of corn in the net weight, %	min. 60	82	83	81	84
Mass fraction of sodium chloride, %	0,8-1,5	1,5	1,4	1,5	1,4
Titrateable acidity, %	no standard value	0,10	0,10	0,12	0,15

1	2	3	4	5	6
Optical density of covering solution at $\lambda = 540$ nm	no standard value	0,083	0,075	0,077	0,075
„Whine natural beans”, S.A. „Alfa-Nistru” Soroca (in metallic cans of 425 ml)					
Mass fraction of iodine, mg/kg	no standard value	0,52	0,17	0,47	-
Mass fraction of beans in the net weight, %	min. 50	56	56	55	53
Mass fraction of sodium chloride, %	1,0-1,8	1,3	1,3	1,3	1,3
Titrateable acidity, %	no standard value	0,10	0,10	0,08	0,07
Optical density of covering solution at $\lambda = 540$ nm	no standard value	0,313	0,310	0,310	0,310
„Canned green peas”, S.A. „Canning Factory of Cosnita” (in glass pots of 720 ml)					
Mass fraction of iodine, mg/kg	no standard value	0,66	0,32	-	-
Mass fraction of peas in the net weight, %	min. 65	60	59	60	59
Mass fraction of sodium chloride, %	0,8-1,5	1,2	1,4	1,2	1,4
Optical density of covering solution at $\lambda = 540$ nm	no standard value	0,047	0,051	0,047	0,051
„Pickled cucumbers”, S.A. „Natur-Bravo” Cupcini in glass pots of 720 ml)					
Mass fraction of iodine, mg/kg	no standard value	0,73	0,25	0,47	-
Mass fraction of vegetables in the net weight, %	min. 50	51	51	51	51
Mass fraction of sodium chloride, %	1,5-2,0	2,0	1,8	2,0	1,8
Titrateable acidity, %	0,5-0,7	0,6	0,5	0,6	0,5
Optical density of covering solution at $\lambda = 540$ nm	no standard value	0,009	0,008	0,009	0,008
„Salted cucumbers”, LLC „Gutarom” Budesti (in plastic barrels of 70 kg)					
Mass fraction of iodine, mg/kg	no standard value	1,54	0,32	0,65	-
Mass fraction of cucumbers in the net weight, %	min. 50	81	80	84	75
Mass fraction of sodium chloride, %	2,5-3,5	2,5	2,6	2,5	2,6
Titrateable acidity, %	0,6-1,2	0,5	0,5	0,6	0,6
Optical density of covering solution at $\lambda = 540$ nm	no standard value	0,055	0,055	0,060	0,060

Table 2. General organoleptic evaluation of samples of canned products

No.	Samples	General tasting mark	
		experimental	Reference
1	Canned Sweet corn	4,7	4,7
2	Red natural beans	4,7	4,8
3	White natural beans	4,8	4,8
4	Salted tomatoes	4,6	4,6
5	Green peas in metallic cans	4,9	4,8
6	Green peas in glass pots	4,8	4,8
7	Pickled cucumbers	4,9	4,9
8	Salted cucumbers	4,9	4,6

Table 3. Integration of iodine in the industry samples of canning products at the end of term of guaranteed storage

No.	Products	Storage period, months	Mass fraction of iodine, µg /100g of product	Preservation of iodine, %
1	Sweet corn	19	34	58
2	Red natural beans	19	28	54
3	White natural beans	14	27	52
4	Pickled cucumbers	10	42	58
5	Salted cucumbers	10	42	44
6	Salted tomatoes	10	62	44

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PROSPECTS OF THE USE OF VITAMIN AND MINERAL COMPLEXES DEVELOPED FOR ELIMINATION OF IRON AND ACID FOLIC DEFICIENCY IN THE REPUBLIC OF MOLDOVA

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Abstract: Practical Scientific Institute of Horticulture and Food Technology performs activities to implement the National Programme 2012 on approval of measures to reduce diseases caused by deficiency of iron and folic acid. There were developed two groups of vitamin and mineral complexes for fortification of the superior quality wheat flour in the Laboratory of Functional Foods: I type - complexes of vitamins and minerals including iron in its various forms with vitamins (B1, B2, B6, B9, PP) and type II - iron complexes including its various forms and folic acid (B9). Under industrial conditions fortified flour was prepared and analyzed for the establishment of shelf life and quality parameters. Tasting industrial bakery samples made from fortified wheat flour and regular flour mentioned the identity of organoleptic characteristics, including appearance (shape, size and color) pulp status, taste and smell. Consumption of 250 g bread made from superior quality wheat flour additionally fortified, will allow to meet the daily needs of vitamins in human nutrition up to 30% and of iron up to 40%.

Key words: flour fortification, vitamins, minerals, complexes

Epidemiological surveys carried by UNICEF and the Ministry of Health of the Republic of Moldova indicate the lack of micronutrients affecting significant part of youth and adult population. Micronutrient deficiency in Moldova hasn't been revealed in a limited category of children and adults; it is typical for almost all populations in all regions of the country:

- More than 30% of children aged 6 to 24 months are at the risk of under-development of the brain due to lack of iron;
- From 75 to 100 infants die each year because of the tough anemia during pregnancy;
- Each year, approximately 50 children are born with abnormalities, including with infantile paralysis due to a deficiency of folic acid;
- Reason for the increase in adult mortality from heart disease and myocardial infarction is a lack of folic acid;
- Because of the adults' loss of productivity due to lack of iron and iodine losses in particular in the Republic of Moldova are about 21.4 million U.S. dollars per year, or 0.7% of GDP [1].

The most effective and affordable way of micronutrients and vitamins availability is further enrichment of foodstuff of mass consumption such as, flour and bakery products with these compounds .

Fortification of food products should not worsen their quality and consumer characteristics, for example to change significantly the taste and digestibility of the other important nutrients found in them, to reduce the period of storage, to change the safety performance of food products, etc.

One of the important micronutrients is iron; the various forms of iron deficiency within Moldovan population are over 60%. This population segment suffers from lack of vitamins B₁, B₂, B₆, PP, folic acid, vitamins C, which are involved in various stages of absorption and metabolism of iron in the body.

Food fortification with iron is a difficult task, since the transition metals, readily catalyzes the oxidation processes, such as lipid peroxidation, thereby expediting the rancidity of fats, flour spoilage during storage and destruction of a number of vitamins [2].

There is a wide range of vitamin and mineral supplements for the enrichment of flour and bakery products in world practice. More often as a source of iron is used: electrolytic iron Fe⁰; sulphate monohydrate Fe₂+FeSO₄•H₂O; Fe₂+sulfate heptahydrate FeSO₄•7H₂O.

It is known that, for example, heptahydrate of iron in the presence of a small amount of moisture (water activity values $a_w = 0.52$) can influence the lipase and lipoxygenase and initiate flour lipid oxidation [3].

The use of iron in this form is undesirable because it can lead to an acceleration of oxidative damage and reduce the shelf life of flour.

The aim of this work was to determine the effect of various forms of iron compounds, in combination with the introduction of vitamins on the quality of enriched flour.

Flour fortification was made in accordance with the recommendations of [4-7], and the doses recommended by the manufacturers, and considering the initial content of iron in flour.

Fortification of flour was carried out based on the satisfaction of 30-50% of the adult daily requirement of iron and vitamins from the consumption of 200-250 grams of bread. The daily recommended dose for adults is: in iron – 14 mg, vitamin B₁ – 1.4 mg, vitamin E – 18 mg and folic acid – 200 micrograms.

For research was used the wheat flour, freshly ground, obtained in a mill plant district from the Chadyr-Lunga district of the Republic of Moldova.

Flour fortification was conducting using the following vitamin-mineral complexes: "Kolosok-1" – Russia (sample 1), "Fortamin" – Russia (sample 2), "KAP-KOMPLEX-1" – USA (sample 3). As a control (sample 4) was used the original flour without added mineral and vitamin complexes.

Premixes "Fortamin" and "Kolosok-1" contain the following components: iron sulphate Fe₂+monohydrate FeSO₄•H₂O, thiamine (B₁), riboflavin (B₂), pyridoxine (B₆), niacin (PP), folic acid; "KAP-KOMPLEX-1" – electrolytic iron Fe⁰, thiamine (B₁), riboflavin (B₂), pyridoxine (B₆), niacin (PP), folic acid, and are additionally enriched with zinc.

These systems are designed for flour fortification at the stage of production, and also for the enrichment of flour at the stage of kneading the dough.

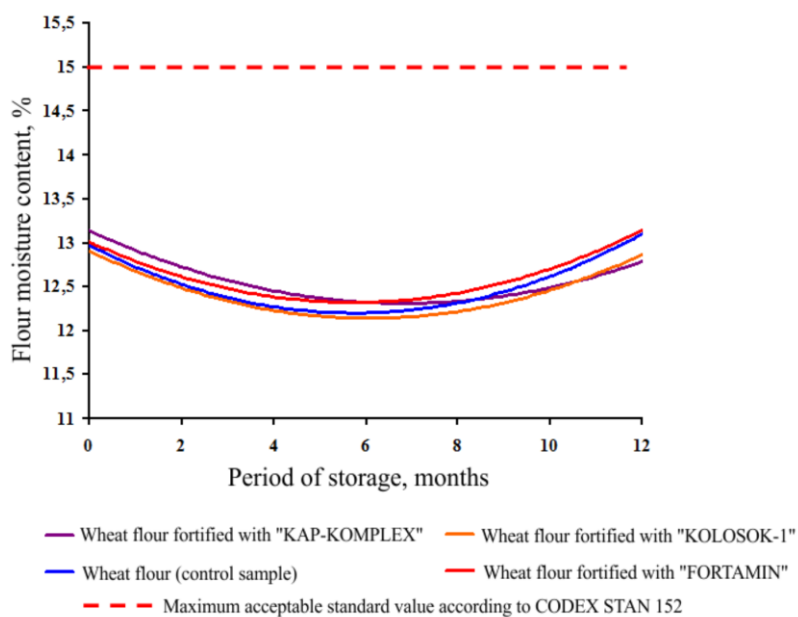


Fig. 1 Changes in moisture content of investigated wheat flour during storage

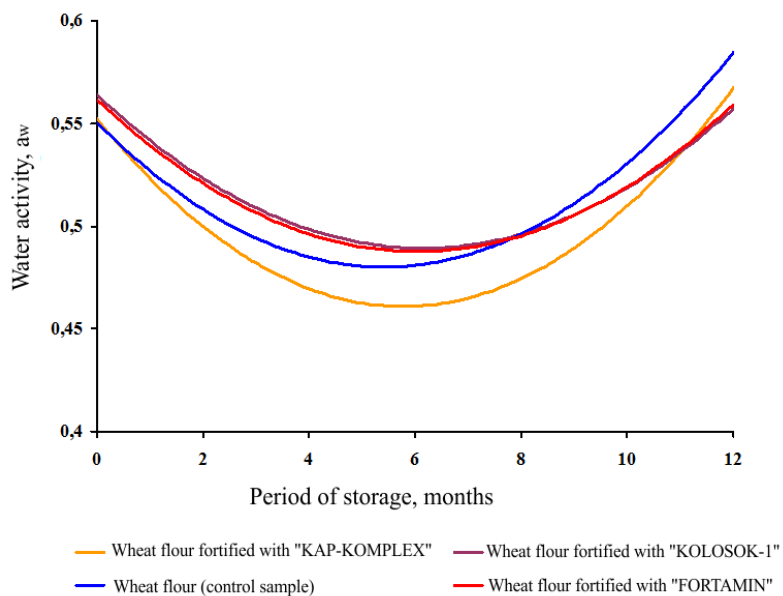


Fig. 2 Changes in water activity level of investigated wheat flour during storage

Storage process is implemented in storage production facilities under unregulated conditions for 12 months of guaranteed storage. Periodicity of the flour quality monitoring (organoleptic, physical, chemical and microbiological parameters) – once in 2 months.

It was found that during the storage of flour organoleptic characteristics (taste, odor, and color) have not changed, both for control and experimental samples.

Microbiological parameters for all the flour samples throughout the research period were similar and did not exceed the standards set for the instant flour for child nutrition, fortified with vitamins and minerals [8].

Flour humidity for all of the samples throughout the storage period was in line with regulatory requirements and was at the level of 12.0% -13.1% (Figure 1).

There was an increase of acidity of the flour from 2.7 degrees to 3.2-3.3 degrees during storage for all samples, including the control samples (Fig. 3), but the organoleptic evidence of food spoilage wasn't revealed.

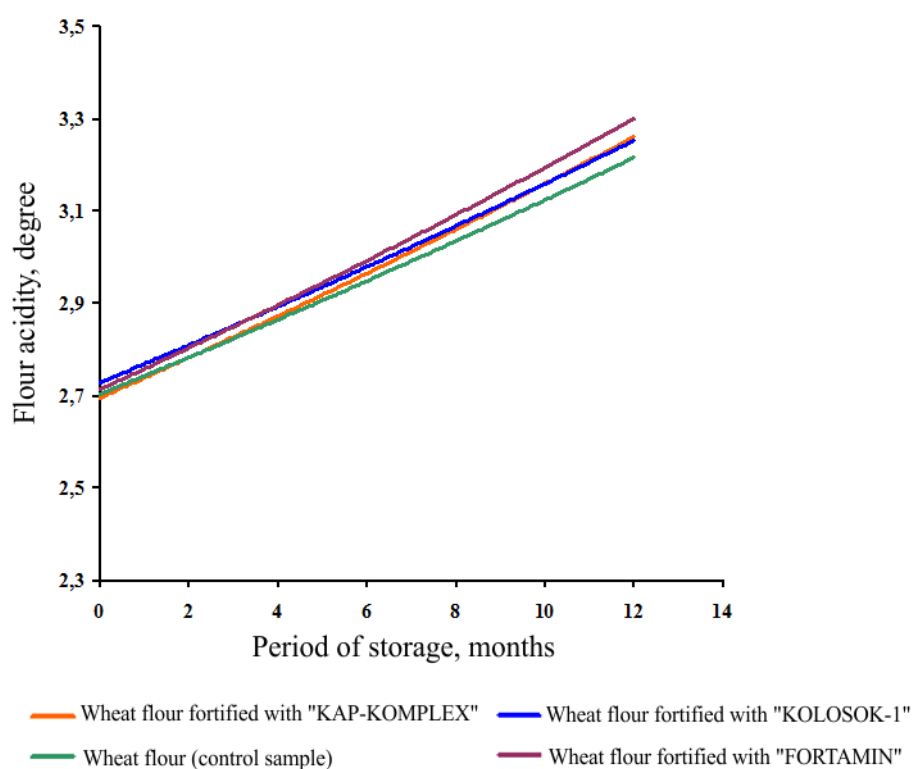


Fig. 3 Changes in acidity of investigated wheat flour during storage

Acid number of fats for wheat flour according to [3] should not exceed 50 mg KOH/100 g dry matter.

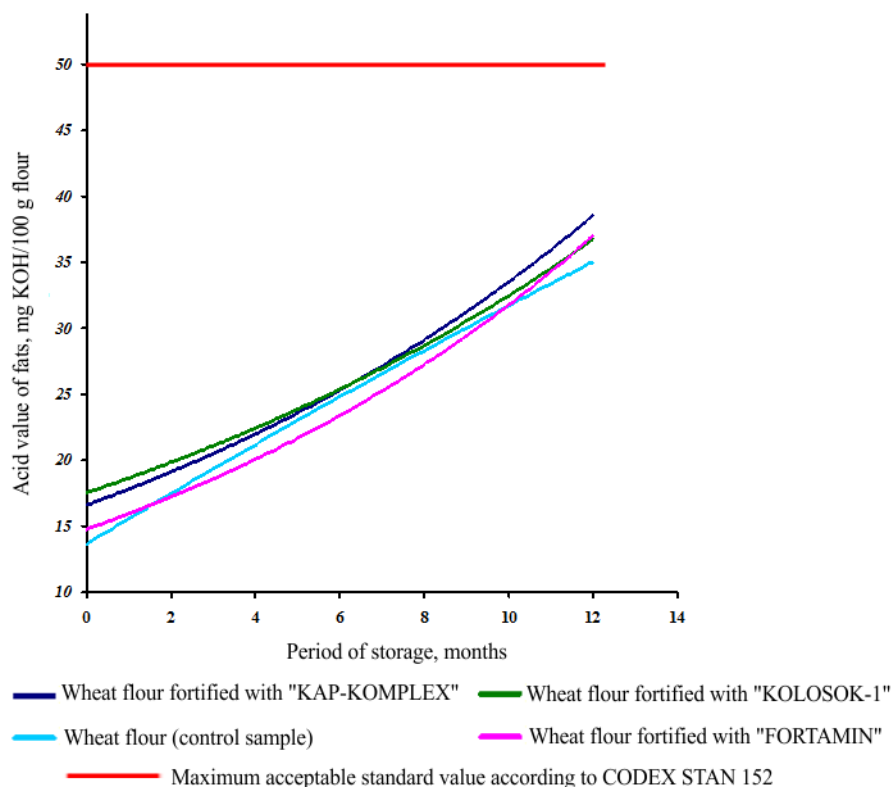


Fig. 4 Influence of vitamin-mineral complexes

There was observed an increase of acid number of fats both for the control and experimental samples of the flour. Acid number of fats of the investigated flour within 12 months of storage increased: for the control by 2.2, fortified flour - by 2.0-2.3 times, while its value is lower than the rated limit (Fig. 4). For the control sample this index does not exceed 65% from the rated value, for the flour enriched with "KolosoK - 1" – 66%, with "Fortamin" – 65%, and with "CAP KOMPEKS" – 68%.

Mass fraction of nicotinic acid during storage varied slightly and was at baseline, both in the control and experimental samples of flour.

During the storage of flour there weren't found any significant effects of the studied vitamin-mineral complexes on the organoleptic and microbiological parameters of the enriched flour.

Storage process is characterized by increasing acidity and acid number of fats in the wheat flour enriched with vitamin and mineral complexes.

Acid numbers of fats during storage increased both for the control and experimental samples, however, for the studied complexes, these indexes do not exceed levels established by CODEX STAN 152 FOR WHEAT FLOUR.

Research is currently being conducted on establishing quality of the wheat flour enriched with a complex containing various forms of iron and folic acid.

Obtained results suggest the possibility of using the studied vitamin and mineral premixes for the fortification of the wheat flour with high consumer properties and guaranteed quality.

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RHEOLOGICAL PROPERTIES OF YOGURT WITH GERMINATEDSORIZFLOUR

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Abstract: Yoghurt with and without the addition of germinated soriz flour and Danisco stabilizer has a behavior similar to that of rheological pseudoplastic newtonian fluids. Soriz yogurt supplementation with germinated soriz flour and Danisco stabilizer changes the network structure and the permeability of curd, reduce essentially the process of syneresis and increases viscosity. The viscosity modification of yogurt supplemented with germinated soriz flour is determined by the weak interactions (hydrogen, hydrophobic, Van der Waals....) of yogurt with the flour proteins, which causes the rearrangement of solids structure of yogurt, particularly in large proportions of flour.

Keywords: yogurt, germinated soriz flour, syneresis, dynamic viscosity, apparent viscosity.

Introduction

The rheological properties are important indicators of the quality of food from the raw material during processing (for choosing the optimal scheme of work) and for consumer.

Structure and rheological properties of fermented dairy products are influenced by a number of factors: the quality of milk, pH, the nature of bacterial cultures, temperature, mechanical processing, fermentation time, additions. (Mullineux, 2008)

In the present paper as filler was used germinated soriz flour. Germinated soriz flour is a natural product and increases essential amino acid content in yogurt, vitamins (especially those from groups B and PP), vegetable fats, easily assimilated carbohydrates, dietary fibre, improves the nutritional and organoleptic properties of enriched dairy products. The flour can also serve as a replacement of some part of the yogurt stabilizers, which ensures the microstructure formation of the finished product. The price of germinated soriz flour is lower than that of the imported stabilizers.

Materials and methods

In studies conducted have used the following raw materials: standard cow's milk (2.5%), skimmed powder milk, sugar-candy, GRINDSTED-stabilizer SB258A (contains native E1422 acetylated diacid adipate and gelatin), starter bacteria (Danisco, Dania), *germinated soriz flour*.

Preparation of yogurt was made by the conventional technology of production, with small changes: *germinated soriz flour* with granular (particle size) 140 μ was mixed with a normalized part of milk at the rate of 1:10. Then flour and milk suspension was thermostated at a temperature of $60 \pm 2^\circ\text{C}$, for 30 minutes, filtered, and then administered in the mass of balanced milk caps along with other components of the recipe.

The study of rheological properties of yogurt were carried out at different concentrations of flour and stabilizer. Experimental variants are shown in Table 1.

Table 1. Experimental variants of recipes used to determine rheological properties of yogurt (2.5% fat)

	Product code	Filler content, %	
		Stabilizer	Germinated sorizflour
1	I+S0	-	-
2	I+S1	0,1	-
3	I+S2	0,2	-
4	I+S3	0,3	-
5	I+F0,5+S0	-	0,5
6	I+F0,5+S1	0,1	0,5
7	I+F0,5+S2	0,2	0,5
8	I+F0,5+S3	0,3	0,5
9	I+F1,5+S0	-	1,5
10	I+F1,5+S1	0,1	1,5
11	I+F1,5+S2	0,2	1,5
12	I+F1,5+S3	0,3	1,5
13	I+F2,5+S0	-	2,5
14	I+F2,5+S1	0,1	2,5
15	I+F2,5+S2	0,2	2,5
16	I+F2,5+S3	0,3	2,5

Yogurt viscosity has been determined by viscometric REOTEST RV. Tension shear of yogurt was calculated according to the formula:

$$\tau = \alpha \times Z, \quad (1)$$

where: τ - tension shear, Pa;

α – viscometric data on screen;

Z – constant cylinder, ($Z= 1,19$)

On the basis of the results obtained was calculated Dynamics viscosity (apparent) η ,

$$\eta = \tau / \gamma \times 100, \quad (2)$$

where: η - dynamics viscosity, Pa·s;

γ - shear rate, s^{-1} .

Determination of synereses index of yogurt included the separation and dosing the quantity of the exudate whey. For this yogurt samples (10g) were placed in centrifuge tubes and centrifuged for 5 minutes at the speed of 1000 revs/min. (H. Г. Меркулова, 2009)

Index of synereses has been determined from the relation:

$$S = m_z / m_p \times 100, \quad (4)$$

where: m_z – quantity of exudate whey, g;

m_p - the sample of yogurt, g;

Results and discussions

Yogurt synereses

Fermented dairy products are structured disperse systems as gels. Their chemical composition does not reflect their physico-chemical properties than at the level of constituent subunits distributed in ensembles with varying degrees of order.

Synereses is a biochemical phenomenon and physico-chemical complex, still unknown and represents a thermodynamic property which consists of reducing the volume of the gel due to expulsion of vast quantities of solvent with its aging. *Syneresis* is actually a continuation of the clotting reaction to the maturing coagulation, which leads to increasing the cross-linking density of links in the contracting gel and the expulsion of solvent.

Synereses intensity and depth of yogurt depends largely on the internal surface of the solid phase, porosity (spaces occupied by whey) and permeability of gel. Gel porosity depends on the size and nature of association of the solid gel and the permeability of solid elements is dependent on the size, shape and pore size. In coagulants formed by acidifying lactic pores have a micellar character (not alveolar, as in the case of curd formed by rennet). In this case during *synereses* curd is relatively slow contracting (absence of forces which create forces of contracting), porosity continuously decreases, but remains relatively high permeability throughout the whole process thanks to the fact that the network is made up of demineralised casein. (Mahaut M., Jeantet R., Brule, 2000)

Supplement of yogurt with germinated soriz flour and stabilizer can change both the network structure and permeability of the curd and may affect the process of *synereses*.

The results characterizing the values of *synereses indexes* yogurt samples analyzed are shown in Figure 1.

Thus the value of *synereses* for plain yoghurt is 23,6%, and the flour with the addition of germinated soriz flour ranges from 8.2 to 14.1% and decreases with the increasing of admixture content. This indicates that the starch (and debatably protein) flour changes the structure and permeability of the gel network.

As for DANISCO stabilizer is a mixture of acetylated distarch adipate (obtained by crosslinking starch with adipic acid and acetic anhydride) and gelatin and is widely used in food industry as an artificial thickening agent, stabilizer, loading agent. Stabilizer in lower concentrations do not form gels, but it moisturizes and increases essentially viscosity and water retention in yogurt curd structure. Therefore, adding up to 0.3% of Danisco reduces the coefficient of *synereses* up to 0. Combining stabilizer with germinated soriz flour produces a synergistic effect and reduces the amount of stabilizer used from 0.3 to 0.2% and in some cases even up to 0.1%.

The ability to move through the pores of the whey is directly influenced by the viscosity of the liquid phase.

Viscosity of yogurt

Viscosity is an important index for technological process but also for the consumer.

Yoghurt is a fluid, i.e. possess a viscous fluid and elastic properties of a solid. The rheological behavior of yogurt is like that of a non-newtonian fluid.

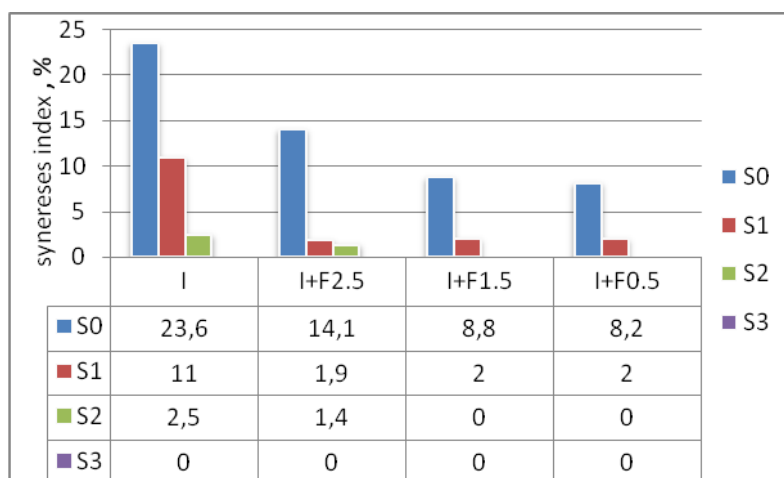


Fig. 1. Variation in the syneresis index (volume of exudate from syneresis) of yogurt samples depending on the amount of stabilizer (S) and added germinated sorghum flour (F)

In this respect the viscosity of product depends on the velocity of exerted shear. In the case of yogurt the viscosity decreases with the increasing shear rate, and the viscosity is characterized by the so-called apparent viscosity at a given shear velocity.

The most common technique used for food rheological fluid is viscometers, which measures the resistance to shear, expressed as the coefficient of viscosity. (Skiver A., 1995).

Analysis of reograms allows the appreciation of the product flow properties in a wide range of shear velocities and the viscometric characterization of the complete product.

Figure 2 shows the change in dynamic viscosity (η , Pa·s) depending on shear velocity ($\dot{\gamma}^{-1}$) yogurt samples 9 to 12 in Table 1. The analog results were obtained and for other types of recipes.

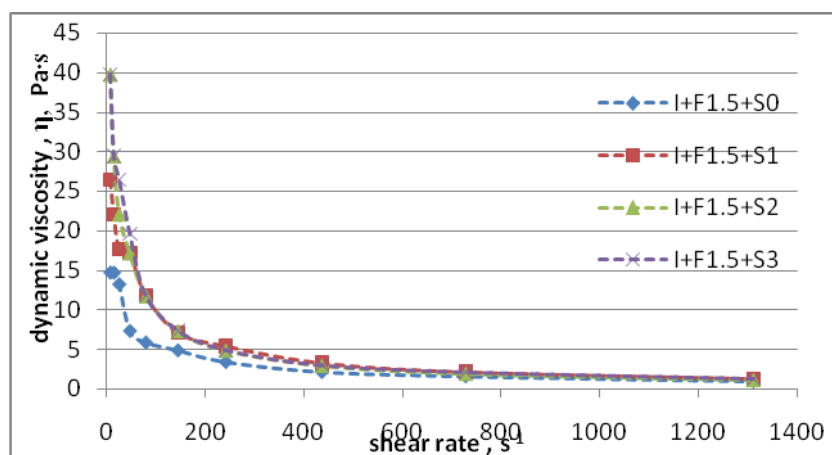


Fig. 2. Variation of dynamic viscosity depending on shear rate for yogurt samples with added flour 1.5% and stabilizer from 0.1 to 0.3%

For all samples of yogurt has found that they have a rheological behavior similar to that of rheological newtonienn fluids, independent of time, that is a pseudoplastic behaviour. Characteristic for a fluid with such behavior is lowering resistance to flow as a result of the increase in the velocity of fluid shear. Values of apparent viscosity to velocity shear 243000 s^{-1} are shown in Figure 3.

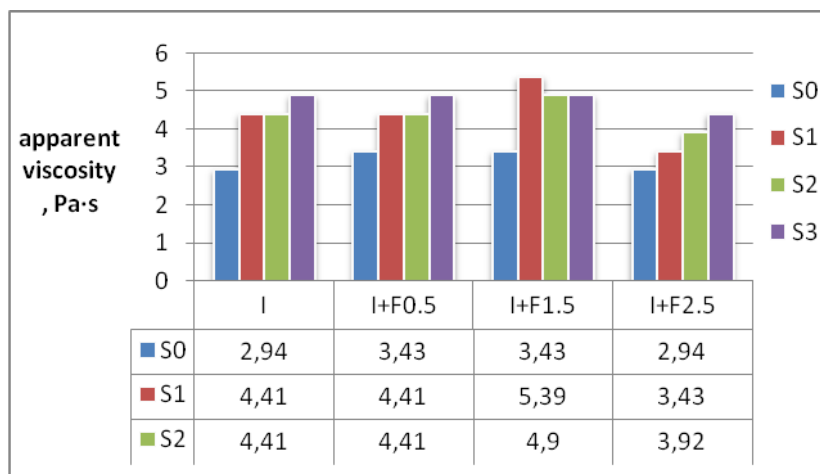


Fig. 3. Variation of apparent viscosity of yogurt samples at shear rate of 243000 s^{-1}

The value of the apparent viscosity of yogurt samples range from 2.94 Pa·s for flour and yogurt without added stabilizer up to 5.39 Pa·s for yogurt with added flour 1.5% and 0.1% stabilizer. The addition of 0.5% flour did not change the apparent viscosity of yogurt, except for the sample without stabilizer. To the addition of flour at the rate of 1.5% is an increase in the viscosity of yogurt. The viscosity of yogurt with 0.2% stabilizer and flour is equivalent to 0.3% sample of yogurt stabilizer (without flour).

Yogurt samples with 2.5% flour have a lower value of apparent viscosity compared to the control sample. The value of the apparent viscosity of yogurt samples range from 2.94 Pa·s for flour and yogurt without added stabilizer up to 5.39 Pa·s for yogurt with added flour 1.5% and 0.1% stabilizer. The addition of 0.5% flour did not change the apparent viscosity of yogurt, except for the sample without stabilizer. To the addition of flour at the rate of 1.5% is observed an increase in viscosity yogurt. The viscosity of yogurt with 0.2% stabilizer and flour is equivalent to 0.3% sample of yogurt stabilizer (without flour). Yogurt samples with 2.5% flour have a lower value of apparent viscosity compared to the control sample.

This variation of yogurt viscosity of can be explained primarily by the presence and quantity of stabilizer with specific hydrophilic properties.

As for the influence of the addition of germinated soriz flour up to 1.5- 2.0%, this is probably due to the fact that the molecules of starch gelatinized flour acts as active molecules that connect water in the casein recipe pores and increase the network of yogurt viscosity. There are probable and weak interactions (hydrogen, hydrophobic, Van der Waals....) of yogurt proteins with those of the flour, which causes strong structural rearrangement of yogurt, especially in large proportions of flour. At higher concentrations of starch in the flour forms a network of its own, incompatible (due to the thermodynamic

incompatibility) with that of casein. As a result casein network is compact and leads to lower viscosity of yogurt.

Therefore the supplementation with fish flour over 2.0 to 2.5% is contraindicated because it causes reduction of viscosity and stability of yogurt. At the same time it leads to the increase content in yogurt and fermenting substances that reduce the duration of fermentation, which also lowers the stability of curd gel.

Conclusions

1. Supplementation of yogurt with germinated soriz flour and Danisco stabilizer changes both the network structure and permeability of curd and essentially reduces the process of *synereses*.
2. Value of the apparent viscosity of yogurt samples range from 2.94 Pa·s flour and yogurt without the addition of stabilizer and flour up to 5.39Pa·s for yogurt with added flour 1.5% and 0.1% stabilizer.
3. Changing of the viscosity and *synereses* coefficient of supplemented yogurt with germinated soriz flour is determined by weak interactions (hydrogen, hydrophobic, Van der Waals....) of yogurt proteins with those of the flour, which causes strong structural rearrangement of yogurt, especially in large proportions of flour.

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STUDY OF HYDRATION GERMINATED SORIZ FLOUR

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Abstract: Hydration capacity and water retention of germinated soriz flour increases with the increasing dispersion of flour, both in case of untreated and heat treated flour, maximum values of hydration were recorded for flour with granulation of 160 -140 μ . Heat treatment of flour (tempering at 180°C) causes partial gelatinization of starch and leads to more pronounced hydration of flour and the increase of water retention. During the hydration of flour occurs partial diffusion of proteins in the hydration environment.

Keywords: flour of germinated soriz, hydration capacity, water retention capacity, diffusion of proteins

Introduction

General trends of dairy products consumption show an increase in the consumption of dairy products, with added fruit, cereal products, vitamins, mineral salts or lactic bacteria. (*B. B. Ключкова, 2011*) The increase in consumption of dairy products and is also supported by changing nutritional habits: consumption oriented by natural products, gradual migration from eating almost exclusively oriented towards meat to dairy products.

In recent years, particular attention is given to obtaining dairy with cereal products (oats, rye, buckwheat, rice and others). Cereal products increase the essential contents of added amino acids, vitamins (especially those from groups B and PP), vegetable fats, carbohydrates, dietary fibre, ameliorate the nutritional and organoleptic properties of enriched dairy products. (*З. Р. Ходырева, 2011*) Cereal components in dairy products are usually administered in the form of flour (in a state of powder) or liquid suspension.

In view of that dairy product containing cereal products are dispersed heterogeneous systems (suspensions), unstable, cereal preparation and stabilization additives requires a series of technical and technological measures. In order to perform complex liquid dairy products, homogeneous and higher stability, as well as the active principle (addition of cereal products) to be distributed uniformly evenly throughout the external phase into turbulences (milk) for a sufficient period of time should be taken into account a number of factors, determining the particle size, wetting ability and water retention. The latter determines the settling velocity of particles dispersed and stability of the finished product.

In the present paper as an additive to fermented dairy products was used germinated soriz flour. Soriz is an annual cereal crop hybrid origin of perspective for the Republic of Moldova because it is resistant to drought, productive, and beans have high nutritional qualities. (*Gh. Moraru, 1998*)

Soriz of malt flour is obtained by grinding and sifting seeds of germinated and dried soriz. The process of hydration of germinated soriz flour depends on the degree of dispersion, size and structure of particles. It was investigated the influence of temperature, duration of hydration and flour granulation on hydration and water retention.

Materials and methods

Germinated soriz flour. Germinated soriz was grinded in the laboratory mill У1-ЕМЛ. Fractional operation after granulosity was achieved by sifting installation РЛ-3М and flours were obtained with different granulation: 1250 μ; 180 μ; 160 μ; 140 μ.

Organoleptic characteristics of flour were:

Color - yellow with caramel hue.

Odor - specific malt flour without strain smell, rancid or mould;

Taste - savory, sweet malt, special malt flour, without strain taste, slightly sour or bitter.

Chemical composition of germinated soriz flour is shown in Table 1.

Table 1. Chemical composition of germinated soriz flour

	Chemical composition	Value
1	Dry matter content, %	9,90
2	Fat content, %	3,88
3	Starch content, %	59,23
4	Sugar content, %	2,25
5	Cellulose content, %	4,52
6	Protein content, %	11,69

Hydration of flour was made in milk (flour ratio: milk = 7: 1). 5 g (± 0,02 g) of flour have been suspended in 35 ml of liquid in centrifuge tubes of 50 ml and incubated for 10 min at predetermined temperatures (20, 30, 40, 50, 60, and 70°C) in water bath. The content of the tubes has been well mixed over every 5 min. The suspension was subsequently centrifuged 15 min to 1000 revolutions per minute. After centrifugation the supernatant was removed, and the precipitate weighed. (Andreas Markus Kratzer, 2007)

Hydration capacity of flour was calculated from the relation:

$$c_{\text{hydration}} = \frac{m_{i1} - m_{i2}}{m_p} \times 100, \quad (1)$$

where: $c_{\text{hydration}}$ – hydration capacity of flour, %;

m_{i1} – the initial quantity of milk, g;

m_{i2} – quantity of milk after centrifugation, g;

m_p – the sample of flour, g.

Water retention capacity (WRC) was determined by planimetric pressing.

0,3 g of wet precipitation of flour were placed on a polyethylene film, which was covered with filter paper and a plate of glass, over what was a mass of 1 kg and 10 min to be under pressure. After pressing the flour was separated from the filter paper and have been marked with a pencil outline of production spot and the contour of wet spot. (Grau R., Hamm R. 1953)

Water retention capacity was calculated from the formula:

$$c_{\text{retention}} = \frac{m_{\text{retained water}} - (S_1 - S_2)}{m_{\text{sample}}} \times 100, \quad (2)$$

where: $m_{\text{retained water}}$ - mass of retained water, g;
 S_1 - surface of wet contour, cm^2 ;
 S_2 - surface of flour contour, cm^2 ;
 m_{sample} - sample mass, g.

Dosing of proteins in milk was performed by the method of titration with formaldehyde, which consists of blocking the protein groups with amino-formaldehyde and release of carboxyl groups, followed by their neutralization with 0.1 n NaOH solution. Volume (ml) of NaOH (0,1 N), consumed in the second titration multiplied by coefficient 1.94 is the amount of total proteins, expressed in % of the total mass of milk product. (ГОСТ 25179-90)

Results and discussions

Influence of granulation and heat treatment of flour on the capacity of hydration and water retention

It was determined the capacity of hydration and water retention of heat untreated soriz flour and heat treated at 180°C for 10 minutes. As a hydration medium has been used milk with fat content of 2,5%. The results are shown in Figures 1 and 2.

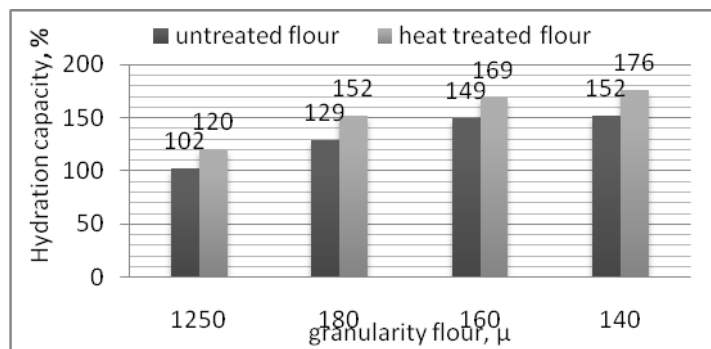


Fig. 1. Influence of granulation and heat treatment of flour on the capacity of hydration

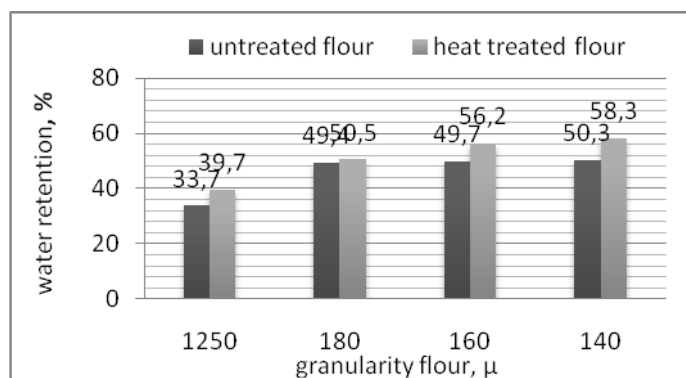


Fig. 2. Influence of granulation and heat treatment of flour on the water retention

Hydration capacity of flour grows with the increase the degree of dispersion of flour, both in the case of untreated and heat treated flour, maximum values of hydration being recorded for flour with granularity of 160-140 μ . Variation of hydration capacity depending on flour granularity is largely determined by specific surface area, which increases with the degree of flour dispersion. It is not excluded that, under the geometrical effect, the capacity of hydration is affected and by the biochemical composition of flour. It is known (*Hebrard A. and of, 2003*) that the fractions of flour with high granulation are richer in protein, pentozans and contain more starch granules damaged during milling, which have a significant contribution in the process of hydration.

Comparing the hydration capacity of untreated and heat treated flour, it is observed that the second has a higher hydration capacity. Heat treatment of flour possibly cause partial gelatinization of starch and leads to stronger hydration of flour.

So, in the case of flour with the granularity of 140 μ preliminary heat treatment applied to the flour leads to an increase in the hydration capacity of flour by 15,8%.

WRC index reflects the water content retained in hydrated product and expressed as a percentage. WRC is influenced by the contents and the structure of proteins. The character of retention capacity of water depending on the degree of dispersion of flour is similar to that for hydration capacity. Heat untreated flour with granularity of 140 μ has a water retention capacity of 50.3% and heat treated flour - 58. 3%.

The influence of temperature and the soaking time on the hydration capacity and water retention

In the production of fermented milk the flour may be introduced to the following technological steps:

- The standardized milk (temperature 60°C) before mixing;
- Before pasteurization (milk temperature: 80-95°C);
- The seeding phase of milk at a temperature of 42-45°C.

The process of flour hydration was pursued for 60 minutes at 20, 30, 40, 50, 60 and 70°C. As a medium for flour hydration was used the milk with fat content of 2,5%. The milk was mixed with the flour in a proportion of 1 to 10.

The results of the influence of temperature on the hydration capacity and water retention of soriz flour with granularity of 140 μ are shown in Figures 3 and 4.

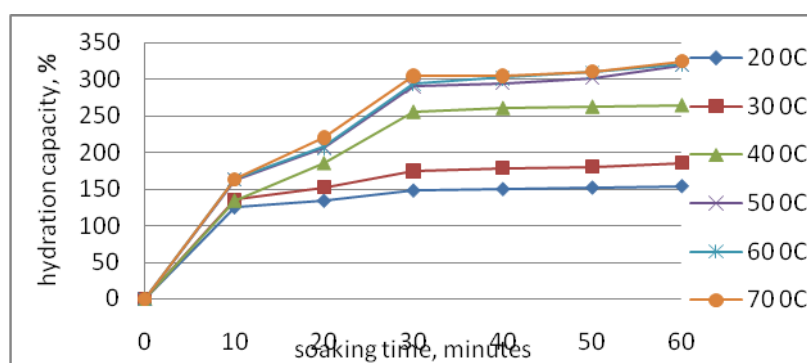


Fig. 3. The influence of temperature and soaking time on the hydration capacity of heat untreated flour

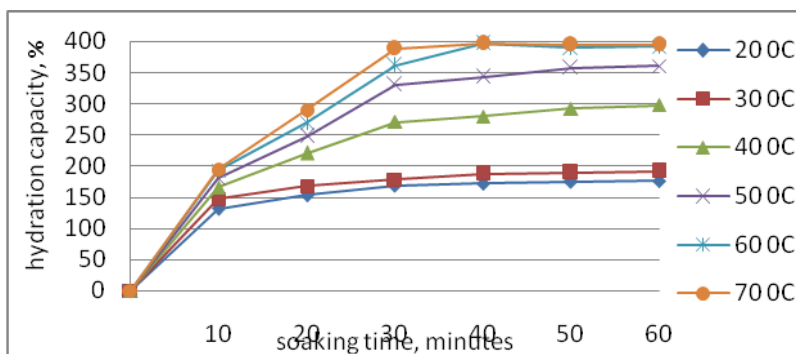


Fig. 4. The influence of temperature and soaking time on the hydration capacity of heat treated flour

So with the increase of temperature from 20°C to 70°C, the degree of hydration of flour rises, reaching the maximum temperature of 70°C to 329% for heat untreated flour and 395% for the hardened (an increase of about 30%).

Note that the process of flour hydration in the first 10 minutes is relatively intense and less dependant on the temperature of ambient liquid. Further hydration rate decreases and the degree of hydration is obviously dependent upon the ambient temperature. The maximum value of hydration is established after about 30 min of soaking.

From the results presented above is that the optimal soaking of germinated soriz flour is:

- Temperature of 60°C, because a higher temperature does not influence significantly the hydration capacity of flour;
- Duration of 30 minutes, which corresponds to the period of establishment of hydration by the balance of flour.

Diffusion of protein substances in the flour in the environment hydration

During the process of flour hydration takes place partial diffusion of milk of soluble substances, including proteins. The mechanism of proteins transfer in the hydration is convective and involves both a mass transfer by molecular diffusion in flour particles and by stagnant fluid layer, as well as the mass transfer through the layer of fluid in motion. (H. B. Pozob, 2000)

To follow the diffusion of protein substances in the flour into the milk was taken 140 μ of granulatory flour. The relationship between protein content in the hydration (steady hydration) and the hydration temperature is shown in Figure 5.

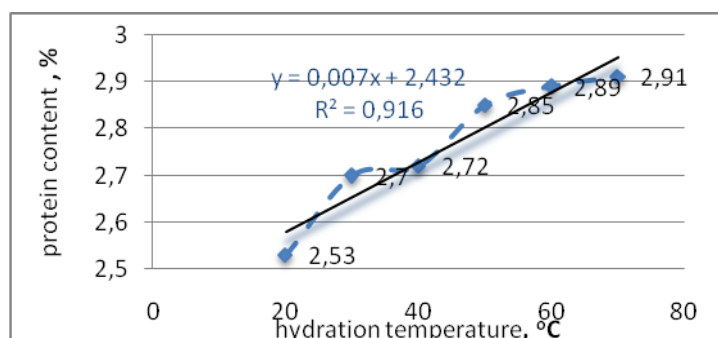


Figure 5. The relationship between the protein content in the hydration (steady hydration) and the hydration temperature

The protein content in the in the space of hydration increases from 2.53% to temperature by 20°C up to 2.91% at a temperature of 70°C. Therefore, we can assume that in the continuous phase passes a part of soluble proteins of ovalbumin-flour.

Conclusions

1. Hydration capacity and water retention capacity of germinated soriz flour grows with the increasing the degree of flour dispersion, both in case of untreated and heat treated, maximum hydration values were recorded for flour granularity of 160 -140μ.
2. Heat treatment of flour (tempering at 180°C) causes partial gelatinization of starch and leads to stronger hydration of flour and the increase of water retention.
3. During the flour hydration occurs the partial diffusion of protein in the hydration environment.

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EFFECT OF NITROGEN TREATMENT ON QUALITY OF COLD PRESSED WALNUT OIL

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Abstract: In this work nitrogen treatment experiment was set up to determine the intensity of oxidation products accumulation in cold pressed walnut oil. The influence of the nitrogen treatment was assessed by measuring the primary and secondary oxidation products in oil samples immediately after treatment up to the 30 weeks of storage. Primary oxidation products of walnut oil samples were evaluated by measuring peroxide value and conjugated dienes content. Secondary oxidation products of walnut oil samples were evaluated by measuring p-anisidine value and 2-thiobarbituric acid value. These data should help us to describe oxidation mechanism of walnut oil and to recommend optimal conditions for walnut oil stabilization. However it is necessary to note that these data are not enough to make a final conclusion about the behavior of walnut oil after nitrogen treatment during storage. Currently we obtained preliminary data about accumulation of primary and secondary oxidation products in walnut oil.

Keywords: walnut oil, nitrogen, oil stabilization, storage, quality characteristics.

1. Introduction

Walnut is a crop of a high economic interest for the food industry [8]. The walnut is highly appreciated nut because of its unique organoleptic characteristics, high level of 18:1, 18:2 and 18:3 fatty acids and hypocholesterolemic, and antihypertensive effects. Walnuts are utilized as shelled whole kernels or ground kernels being used as ingredients of many foodstuffs such as bakery products and confectionary as well as flavoring agents in beverages and ice-cream [9].

Walnut kernels have a lipid content of 65% of which 73% consists of polyunsaturated fatty acids, although values do vary between cultivars. Walnut kernels are consumed fresh or toasted, alone or in other edible products. Among the by-products of the walnut industry the oil has not yet obtained popularity, although it has been demonstrated that its consumption has a lot of nutritional benefits. The consumption of walnut oils from different geographic origins has been reported extensively. Their major constituents are triglycerides, in which monounsaturated (oleic acid mainly) and polyunsaturated fatty acids (linoleic and α – linolenic acids) are present in high amounts. The presence of other bioactive minor components, such as tocopherols and phytosterols, has been also documented. Walnut oil is appreciated as specialty oil also because of its characteristic flavor, aroma and health benefits [7 - 9].

A major goal in walnut production is to find an appropriate method to stabilize lipids from walnut kernels. The oxidative stabilization of walnut oil is imperative to determine the feasibility of bringing it into commercial production. A number of storage experiments have been carried out on the storage of in-shell walnuts, walnut kernels and walnut oil [5]. Mexis et al. (2009) investigated the effect of packaging and storage conditions on quality of raw shelled walnuts. It was established, that the effect of parameters investigated are followed the sequence: temperature > degree of O₂ barrier > lighting conditions. Savage et

al. (2001) reported that fresh walnut kernels could be stored in-shell at room temperature (mean 24 °C) for up to 12 months with only modest rises in peroxide levels. Stark et al. (2000) found that walnut oil stored at room temperature (mean 24 °C) in the dark, in sealed bottle, showed only small rises in peroxide levels after four months storage and remained an acceptable product in terms of its organoleptic properties.

However, the addition of this type of oil in processed food is not usual due to its low stability. To avoid this stability problem, researcher Calvoa et al. (2012) investigated the possibility to introduce walnut oil to the processed food as its microencapsulated form. As result of this research work, walnut oil was protected while food processing and released after consumption by the digestive process. Tsamouris et al. (2002) extracted and analyzed total lipids of walnut oil by high performance thin layer chromatography (HPTLC)/FID and GC-MS. Unsaturated fatty acids were found as high as 85%, while the percentage of the saturated fatty acids was found 15%. On the base of this data, researchers decided to prepare liposomes, which were characterized as new formulations. As authors declare, these formulations may have future applications for encapsulation.

In view of the fact, that walnut oil is to be effectively used in the food industry and human nutrition, it is extremely important to determine how long it can be stored for without any deterioration. This storage trial was set up to determine the storage life of walnut oil with particular reference to the walnut oil treated with nitrogen. The effectiveness of the nitrogen treatment was assessed by measuring the primary and secondary oxidation products accumulation in the walnut oil during storage up to a total duration of 30 weeks.

2. Materials and methods

2.1. Samples and extraction of oil

Walnuts (*Juglans regia* L.) were collected from the faculty yard Chisinau, the Republic of Moldova in October 2011. Walnuts were cracked and shelled. Oil extraction was carried out with mechanical press. Immediately after extraction oil was filtered, treated under different conditions and used for experiments.

2.2. Chemicals

Ethanol (99.9%), methanol (99%), potassium hydroxide, phenolphthalein, potassium iodide, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3 \times 5\text{H}_2\text{O}$) and starch were supplied by Eco-Chimie (Chisinau, Moldova). Chloroform, 1-butanol and glacial acetic acid were purchased from Sigma-Aldrich. 2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine) and p-anisidine were obtained from Alfa Aesar. All the chemicals used were of HPLC or analytical grade. Distilled water was used throughout.

2.3. Peroxide Value

Oxidation rate was studied by determination of the peroxide value (PV). This was determined according to AOCS Official Method Cd 8-53 (AOCS, 2003). Peroxide value was expressed as millimoles peroxide per kilogram of walnut oil [1].

2.4. Conjugated dienes

The experiment was carried out according to the AOCS Official method Ti la 64 (AOCS, 1993) with minor modifications [2, 10]. Approximately 0.02 g of walnut oil was placed into a 25 ml volumetric flask. The sample was dissolved in chloroform, brought to volume and mixed thoroughly. Absorbance of the dissolved walnut oil was measured in UV/Vis spectrophotometer HACH-LANGE DR-5000 (Germany) at 236 nm using quartz cuvette 10×10 mm. Results were expressed in micromole conjugated dienes per gram of walnut oil.

2.5. *p*-Anisidine Value

The *p*-anisidine value of walnut oil samples was measured following the methodology described in AOCS Official Method Cd 18-90 (AOCS, 1997) [3, 11]. This value was determined by the amount of aldehydes (principally 2-alkenals and 2,4-dienals) in walnut oil samples after reaction in an acetic acid solution of the aldehydic compounds in the walnut oil and the *p*-anisidine mixture. Absorbance of the samples was measured in UV/Vis spectrophotometer HACH-LANGE DR-5000 (Germany) at 350 nm using quartz cuvette 10×10 mm.

2.6. 2-Thiobarbituric acid Value

The 2-thiobarbituric acid was determined according to the AOCS Official Method Cd 19-90 (AOCS, 2009) [4]. The method is based on the spectrophotometric quantitation of the pink complex formed after reaction of one molecule of malondialdehyde (MDA), product of oxidation, with two molecules of 2-thiobarbituric acid added to the walnut sample.

2.7. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program version 2007. Differences were considered statistically significant if probability was greater than 95% (*p*-value <0.05). All assays were performed by triplicate at room temperature 20 ± 1 °C. Experimental results are expressed as average \pm SD (standard deviation).

3. Results and discussion

The health benefits of walnut oil are attributed to its chemical composition. Walnut oil contains approximately 7% saturated, 20% monounsaturated and 73% polyunsaturated fatty acids [16]. These high levels of polyunsaturated fatty acids make walnut oil prone to oxidation and may mean that oil has a limited shelf-life, i.e. nutritional and organoleptic changes due to losses of essential fatty acids and formation of volatile compounds from subsequent degradation of hydroperoxides [12].

It is well known, that primary oxidation products of vegetable oils are peroxides, which can be transformed induced by environmental factors such as humidity, temperature and oxygen content into secondary oxidation products such as aldehydes, ketones, oxidized fatty acids and other compounds [9].

In order to prevent high level of walnut oil oxidation was proposed technological treatment of walnut oil immediately after oil extraction. In this study the benefits of combined treatment with temperature and nitrogen of walnut oil was investigated. Quality of walnut oil samples was characterized by determination of peroxide value (PV), conjugated dienes content (CD), *p*-anisidine value (*p*-AV) and 2-thiobarbituric value (2-TBA). Obtained values of PV are shown in figure 1.

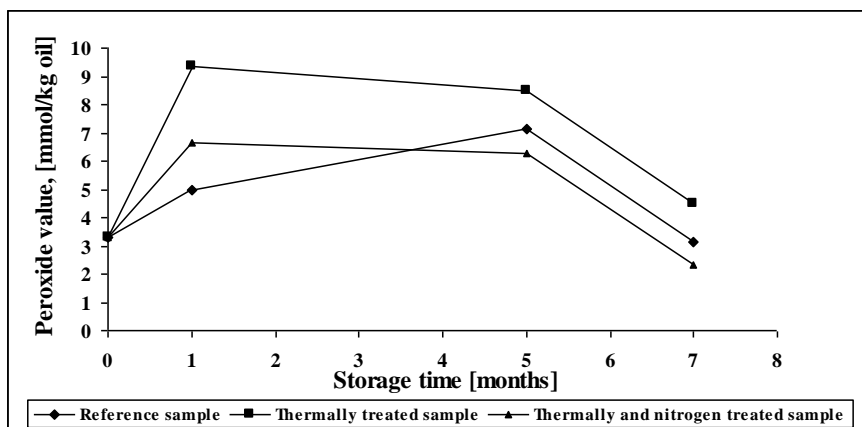


Fig. 1. Changes in peroxide value of walnut oil samples as a function of treatment conditions and storage time

The oil quality data from walnut kernels pressed at cold indicated significant variations for all parameters evaluated. The most outstanding feature was the decrease of PVs with increasing storage time of all investigated oil samples.

It can be explained, that peroxides represent unstable intermediate compounds of lipid oxidation process. These trends were registered for conjugated dienes content of the investigated walnut samples (Figure 2).

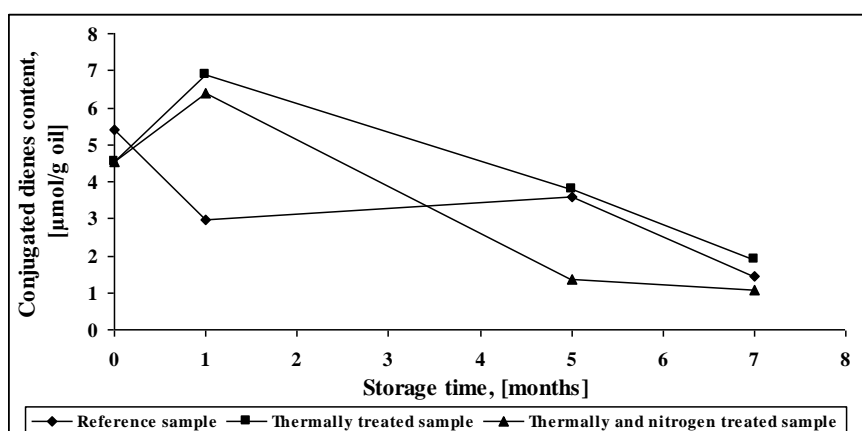


Fig. 2. Changes in conjugated dienes content of walnut oil samples as a function of treatment conditions and storage time

Further secondary oxidation products accumulation (p-anisidine and 2-thiobarbituric values) in walnut oil samples was investigated. Figure 3 shows the results of p-anisidine values changes. It can be seen, that significant increase of p-anisidine values were registered after 5 months of storage in all investigated oil samples. Considerable change of p-anisidine value was characterised for thermally treated oil sample, which is in accordance with theoretical data.

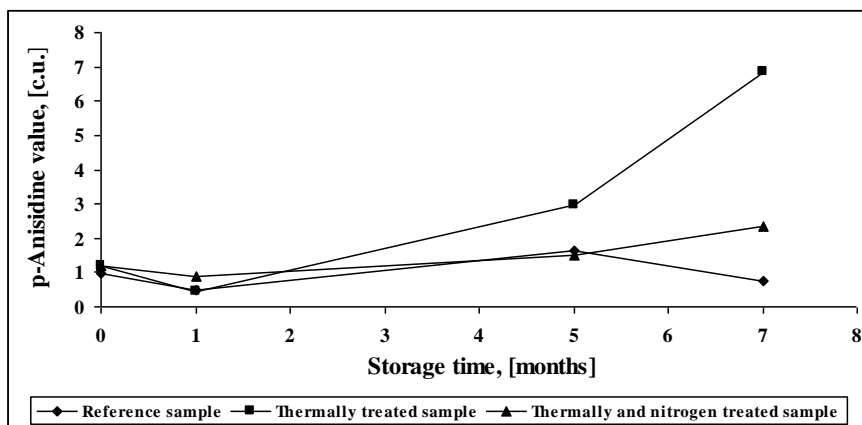


Fig. 3. Changes in p-anisidine value of walnut oil samples as a function of treatment conditions and storage time

For better understanding of obtained experimental results there was calculated total oxidation value (TOTOX) for all walnut oil samples, TOTOX value represents the sum of primary (peroxides) and secondary (aldehydes) oxidation products accumulation in vegetable oils. This value ranged from 7,58 to 7,81 c.u. for fresh oils and from 7,04 to 15,77 c.u. for walnut oils after 7 months of storage. It was obvious, that application of heat treatment of walnut oil led to a significant increase of the TOTOX value. But combination application of thermally and nitrogen treatment led to decrease of all investigated parameters (PV, CD, p-AV and 2-TBA).

4. Conclusion

The results of this research showed the influence of heat, nitrogen and their combination treatment on the intensity of primary and secondary oxidation products accumulation in cold pressed walnut oil as a function of storage time. It was demonstrated that walnut oil retains acceptable quality after combined heat and nitrogen treatment and quality deteriorates for reference sample (untreated walnut oil) and thermally treated sample. It is important to underline, that obtained results of this study are intermediate and could help authors to describe the scheme of walnut oil oxidation process and also to elaborate improved technology for walnut oil stabilization.

5. Acknowledgements

This work was benefited from support through the 2817/16.04 project, “*Elaboration of methods to protect walnut lipids (Juglans regia L.) from oxidative degradation*”, funded by the Academy of Science of Moldova and Moldavian Government.

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SUPERCRITICAL FLUID EXTRACTION OF BIOACTIVE COMPOUNDS FROM WALNUT LEAVES

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Abstract: The extractive efficiency of bioactive compounds from plant material is greatly depended on the extraction techniques. In this study, supercritical fluid extraction (SFE) of bioactive compounds from walnut leaves was used. Work conditions of supercritical fluid extraction were pressure level at 200 bar, temperature at 50 °C and flow rate of CO₂ - 20 kg/h. Efficiency of the SFE was evaluated by measuring total polyphenol content (Folin-Ciocalteu assay), reaction kinetics and reducing power of the extract towards DPPH[•] free radical. Walnut leaves extract obtained by supercritical fluid extraction showed the good antioxidant properties and could be used as a natural, low cost source of bioactive compounds.

Keywords: walnut leaves, supercritical fluid extraction, bioactive compounds, Folin-Ciocalteu's and DPPH[•] assays

1. Introduction

Walnut leaves are considered a source of healthcare compounds, and have been widely used in traditional medicine [5]. In some European countries, especially in rural areas, dry walnut leaves are frequently used to prepare infusions for their antiradical and antibacterial properties [1, 8].

In walnut leaves, naphthoquinones and flavonoids are considered as major phenolic compounds [12]. Juglone (5-hydroxy-1,4-naphthoquinone) is known as being the characteristic compound of *Juglans* spp. and is reported to occur in fresh walnut leaves [4, 6, 7, 11, 12]. Nevertheless, because of polymerization phenomena, juglone only occurs in dry leaves at vestigial amounts [12]. Several hydroxycinnamic acids (3-caffeoylquinic, 3-p-coumaroylquinic and 4-p-coumaroylquinic acids) and flavonoids (quercetin 3-galactoside, quercetin 3-arabinoside, quercetin 3-xyloside, quercetin 3-rhamnoside and two other partially identified quercetin 3-pentoside and kaempferol 3-pentoside derivatives) of different walnut cultivars collected at different times were studied [1]. In addition, the existence of 5-caffeoylquinic acid was also reported [12].

Supercritical fluid extraction with CO₂ is an environmentally benign and efficient extraction technique for solid materials and has been studied for the separation of bioactive compounds from plant materials. Organic solvent-free extract can be obtained and the low operating temperature makes it possible to preserve all natural properties of plant materials [2].

In this paper, the extraction of bioactive compounds from walnut leaves was effectuated by applying supercritical fluid extraction. This method has been proven to be desirable for phenolics extraction and has many advantages such as increasing extraction yield, shortening extraction duration. Evaluation of the efficiency of supercritical fluid extraction process application was measured by total polyphenol content and antioxidant activity assays.

2. Materials and methods

2.1. Plant material

Walnut (*Juglans regia* L.) leaves were harvested during Autumn, October 2011, in Chisinau, Central Moldova. Fresh and healthy leaves were manually collected from the middle third of branches exposed to sunlight. The leaves were dried at room temperature, powdered and packed in paper bags in order to protect them from light. Voucher specimens were transported to the laboratory of Faculty of Food Science and Engineering, University Dunarea de Jos Galati, Romania.

2.2. Chemical and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH[•]) as free radical form (95%), Folin-Ciocalteu's phenolic reagent, sodium carbonate were supplied by Sigma-Aldrich. 3,4,5-trihydroxybenzoic acid were obtained from Alfa Aesar. Methanol (99,8%) and ethanol (96%) were provided by Eco-Chimie (Chisinau, Moldova). Carbon dioxide gas (99,92%) supplied by Technic Gaz (Buzau, Romania) and delivered in cylinders with siphon tube for feeding with liquid solvent of the extraction plant.

2.3. Supercritical fluid extraction

Equipment used for supercritical fluid extraction (SFE) of walnut dried leaves was designed and supplied by Natex Prozesstechnologie GmbH (Ternitz, Austria). Extraction parameters used in this work are shown in table 1.

Table 1. Parameters of supercritical fluid extraction process of walnut leaves

Extract	Pressure [bar]			Temperature [°C]			Flow rate of CO ₂		Dynamic extraction time [min]
	C 30	S 40	S 45	C 30	S 40	S 45	kg/h	kg (Total)	
Walnut leaves	200±4	100±1	50±1	50,8±0,9	36,6±0,2	17,4±2	20±1	81	240,067

The pressure level was set at 200 bar as is above the critical pressure of the CO₂ solvent (73,8 bar), and as suggested by previous workers for extraction of phenolic compounds from plant material. Temperature of 50 °C is above the critical temperature for CO₂ (31,06 °C) and this temperature is generally used in the extraction of plant materials by SC-CO₂. The selected value of the temperature (50 °C) was low enough to avoid the damage of heat sensitive compounds.

2.4. Total polyphenol content measurement

For quantification of total polyphenol content, the Folin-Ciocalteu's method was used [10]. A volume of 0.5 ml of Folin-Ciocalteu's reagent was added to a dark flask, containing 0.5 ml of the each extract sample and 10 ml of distilled water. After 5 min, 8 ml of a 7.5% aqueous sodium carbonate solution was added to the mixture and the content was mixed thoroughly. The samples were kept in dark for 2h and then the absorbance was measured at 765 nm with HACH LANGE DR-5000 UV/vis spectrophotometer. Three parallel samples were analyzed. Gallic acid was used for constructing the standard curve, obtained in advance. Concentration range of gallic acid was of 0.05-0.5 mg/ml. The results of total polyphenol content were expressed as mg of gallic acid equivalents per ml of extract (mg GAE/ml).

2.5. Determination of DPPH radical scavenging activity

The antioxidant activity of walnut leaves extracts as well as the kinetics of inhibition of free radicals were studied in terms of radical scavenging ability using the stable DPPH[•] method [3]. 0.1 ml of the extract sample was added to 3.9 ml of 60 µM solution of DPPH[•] in methanol. The reaction was carried in dark and the absorbance was recorded at 515 nm to determine the concentration of remaining DPPH[•]. Methanol as instead of DPPH[•] solution was used as blank solution. The values of [DPPH[•]]_t at each reaction time were calculated according to the calibration curve (in the concentration range of 0.38-38 µg/ml): $A_{515\text{ nm}} = 0.0293 [\text{DPPH}^{\bullet}]_t - 0.0072$, where the concentration [DPPH[•]]_t is expressed in µg/ml. The coefficient of linear correlation of the above relation is $R = 0.9999$. The radical scavenging activity (RSA) was calculated using the equation [9]:

$$\text{RSA} = 100\% \cdot ([\text{DPPH}^{\bullet}]_0 - [\text{DPPH}^{\bullet}]_{30}) / [\text{DPPH}^{\bullet}]_0$$

where [DPPH[•]]₀ is the concentration of the DPPH[•] solution (without sample) at $t=0$ min and [DPPH[•]]₃₀ is the remained DPPH[•] concentration at $t=30$ min. Lower [DPPH[•]]_t in the reaction mixture indicates higher free radical scavenging activity.

2.6. Statistical analysis

Variance analysis of the results was carried out by least square method with application of coefficient Student and Microsoft Office Excel program version 2007. Differences were considered statistically significant if probability was greater than 95% (p-value <0.05). All assays were performed by triplicate at room temperature 20 ± 1 °C. Experimental results are expressed as average \pm SD (standard deviation).

3. Results and discussion

Walnut leaves are inedible by-products in walnut plantations, which may be a potent source of antioxidants, and have a potential as a value-added ingredients for functional foods.

Several studies on the extraction of bioactive compounds from different cultivars of walnut leaves have been published [1, 8]. Knowledge of the behavior of the factors influencing the process conditions is necessary to enhance the optimization extraction efficient for any bioactive compound. Previous findings have reported the influence of many independent variables, such as extraction method, solvent composition, pH, temperature, pressure and extraction time on the yields of bioactive compounds which can be extracted from diverse natural products. The positive or negative role of each factor in the mass transfer of the process is not always clear; the chemical characteristics of the solvent and the diverse structures and compositions of the natural products mean that each material-solvent system has a different behavior, which cannot be predicted. In this study walnut leaves extract was obtained by supercritical fluid extraction with carbon dioxide as a solvent and showed the polar properties. Chloroform as a solvent was used to analyze the antioxidant potential and content of bioactive compounds analysed extract.

In this study, the UV/Vis spectra of the walnut leaves extracts were analysed in the wavelength range 270 - 710 nm. From identification of bioactive compounds by UV/Vis spectra, it clearly revealed that studied extract contain phenolic acids (237, 245, 270 and 290 nm), flavonoids (333 nm) and carotenoids (417, 457, 484 and 537 nm). The total phenolic content (by Folin-Ciocalteu assay) was 477,59 mg/g in walnut leaves extract. Obtained UV/Vis spectra of walnut leaves extract with concentration of 1 mg/ml is presented in figure 1.

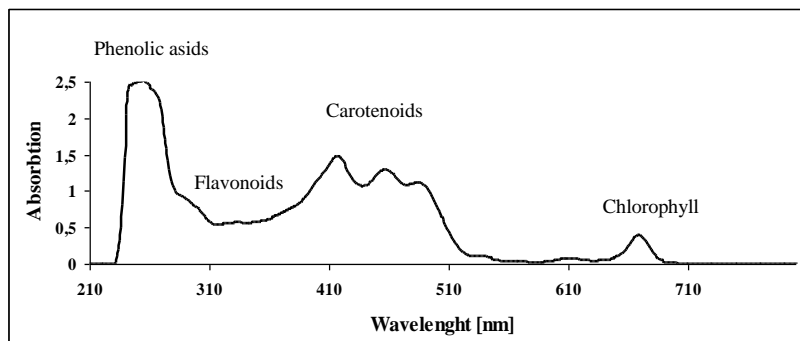


Fig. 1. UV/Vis spectra of walnut leaves extract

The knowledge of the kinetics of atom transfer is important because free radicals in the organism are short-lived species, what implies that the impact of a substance as an antioxidant depends on its fast reactivity towards free radicals. In this study the antioxidant capacity of the walnut leaves extracts were analyzed as the kinetics of inhibition of free radicals (the percentage of DPPH[•] remaining at steady state). The work concentrations of the walnut leaves extracts were between 0,1 and 10 mg/ml. Reaction kinetics of DPPH[•] free radical with walnut leaves extracts are shown in figure 2.

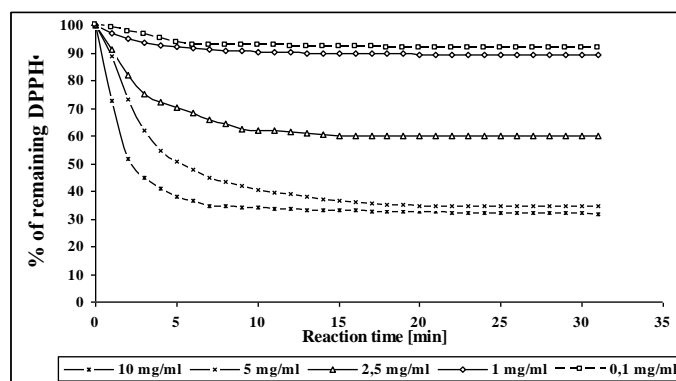


Fig. 2. Reaction kinetics of DPPH[•] free radical with different concentration of walnut leaves extracts.

It is well known that the absorbance decreases as a result of a colour change from purple to yellow when the radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH-H molecule. A more rapid decrease of the absorbance means more potent antiradical activity, expressed in terms of hydrogen donating ability of the compounds.

Walnut leaves extract obtained by SFE possess good amounts of bioactive compounds and a significant radical scavenging activity towards stable DPPH free radical (Figure 3).

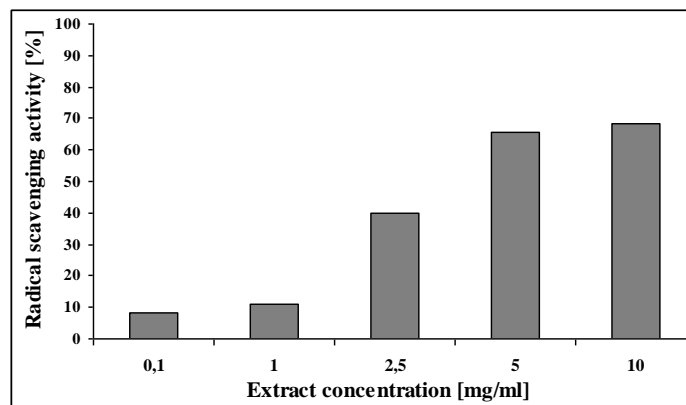


Fig. 3. Scavenging activity of walnut leaves extracts on the DPPH[•] free radical

The antioxidant activity analyses were performed with the walnut extracts of different concentration in chloroform. The antioxidant activity value of tested extracts was expressed as radical scavenging activity and this parameter was in the range of 8,16 – 68,16%. The amount of extract needed to decrease the initial DPPH[•] concentration by 50% is usually used for antioxidant activity appreciation of studied extract. In this study EC₅₀ for walnut leaves extract was also determined. Thus value was 3,74 mg/ml for walnut leaves extract (Figure 4).

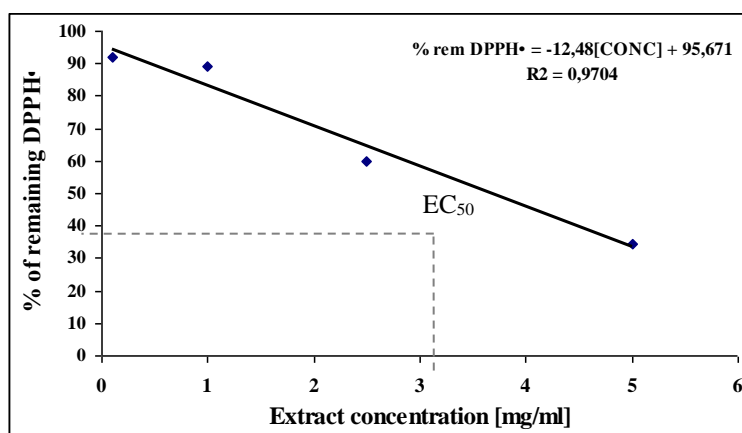


Fig. 4. Reducing power (EC₅₀) of the walnut leaves extracts towards DPPH[•] free radical

4. Conclusion

The study suggests the walnut leaves, as a by-product, can become the raw material for bioactive compounds extraction. To increase the extraction efficiency, and consequently, reduce the extraction time of bioactive compounds and extraction yields from walnut leaves it can be proposed to increase the polarity of carbon dioxide solvent by addition of small amount of a liquid co-solvent (modifier). Ethanol is more preferable as a co-solvent in supercritical fluid extraction because of its lower toxicity.

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STUDY ON MICROFLORA OF VEGETABLE SALADS

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Salads are prepared with a special role in human ration due to beneficial advantages on the body. These are reliable sources of minerals (K, Na, Ca, P, Mg, Fe), vitamins (vitamin C, vitamin. B1, vit. B2, vit. B6, vit. PP), easily digestible and not the least prevent constipation, obesity and helps lower cholesterol in the body, and not least, reduce the risk of diabetes. Unlike other dishes, salads are kept at $t = 6.4^{\circ}\text{C}$ no longer than 1 h, and salads of raw-for 0.5 h [2].

Reduced during storage of salads is explained by the variety ingredients and different sources of contamination with unwanted microflora with during other operations: cutting components, their mixing and portioning transportation, marketing. Purpose of the thesis was to study the microflora of raw materials used in the preparation of 2 types of salads - "cabbage salad" and No.100 "meat salad" no. 89 [1], and their microflora during storage at $t = 4-8^{\circ}\text{C}$ for 5 h. To identify the appropriate microflora samples were inoculated on culture media, Saboureaud, for detecting yeasts and molds and Agar meat peptonate - for bacteria.

Cultures were incubated in Petri plates under appropriate rules on culture media indicated pH of 2.5 to 5.5 which were subsequently kept in thermostat for 48-72 h at $t = 32-37^{\circ}\text{C}$. Paralel to this was used citric acid solution 5% as control and preservative on the same period of cultivation.

Findings microbiota of raw materials used for preparing salads are presented in Table 1.

Table 1. Microbiota identified in untreated and heat-treated materials

Raw material	Culture medium	Total number of colonies n/g	Identified microflora
1	2	3	4
Raw material for "Cabbage salad"			
Cabbage	Agar Saboureaud Meat peptone agar	$1,1 \cdot 10^2$ $1,84 \cdot 10^2$	- Gen. Bac. mesentericus
Lettuce (leaves)	Agar Saboureaud Meat peptone agar	$0,73 \cdot 10^2$ $0,6 \cdot 10^2$	Lees, gen Torulopsis Micrococci
Green onion	Agar Saboureaud Meat peptone agar	$0,3 \cdot 10^2$ $0,65 \cdot 10^2$	- Gen Bacillus
Raw material for "Meat salad"			
Raw potatoes	Agar Saboureaud Meat peptone agar	$0,64 \cdot 10^2$ $3,0 \cdot 10^2$	Micrococcus
Boiled potatoes $T=30^{\circ}$	Agar Saboureaud Meat peptone agar	$0,4 \cdot 10^2$ $0,55 \cdot 10^2$	Gen Bacillus
Raw meat	Agar Saboureaud Meat peptone agar	$0,38 \cdot 10^2$ $9,0 \cdot 10^2$	- Micrococci, gen Baccilus, Streptococcus Lactis

1	2	3	4
Boilead meat T=60-90°	Agar Saboureaud Meat peptone agar	0,2*10 ² 1,5*10 ²	- Micrococci, Streptococcus Lactis
Raw egg	Agar Saboureaud Meat peptone agar	0,7*10 ² 0,8*10 ²	- Gen. Potcus
Boileg egg T=10°	Agar Saboureaud Meat peptone agar	0,25*10 ² 0,65*10 ²	- Sac. subtilis

From the data presented in Table 1 is observed that the number of colonies of microorganisms appeared on both culture media of heat untreated raw is higher than the boiling feedstock and is included within $(0,3 \div 9,0) \cdot 10^2$. On the raw material heat treated microbes we can remark, that the overall number of colonies is reduced virtually for all types of environmental samples analyzed by $1.5 \div 6.0$ times.

It is also noteworthy that the microflora identified for untreated and heat-treated material is representing, especially Peptone Agar culture medium meat by: Bac.gen Bacillus, Micrococci, Streptococcus lactis. Genus Torulopsis yeasts were found in lettuce (leaves) on agar culture medium Saboureaud in number of $0.73 \cdot 10^2$ colonies. Microbiota of "cabbage salad" and "meat salad" in dynamic storage for 4 h at temperature 5-10 ° C is shown in Figure 1 and 2.

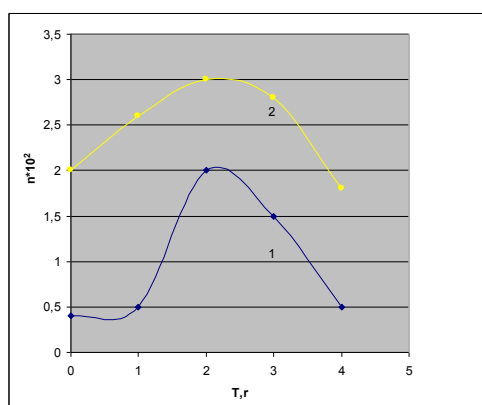


Fig. 1 Microbiota evolution in "cabbage salad": 1 - Agar Saboureaud culture medium; 2 - Meat peptone agar culture medium

The data fig.1 notice that the initial number of colonies salad "cabbage salad" appears in the culture medium, the microbiota exceeds the number of colonies on Agar culture medium Saboureaud on average 1.5 to 6.0 times during 4 h of storage.

But comparing the microbiological standards for salads of crudités with laboratory results is confirmed the fact that the total number of bacteria (N TG) and standards for yeasts and molds respectively equal to $5 \cdot 10^4$ and $1.0 \cdot 10^2$ microorganisms / g, were not exceeded and Salmonella and Listeria in 25 g sample type - were not detected.

Thus, the data obtained for "cabbage salad" kept at low temperatures to 5 h, shows that it can be consumed without danger human health. Similar results were obtained for "meat salad" (Fig. 2).

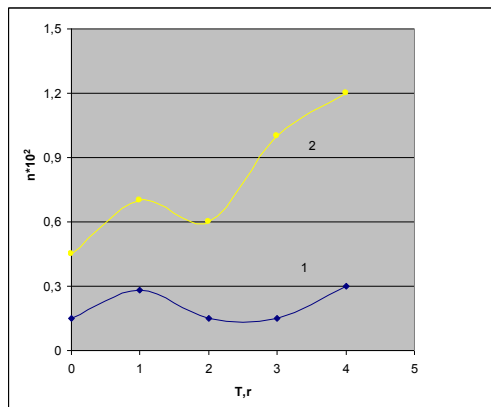


Fig. 2 Microbiota evolution in "Meat salad": 1 - Agar Saboureaud culture medium; 2 - Meat peptone agar culture medium

Insemination on both culture media of "meat salad" of heat treated components was carried over each hour of storage period at temperatures of 4-10 °C. The results presented in Figure 2 show insignificant deviations in the number of colonies on Agar culture medium Saboureaud (curve 1) the salad immediately prepared and examined over 2 h of storage, maintained at $t = 4.2$ °C.

NTG values $0.18 \cdot 10^2$ for every prepared salad and after 2 h of storage are practically identical, which confirms that temperature is the important factor, influencing storage time. At this temperature - 2-4 °C the speed of metabolic processes of microorganisms is reduced. In these cases low storage temperatures of salads help reduce the number of microorganisms and possible increase of a limited duration of storage of foodstuffs. (17)

Microflora of studied salads was identified by examining the salad samples at microscopic size $16 \cdot 100$ by emersion.

In "cabbage salad" during storage were found and identified the following types of organisms presented in Table 2.

Table 2. Salad microorganisms

Time of storage, h	Temperature, °C	Cabbage salad		Meat salad	
		Agar Saboureaud	Meat peptone agar	Agar Saboureaud	Meat peptone agar
0,0	4-10°C	Lactobacillus, Dj.Torulopsis	-	Bac. subtilis	
1,0	4-10°C	-	Gen.Bacterii	-	Gen. Bacillus
2,0	4-10°C	-		-	Gen. Bacillus
3,0	4-10°C	Gen. Bacillus	-	-	-
4,0	4-10°C	Gen. Bacillus	-	Gen. Bacillus	-
72	37°	-	Streptococcus	-	Bacillus with spores

From Table 2 data it is observed that the "meat salad" inseminated on Peptone Agar contains *Bacillus* with spores, detected after maintaining the samples at 37 ° C for 72 h. These results confirm that the prepared salad including raw material heat treated and used for cooking presents the danger of food poisoning because contamination with microorganisms cooked meat occurs after cutting it. It should be noted that such Bacillaceal family includes over 36 types of species: *Bac.anthraxis*, *Bac.cereus*, *Bac.subtilis*.

On "cabbage salad" were found streptococcus, which group includes *S.Aureus*, *S.Pseudomonas*, *S. faecalis* and.

Remarkably, the salads prepared and inseminated on medium Eudo for detecting microorganisms such as *E. coli* has not confirmed their presence.

In order to protect salads of influence of pathogenic microorganisms and increase the retention period were used cloth impregnated with citric acid solution with a concentration of 5%, covered with culture media.

Analysis of samples inoculated with suspension of salads formed sterile areas of microorganisms around fabrics impregnated with citric acid solution, indicating inhibition of microorganisms by acid.

The study of process preparation of 2 types of salads - "cabbage salad" and "meat salad" allow for molding the following conclusions:

- Perishable salads are prepared with a shortened retention due to the large number of components and mechanical processes;
- Raw food salads are more likely for microorganisms invasion and their slightly contaminated during preparation (washing with water, and cut);
- Heat treatment of raw materials reduces initial microflora:

In potato - 5.5 times, meat - 6.0 times, eggs - 1.2 times.

- reducing the contamination of salad of raw is achieved by substituting mass vinegar with lemon salt solution
- Microflora of raw materials used to make "cabbage salad" is represented by conditioned pathogenic bacteria like: *Bacillus* and pathogens such as salmonella, *E. coli* - were not detected.

Using lemon salt solution (C6 H8 O7) in concentrations of 5% or lemon juice as a substitute of vinegar for salads of raw greatly reduce their contamination with microorganisms.

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DEFINING OF ASEPTIC TANK POSITION IN THE PRODUCTION PROCESS OF COSMETIC AND PHARMACEUTIC PRODUCTS

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Abstract: A survey for the use of aseptic processes in the production of cosmetic and pharmaceutical products is carried out. It is turned out that the aseptic filling is one of the most critical processes in these industries. The requirements of this process and for establishing of the aseptic conditions for its conducting are examined. An investigation of the installations for production of liquid cosmetic and pharmaceutical products, their main components and characteristics and the technology for production of studied products is carried out. The place and the function of the aseptic tank in these installations are defined. The advantages of the aseptic tank, its technical characteristics and requirements for its production are presented.

Keywords: aseptic tank, aseptic filling, cosmetic and pharmaceutical products

Introduction:

Aseptic filling of sterile medicines (also known as a sterile filling) is one of the most critical processes in biopharmaceutical industry. This is due to its highly technical driven process and the potential impact of the security to the end user, usually placed in an advance risk patients. There are only indirect protective measures for sterility of the filled medicine after its filling and sealing in a clean room.

In the method of aseptic filling, the product and the packaging are sterilized separately, and then cooled product is filled ("cold filling") and hermetically sealed under conditions that exclude re-contamination by microorganisms (aseptic conditions). This method is characterized by two specific process stages - pasteurization (sterilization) of the product and creating of the required conditions for a sterile transport, packaging and storage of the product. Heat treatment of the product intended for aseptic filling is carried out according to the principle of the high temperature short-term heating. Two methods of product heating (indirect and direct) have application in this process.

Aseptic filling is an aseptic process which requires good coordination and complex interaction between personnel, sterilized product, technological equipment for filling (final treatment), clean rooms, attaching equipment and sterilized filling components. Micro contaminations have infinitesimal dimensions and the surfaces, which look clean and sterile, in fact may are not so clean and sterile. Therefore the aseptic filling (final processes) are highly dependent on the equipment, detail technological procedure and control. The more unique are the products or the packing system, the greater technical and operational requirements have to be provided.

Creating of aseptic conditions

A necessary precondition for successful implementation of the method of aseptic filling is to create sterile conditions for transport, packaging and storage of the product. The secondary microbiological contamination of sterile products should be totally excluded.

A specific preparation of the technical equipment for the process of aseptic filling of the packaging is required to achieve this purpose. The achievement of absolute purity and

sterility of all industrial facilities that come into contact with the sterile product during the process of aseptic filling is extremely important. The sanitary processing should ensure the implementation of two major tasks: 1 - mechanical separation of the dirt - product residue, sludge, scale, scaling, etc., 2 - separation of the residual micro flora.

Installations for liquid pharmaceutical or cosmetic products:

Different liquid pharmaceutical and cosmetic products are manufactured and stored in fully automated installations (Fig. 1 shows an installation for liquid pharmaceutical products, and Fig. 2 shows a scheme of the operation of such unit.) Each of the three production vessel with content of 500, 1000 and 2000 liters respectively, can be connected with each of the five vessel for product's storage (2x1000, 3x2000 liters), which means possibility for change and a flexible system. Up to three different manufacturing steps are possible.

The installation is designed in accordance with good manufacturing practice (GMP) and is approved by the Administration of food and medicines (FDA). The design and construction of the installation are in accordance with the latest directives and standards. The product transfer from the preliminary tank to the storage tank, cleaning in place (CIP), sterilization in place (SIP) and drying up in the different sections of the aseptic installations could be performed at the same time. Each preserver vessel is set for a filling machine.



Fig. 1. An installation for liquid pharmaceutical products

An automatic cleaning and drying up process of all, wetted from the operating liquids, components and sections is provided.

The full transfer length of the installation is 1,000 meters. The equipments are made from a high-quality stainless steel. All components, which could be wetted from the working fluids are mechanically grinded or electrolytic polished with aim to achieve a smoothness of the inside surfaces, and all links are orbital welded.

Each of the preliminary tanks is placed on a bearing construction in an individual clean room. In such way cross-contamination with active substances from other products is eliminated. The active substances and the working liquids are added to the aseptic vessels through a direct connection or through vacuum suction from the reservoirs. All installed, manually or pneumatically controlled, valves from DN8 to DN50 (with the exception of a few valves in the transferring section) are membrane (diaphragm) valves.

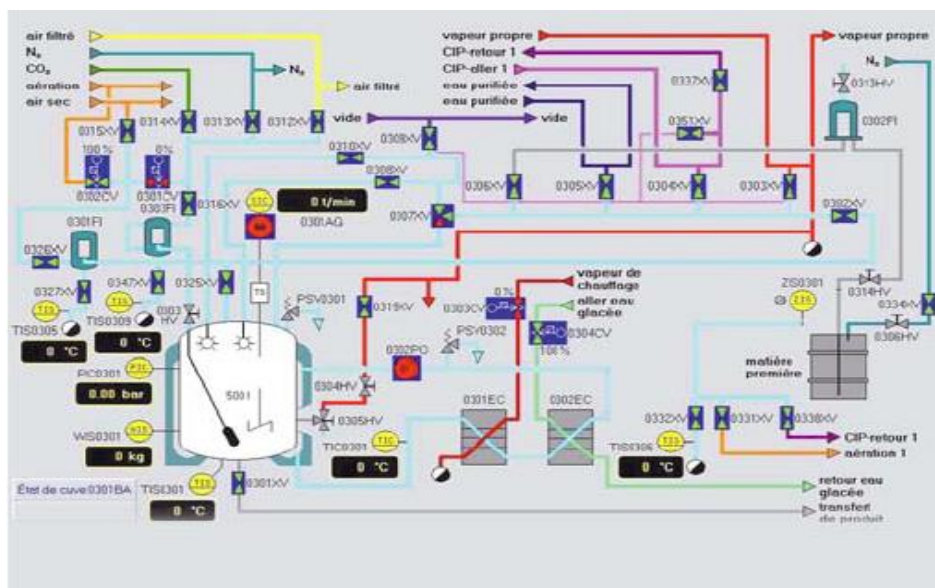


Fig. 2. Operation scheme of the installation for liquid pharmaceutical products

Aseptic tank for pharmaceutical and cosmetic applications (Fig. 3 and 4):

Aseptic tank, as a part of these installations, is widely used for intermediate hermetic storage of different pharmaceutical, biotechnological and cosmetic semi-manufacture products under aseptic conditions or as a process vessel. It may be used as a one separated piece or as a component in an aseptic line. In the second case it is located after the sterilization equipment and before the filling machines.

The aseptic vessel, used in the pharmaceutical industry, must meet certain requirements depending on the stored in it product. These vessels require optimal outside species, easily maintained surface of stainless steel with a polished inner surface and the roughness height of the surfaces, which are in contact with the manufactured product from 0.1 μm to 0.3 μm .

The aseptic vessel must be produced according to requirements of the specific technological regime, consistent with the process thermal parameters (temperature and pressure). Strength design of the tank must meet the corresponding absolute pressure in the vessel designated by the given technological regime. The pressure in the tank ranges from perfect vacuum to 0.3 MPa gauge pressure. The vessels usually have a double jacket for cooling water circulation and it is sterilized with steam. They must have safety valves on steam and sterile air lines, cleaning in place (CIP) system and an agitator at customer's request. The aseptic tanks are also equipped with all the necessary accessories – an access hatch, system of inlets and outlets with taps for loading and unloading of the products, for steam or compressed air supply, for mounting of the measuring devices.



Fig. 3. Aseptic fermentation vessel



Fig. 4. Pharmaceutical aseptic tank

The spectrum of demand is from small mobile vessels with working volume of 10 l to larger volumes of technological and storing vessels with a diameter from 1.8 to 4.2 m and a height from 3 to 7.8 m. The large aseptic containers are mainly used for storage of various pharmaceutical and biotechnology products as a semi-manufactured product with aim of their subsequent processing into finished product pieces. This allows to be extended the production season and sharply reduces production costs. Furthermore this allows to be eliminated the need of expensive additional equipment for creation of artificial cold.

So the advantages of the aseptic tank as a buffering vessel are: The production season could be prolonged and the production costs could be sharply decreased; There is no requirement from the additional use of expensive apparatuses for product cooling; The aseptic tank secures safety of the sterile products through constant pressure and temperature, and guarantees products stabile quality during the whole production season; The repeatedly product heating, due to the stopping of the production process or to the shortage of the filling machine capacity could be avoided; The sterile intermediate storage of the aseptic product ensures continuously operation of the filling machine, even during the process of cleaning in place; The aseptic tank ensures flexible connections between one or more technological processes and filling lines, and also different products could be filled and packed at the same time without manual intervention; Excellent quality of the product; Steady regulation of the sterilization process at using of different storage volumes; Possibilities for manufacturing of more than one product.

The tank (Figs. 5 and 6) is designed for operation at pressures up to 0.31 MPa and at a perfect vacuum. All interior surfaces are polished with a maximum roughness of 25 Ra. The vessel is sterilized with steam and has double walls for circulating of cooling water under atmospheric pressure. There is a high speed washing turbine for washing out of the vessel and an agitator at the bottom of the vessel. An aseptic connection and an aseptic filling hole with a steam barrier are also provided. The sterility of the vessel is provided by its connecting and inlet openings. The connecting holes have size from 1 to 6 inches, and the feeding hole is 20 inches.



Fig. 5 & 6. Aseptic tanks

Technical specification of an aseptic stainless steel vessel (Fig. 7): Volume - 2000 liters; Material - stainless steel; Orientation - horizontal; Shape - round; Supports - 4; Working pressure - 0,5 bar; Operating temperature - 111 0C; Agitator - yes; Dimensions: 2700 mm x 1500 mm x 2400 mm overall height.



Fig. 7. Aseptic tank

Conclusion:

1. The process of aseptic filling of sterile medicines is one of the most critical processes in the biopharmaceutical manufacturing. The advantage of the aseptic filling of different products over the methods of hot filling or refrigeration is proven.

2. The role of the aseptic tank in cosmetics and pharmaceutical industry is for intermediate storage of different products in aseptic conditions or to be used as a technological vessel. It could be used as a separate vessel or as a component of an aseptic line. In the second case the aseptic vessel is placed after the sterilization equipment and before the filling section.

3. The advantages of the aseptic tank for storages purposes are:
- Excellent product quality;
 - Efficiency of utilization line (possibilities for running of the sterilization process simultaneously with the filling process or the process stopping);
 - High-flexibility of the production process (easy transfer of the product between the sterilization equipment and filling line);
 - Possibility for more than one product processing;
 - Energy consumption and product loss reduction;
 - The aseptic buffered product prevention;
 - Steady regulation of sterilization process, when aseptic vessels with different volume are used.
4. A module for aseptic storage of liquid food products has been worked out and a prototype has been developed in the company “Biomashinostroene AD” - Plovdiv on the base of this study.

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POSSIBILITIES FOR ENERGY SAVING IN A STUDENT HOSTEL “MARITZA 4” OF UNIVERSITY OF FOOD TECHNOLOGIES

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Abstract: Energy audit of the student hostel “Maritza 3” of University of Food Technologies – Plovdiv is done. The data about the building energy consumption and about the present state of the building installations and enclosing elements are collected and analyzed. Computer model of the real energy consumption is created and the appropriate measures for energy saving are proposed. The potential for reducing of energy consumption and CO₂ emissions are defined.

Keywords: energy efficiency, energy audit, buildings.

Introduction:

Energy efficiency is vital for the European Union. More and more citizens and firms feel the impact of increased energy bills. The dependence of Europe and Bulgaria in particular from the import of fuels will grow and the security of fuels supply is going to get worse. Reducing energy consumption is the most appropriate long-term answer to that. Energy saving is the most immediate and cost efficient way to overcome the key energy challenges regarding sustainable development, security of supply and competitiveness. Avoided energy consumption through energy savings is becoming the most important energy source. Buildings are the biggest consumers of energy. At the same time they have enormous potential for energy savings. If we reduce their energy intensity the quotas of CO₂ emission will be release and then countries can sell them.

The aim of this study is to establish the possibilities for reducing energy consumption of the student hostel at keeping the normative parameters of the living areas.

Materials and methods:

The building of the student hostel “Maritza 4” is studied. It was put into exploitation in 1986. The building has central heat supply. The building’s facade is shown on fig.1.



Fig. 1. Facade of the student hostel

The building model investigation is done with the “EAB Software” in accordance with the regulations on energy efficiency, and the economic analysis of the proposed energy saving measures (ESM) – with the “ENSI Economy” software in real interest rate - 6.73%. The building falls into climate zone 6 – Plovdiv. The current price of heat energy is 110 BGN per MWh with VAT. The economic life of the proposed ESM is set according to [4].

Results and discussion:

The building has a basement and five floors. The basement is partially heated. The building construction is a large-area formwork. The total built-up area is 947.3 m² the developed area - 5846.47 m² heated area - 4938.65 m² and gross heated volume is 13882.75 m³. For types of outside walls are established. The basement's exterior walls are made of reinforced concrete, double plastered and with a bush-hammered outside surface. Their whole thickness is 43 cm. The walls of the 5 floors are both sides plastered multilayered panel with a thickness of 30 cm of the north and south facades and 25 cm of the western and eastern facades. All three facades of niches under the windows along the eastern and western facades are both sides plastered reinforced concrete panel with a total thickness of 20 cm. The windows are mainly wooden with double glassing, and in the foyer there is a metal window, starting at 40 cm from the ground and get to the roof. The woodworks are in poor condition, frames are deformed, some of them are rotten and can't be repaired. There are broken and missing windows on the facades. The building's roof is a flat cold roof with an air layer with 1, 1 m thickness. The roof of the window niches is a hot roof. The building has three types of floors – floor, bordering with outside air (bay); floor of a heated basement; floor above a nonheated basement.

The heat supply substation is mounted in the basement. It is recently renovated and is in good condition. The circulating pump and the heat exchanger are new. There are installed and a new closed expansion tank. For metering is installed new ultrasonic heat meter. The heating operates 20 hours a day at 22 °C inside temperature and 4 hours a day (twice in 2 hours a day) at 18 °C. Domestic water is heated all year round. The condition of the pipe work in the substation is good. The pipes are well insulated. At very few places there is partially damaged insulation of pipelines. The heating installation has a scheme with a lower distribution and radiation pattern. The isolation of the pipes in the basement is destroyed only in a few places, but aside from that, it is in relatively good condition. In the room for celebrations the pipes are not insulated.

Energy consumption: The energy consumption for 3 years (2008 ÷ 2010) is analyzed. The specific heat consumption for heating is between 175 и 201 kWh/DD. The consumption of electric energy is average 34 % from the total energy consumption.

The computer model of the building [2, 3, 4] is worked out. The reference models of energy consumption of building are composed in accordance with the legal standards, operating during the year of bringing of the building into exploitation (1980), and the legal standards operating at the time of the audit doing (2011).

The model calibration is done considering the energy consumption for domestic hot water, lighting and electrical appliances with a referent heat consumption for space heating of 104, 4 kWh per year. Unknown model parameters (infiltration - 0, 96 h⁻¹, design temperature - 22 °C, temperature with reduction - 18 °C) are established. The baseline energy consumption for space heating is obtained through normalization of the model and it is 104.4 kWh per year. The current class of energy consumption is “F” according to [6].

Establishing the potential for reducing of energy costs, simulating of energy saving measures and economic analysis:

The building's energy saving potential is established from the model analysis. The results from calibration of the model, reflecting the actual state of the object, base model state and the obtained impact of the introduction of the ESM are presented in Figure 2.

Параметър	Еталон	Състояние	Базова линия	Чувствителност kWh/m ² a	ЕС мерки	Спестяване
1. Отопление 16,6 kWh/m²a						
U - стени	0,38 W/m ² K	1,78 >	1,78 >	+ 0,1 W/m ² K = 2,02	0,45 >	23,25
U - прозорци	2,00 W/m ² K	2,95 >	2,95 >	+ 0,1 W/m ² K = 1,32	2,00 >	10,91
U - покрив	0,24 W/m ² K	0,68 >	0,68 >	+ 0,1 W/m ² K = 1,56	0,23 >	6,11
U - под	0,32 W/m ² K	0,93 >	0,93 >	+ 0,1 W/m ² K = 1,57	0,68 >	3,42
Фактор на формата	0,33 -	0,33	0,33		0,33	
Относ. площ прозорци	17,8 %	17,8	17,8		17,8	
Коеф. на енергопрем.	0,45 -	0,45 >	0,45 >		0,45 >	
Инфилтрация	0,50 1/h	0,96 >	0,96 >	+ 0,1 1/h = 6,60	0,50 >	26,16
Проектна темп.	20,0 °C	22,0 >	22,0 >	+ 1 °C = 7,50	20,0 >	12,82
Темп. с понижение	18,0 °C	18,0 >	18,0 >	+ 1 °C = 1,49	18,0 >	
Приноси от						
Вентилация (отопл.)	kWh/m ² a	0,00 ...	0,00 ...		0,00 ...	
Осветление	kWh/m ² a	4,09 ...	4,09 ...		3,34 ...	
Други	kWh/m ² a	16,55 ...	16,55 ...		13,53 ...	
Сума 1	kWh/m²a	90,2	90,2		18,8	
Ефект. на отдаване	100,0 %	100,0 >	100,0 >		100,0 >	
Ефект. разпред. мрежа	95,0 %	94,6 >	94,6 >		94,6 >	
Автом. управление	97,0 %	97,0 >	97,0 >		97,0 >	
Е П / ЕМ	96,0 %	96,0 >	96,0 >		96,0 >	
Сума 2	kWh/m²a	102,4	102,4		21,3	
КПД на топлоснабд.	98,0 %	98,0 >	98,0 >		98,0 >	
Сума 3	kWh/m²a	104,4	104,4		21,8	

Fig. 2. Results from the energy consumption model before and after ESM

The next ESM are proposed:

Replacement of existing wooden windows with aluminum windows with a thermal break, and double glass package and "k" glass. The summary heat transfer coefficient of windows reduces to 2 W/(m²K). The effect is saving of 20 140 BGN, or 17.57% reduction in heat consumption and results in reduced infiltration and heat transfer through windows. At a new windows price of 160 BGN per m², the necessary investments are 140 480 BGN and payback period is 7.0 years;

Thermal insulation of external walls and jetty with 7 centimeters fiber. The effect is saving of 14 490 BGN per year, or 12.64% reduction in heat consumption and a reduction of the thermal transfer through the walls and jetty. At a new insulation price of 50 BGN per m² the necessary investments are 75 779 BGN and payback period is 5.23 years;

Thermal insulation of roof, made of mineral wool mattresses with 10 cm thickness placed on the attic floor in under roof space and thermal insulation of the roof of the sub window niches with 7 cm fiber. The effect is saving of 3 320 BGN, or 2.9% reduction in heat consumption and a reduction of the thermal transfer through the roof. At a new

insulation price of 30 BGN per m² for mineral wool and 50 BGN per m² for fiber the necessary investments are 33 181 BGN and payback period is 10.0 years;

Бюджет "Разход на енергия"		ЕС мерки	Мощностен бюджет	ЕТ крива	Годишно разпределение	Топлинни загуби	
Тип сграда		Жилищенблокбет_COBlock4.		Клим. зона	Клим. зона 6 - Пловдив, Ямбол		
Референтни стойности		2012					
Параметър	Еталон kWh/m²	Състояние		Базова линия		След ЕСМ	
		kWh/m²	kWh/a	kWh/m²	kWh/a	kWh/m²	kWh/a
1. Отопление	16,6	104,4	515 866	104,4	515 866	21,8	107 570
2. Вентилация (отопл.)	0,0	0,0	0	0,0	0	0,0	0
3. БГВ	60,6	60,6	299 448	60,6	299 448	60,6	299 448
4. Помпи. вент.(отопл.)	1,0	1,0	5 093	1,0	5 093	1,0	5 093
5. Осветление	8,9	8,9	43 915	8,9	43 915	8,9	43 915
6. Разни	36,0	36,0	177 662	36,0	177 662	36,0	177 662
Общо (отопление)	123,1	211,0	1 041 984	211,0	1 041 984	128,3	633 688
Обща отопляема площ		4 939					

Fig. 3. Results from building energy consumption's model, before and after ESM

Table 4. The economic indicators from the implementation of proposed ESM

ENERGY SAVING MEASURES (ESM)	CURRENT SITUATION	SITUATION AFTER IMPLEMENTATION OF ESM	ENERGY SAVINGS	
	kWh/year	kWh/year	kWh/year	%
Replacement of 878 m ² windows with double glazed aluminum windows	1 041 984	927 151	183 068	17,57
Thermal insulation of external walls and jetty	1 041 984	910 248	131 736	12,64
Thermal insulation of roof	1 041 984	1 011 818	30 166	2,9
Installing of radiator's thermostatic valves	1 041 984	978 658	63 326	6,08
Total for the whole package of ESM	4 167 936	3 827 875	408 296	39,19

Installation of thermostatic valves on heating units. The effect is saving of 6 970 BGN, or 6.08 percent reduction in heat consumption and it is expressed in control and maintenance of the set temperature in the rooms. At price of 30 BGN per valve the necessary investments are 4 800 BGN, and payback period is 0.7 years.

The economic indicators from the implementation of proposed ESM are presented in Table 4 and Fig. 4. The assessment of the individual indicators of the individual ECM shows that the measure with the highest percentage of energy savings is the replacement of windows, and a measure with the shortest return period is a measure of improved room temperature management by installing of thermostatic radiator valves.

Мерки

Проект: SO BLOK 4I

Всички мерки

Рентабилни мерки

Мерки за реконструкция

Мерки по вътрешния микроклимат

PIR

Нерентабилна мярка

Мерки	Инвестиция	Нето икономии	PB	PO	IRR	NPV	NPVQ	Макс. инвестиция		ОБЩО
								1)	2)	
Монтиране на термостатични	4.800	6.870	0,7	0,7	143%	44.058	9.18	37.418	7,0	Инвестиция: 254.240 BGN
Изолация под еркер	5.648	1.860	3,0	3,5	33%	14.476	2,56	10.131	7,0	Икономии: 44.820 BGN
Изолация стени	70.131	12.630	5,6	7,2	17%	66.517	0,95	68.791	7,0	
Смяна дограма	140.480	20.140	7,0	9,7	13%	77.421	0,55	109.695	7,0	
Изолация покрив	33.181	3.320	10,0	17,2	9%	6.466	0,19	18.083	7,0	Срок на откупуване: 5,7 години
										Срок на изплащане: 7,4 години

Мерки

Нов

Промяна

Изтрий

Реален лихвен %: 6,7 %

1) Макс. инвестиция с 2) год. срок на изплащане

Печат

Затвори

Fig. 4. Results from the economic analysis of proposed ESM

Conclusion:

From the obtained results can be drawn the following conclusions:

1. By the building's model study it is established that the potential of thermal energy saving is over 39 %, which is 448 726 kWh saved primary energy annually. Full utilization of this potential requires investments of 254 240 BGH with payback period of 5.7 years. The building will have class of energy consumption B [6] and energy certificate class A [5] after ESM introducing.

2. Environmental impact in results of realized heat savings will be reduction of CO2 emissions in the atmosphere with 139, 6 tons annually.

3. Additional investments will be necessary for isolation the walls (238.57 m2) and jetty (226.22 m2) of under roof space and for hydro insulation of the roof.

4. The results show that the consumed heat energy for domestic hot water heating is 28.74 % from the building's total energy consumption. This energy could be supplied from renewable (solar or geothermal) energy source. Furthermore, the existing incandescent lamps can be replaced with more energy efficient lamps. This will result in savings of 1.62 % electric energy.

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FEASIBILITY EVALUATION OF SOME METHODS AND INSTALLATIONS FOR CLEANING OF WORKING AIR FROM INDUSTRIAL PLANTS

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Abstract: The techno-economic evaluation of four of the most used methods for cleaning of air by industrial work areas, polluted with organic vapor compounds, is done. The operating costs and the value of the energy sources needed for these methods implementation and the value of the recovered solvents are defined. Their advantages and disadvantages are established.

Keywords: air cleaning, volatile organic compounds, ethanol, methanol, ethyl acetate

Introduction:

The air in work zones in many companies from food, biotechnological and other industries is polluted with vapors of different volatile organic solutions (VOS), used in technological process. Under the Law on protection of the environment [7], synchronized with the European legislation on environmental protection and the Regulation for the procedure of issuing complex permits for building and exploitation of existing industrial plants [8] it is required the application of best available techniques in the field of environmental protection and air protection in the working premises. Therefore, such companies need to build plants for working air purification. In addition to the realized environmental effect the economic effect can be achieved through these solvents recovery [1].

In the selection of appropriate technology for purification of air the origin, concentration and other parameters of contaminants such as corrosivity, abrasiveness, toxicity, flammability, temperature, relative humidity, pressure, flow and content of particles in the emissions, efficiency, maximum admissible concentrations (MAC), opportunities and economic efficiency for recycling or recovery of the respective pollutant, energy efficiency, costs of installation and maintenance, etc. must be taken into account. In practice, different methods and technical equipment for purification of polluted with vapors of organic substances air, which have several advantages and disadvantages [12.13] are used.

The purpose of this study is analysis and evaluation of four of most used in practice methods for purification of air from the working premises from vapors of the most used volatile organic solvents (VOS).

Materials and methods:

The possibility and the costs for separation of vapors of the most widely used in industry organic solvents ethanol, methanol and ethyl acetate from their respective mixtures with air in the working premises was examined.

The following technical solutions were studied:

1 method - Condensation. Solvent's vapors were separated from air by installation, which consists of condenser, fan and heater. The contaminated air is sucked by the fan and passes through the condenser, where is indirectly cooled by water from the environment.

methods - 1 kg/h. The actual prices with VAT for industrial consumers in force at 1.1.2012 (electricity - 0.179 BGN/kWh, water - 1.98B GN/m³, heat energy - 110 BGN/MWh) were used for determination of the economic efficiency of the proposed methods and installations. The prices of the investigated organic solvents including VAT are: ethanol - 19.50 BGN/l, methanol - 1.60 BGN/l, the ethyl acetate - 4.70 BGN/l, according to [14].

Results and discussion:

At the first method part of the solvent vapors from the polluted air condense by cooling with water. The disadvantage of this method is that the used cooling water is has an initial temperature of about 14 °C, thus the mixture "humid air - organic vapors" can not be cooled below 17 °C. This does not allow the concentration of organic vapors in air to be reduced to a minimum limit from the point of view of admissible limits of ignition. If it is assumed that the content of organic vapors in air before cooling is d_3 (Fig. 1, Table 1), from the i, d - diagram [3, 9, 10] is read that at 17 °C the ethanol vapor content in the gas mixture is $d_4 = 0.08$, the methanol vapor content - $d_4 = 0.13$, and in the case of ethyl acetate the temperature at all did not reaches the dew point and it is impossible to separate its vapor from the gas mixture. Therefore, this method can't be used for cleaning of the air polluted with ethyl acetate vapors. The operating costs of this method are: cost of cooling water and steam consumption for heating of the purified air into the heater.

At the second method the cooling of the mixture "air - vapor of organic solvent" is carried out with refrigerating machine. Depending on the considered organic solvent it is assumed that for the ethanol $t_4 = 7.2$ °C; for the methanol - $t_4 = 8.5$ °C and for the ethyl acetate - $t_4 = - 7.1$ °C. These temperatures are the dew point temperatures at the minimal limit of ignition of the corresponding organic vapors in air, according to [2]. Operating costs for this variant are: electricity for the fan, compressor and pump for cooling water, as well as the consumption of cooling water and steam for heating of the purified air into the heater.

At the first and second methods the specific enthalpy of conditions 1 and 2 are read by the respective i, d -diagrams. The enthalpy of state 3 is calculated from the energy balance of the corresponding apparatus, and the enthalpy of the state 4 - analytical (the point is located in the area of the fog). The heat flux \dot{Q}_{34} which must be discharged from the condenser - evaporator for cooling of the gas mixture is determined.

$$\dot{Q}_{34} = \dot{m}_B \cdot (i_4 - i_3), \quad (1)$$

where: i_3 and i_4 are the specific enthalpies of the gas mixture before and after the condenser-evaporator [J/kg];

- mass flow rate of the humid air, [kg/s].

From the energy balance of the heater is defined the heat flux \dot{Q}_{12} for heating the humid air from state 1 to state 2.

$$\dot{Q}_{12} = \dot{m}_B \cdot (i_2 - i_1), \quad (2)$$

where: i_1 and i_2 are the specific enthalpy of moist air before and after the heater [J/kg].

For the second method - the output of the compressor and the efficiency ratio of the refrigeration machine are calculated. The consumption of electricity, steam and cooling water, required for separation of the organic vapors from humid air are defined [5]. The

economic effect of the work of these installations in industrial environments to purify contaminated with organic vapor moist air is estimated. The recovered organic solvents are returned to the main production process.

For the *IIIrd* method it is accepted that the mass flow rate of gas mixture is 51 kg/h (mass flow rate of air - 50 kg/h and mass flow rate of organic vapors - 1 kg/h). For the mixture "air - vapors of ethanol" the required mass flow rate of cooling water is 104.5 kg/h, and its cost is 0.21 BGN/h. For the mixture "air - methanol's vapors" the required mass flow rate of water is 196.3 kg/h, and its cost is 0.39 BGN/h. Calculations for the change in temperatures of the mixture "air-organic solvent vapors" and of the cooling water for each stage of the scrubber's height are done [6]. The results are presented in Fig. 3 and 4. A pump with output of 0.37 kW and a fan with output 0.245 kW are chosen.

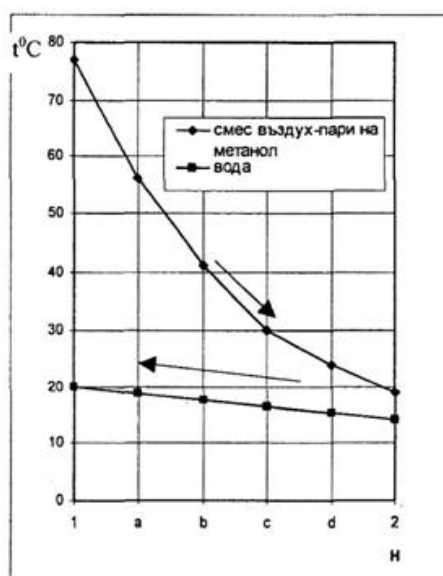


Fig. 3. Change in temperatures of the mixture "air-methanol's vapors" and of the cooling water for each stage of the scrubber's height, if the outgoing water temperature is 20 °C

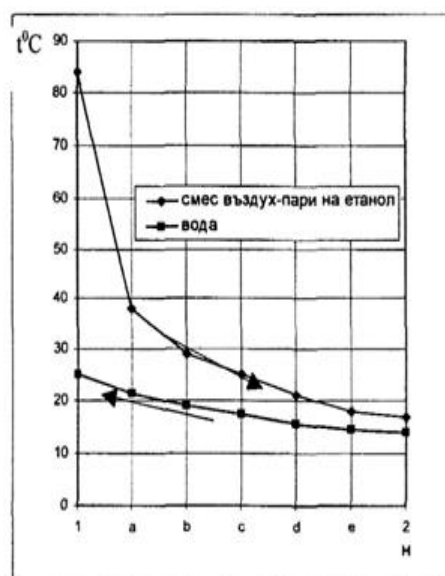


Fig. 4. Change in temperatures of the mixture "air-ethanol's vapors" and of the cooling water for each stage of the scrubber's height, if the temperature of the outgoing water is 25 °C

For the *IVth* method - the organic vapors from the air are adsorbed of activated carbon and then the organic solvents are recovered by desorption. This method is used at low concentrations of organic vapor in air (50 g/m³). It uses electricity for fans, steam, hot and cold air for desorption process and additional energy for distillation of solvents. The average energy consumption for 1 t recovered solvent is: water vapor - 3 ÷ 5 t, cooling water - 30 ÷ 100 m³, electricity - 50 ÷ 600 kWh and activated carbon - 0.5 ÷ 1 kg [11]. The results of the data processing for the four studied methods are presented in Table 1.

Table 1. Parameters of the process cleaning of polluted with volatile organic components air

Method	Parameter	Ethanol	Methanol	Ethyl acetate
I	d_4 , kg/kg	0,10	0,15	0,185
	Mass flow of recovered solvent, kg/h	1	1	1
	Value of recovered solvent, BGN/h.	15,31	1,26	4,20
I	t_1 , °C	17	17	-
	d_1 , kg/kg	0,08	0,13	-
	t_3 , °C	84	77	-
	t_4 , °C	17	17	-
	\dot{Q}_{12} , W	1306	1360	-
	\dot{Q}_{34} , W	1328	1369	-
	Steam consumption, kg/h	2,13	2,22	-
	Cooling water consumption, kg/h	19,0	23,4	-
	Operating costs, BGN/h	0,18	0,194	-
II	t_1 , °C	7,2	8,5	-7,1
	d_1 , kg/kg	0,042	0,08	0,068
	t_3 , °C	51	27	57
	t_4 , °C	7,2	8,5	-7,1
	\dot{Q}_{12} , W	479	400	285
	\dot{Q}_{34} , W	499	410	316
	Refrigerating ratio - ε	3,1	3,2	3,16
	Compressor consumption, kW	0,16	0,13	0,1
	Steam consumption, kg/h	0,78	0,65	0,47
	Cooling water consumption, kg/h	18,7	15,48	12,24
	Operating costs, BGN/h	0,12	0,10	0,0734
III	Cooling water consumption, kg/h	104,5	196,3	-
	Pump and fan consumption, kW	0,615	0,615	-
	Operating costs, BGN/h	0,32	0,50	
IV	Steam consumption, kg/h	4	4	4
	Water consumption, kg/h	0,065	0,065	0,065
	Electricity consumption, kWh/h	0,3	0,3	0,3
	Activated carbon consumption, kg/h	0,00075	0,00075	0,00075
	Operating costs, BGN/h	0,46	0,46	0,46

Conclusion:

1. Analysis and evaluation for four of most used in practice methods for cleaning of polluted air with VOS vapors (condensation, cryocondensation, absorption and adsorption) have been done.

2. Operating costs for the I, II and III method are calculated, and these for the IV method are taken according to the literature. The obtained results show that the economic effect (profitability) from regeneration of 1 kg VOS from the polluted air in installation according to the II method is considerably higher in comparison with the rest three methods.

3. The I and III methods couldn't be applied to cleaning of polluted with ethyl acetate vapors air. The IV method could be used only when the organic vapors content in air is up to $0,05 \text{ kg/m}^3$, and except of that these installations are too large and complicated [11].

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TECHNOLOGY FOR PRODUCING VEGETABLE OIL FOR PREVENTIVE

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Abstract: The technology of obtaining vegetable oil for preventive was research. It is established that the higher pulp temperature and below it is moisture content, the higher content of biologically active substances in vegetable oil.

Keywords: Vegetable oil, bioactive substances, technology.

Introduction

Processing of oil-yielding crops receiving vegetable oil, possessing medicinal properties, is the social and technological issue of the day of fat-and-oil branch of food industry.

Matters of vegetable oil which concomitant threeatsilglicerid, namely: phospholipides, liposoluble vitamins and provitamins (tocopherolss, carotins), waxes, odorizing matters and free fatty acids add characteristic for every vegetable oil organoleptic features by which it is possible to identify the source of its obtaining.

Modern technology of vegetable oil receiving at industrial enterprises of Ukraine foresees realization of affinage with the purpose of moving away of concomitant matters which accelerate the processes of oil spoilage, because of flowing of oxidizing and hydrolysis reactions during storage of vegetable oil. After affinage realization vegetable oil becomes depersonalized, without taste, smell, almost colourless. On the stage of adsorption affinage with whitening clays liposoluble vitamins are deleted from oil and phospholipides are destroyed using hydratation by bringing of runback or acids solutions in. The refined vegetable oil impoverished for bioactive matters, which showing medicinal properties and determining prophylactic setting of food product, which they contain. Vegetable oil and oil-bearing seed contain pigments having vitamin and antioxidant properties and which are used in medicine as a general tonic mean. Pigments are soluble in oil and colour it. Tokopherol (vitamin E) and carotins (provitamin A) colour oil yellow-and-red, and chlorophyll green. Pigments absorb electromagnetic waves in visible light range in different ways, that gives the possibility to identify and to amount them.

Modern major concerns obtain vegetable oil from seed of oil-bearing cultures following the classic plan of forepressing and extracting, thus on the forepressing stage they get up to 60 % lipids from their primary content in oil-bearing raw material. The classic plan foresees cleaning of seeds from admixtures, decortication, dividing of cortical substance into fractions, crushing of the cleared kernel, wet-thermal treatment, forepressing.

Changing the technological modes of press vegetable oil obtaining it is possible to get oil with certain content of bioactive matters. A.I. Skipin [1] showed the possibility of getting high-cleared press vegetable oil by means of fiber moistening to humidity more than 10–12 %. Shortcoming of Skipin's method is the necessity of moist cake drying that draws power expenses and law quality of the dried up cake because of denaturizing of albumens, oxidization and hydrolysis of threeacetilglicerids.

High temperature of the granulated crushed of the kernel promotes lipids coalescing by spheroids of exposed vegetable cages, as a result of which oily superficial films appear. Lipids viscosity diminishes and the material becomes plastic. The brought in steam condenses and is sorbed by cellulose, creating unwettable oil capillaries. Fiber temperature at the exit from a fire-pan, in which conduct wet-thermal treatment is made, is about 105 °C, that promotes evaporation of free moisture and inactivates lipase, lipooxidase and other enzymes of the pressed material.

Materials and Methods

The purpose of our work consisted in creation of technology for vegetable oil enriched by the bioactive matters obtaining.

In our research we reproduced moisture-temperature treatment of sunflower crushed of the kernel, by moistening material before pressing and heating it at a laboratory press during pressing. The cleared sunflower kernel was moistened by water, and then left for three hours with further chopping and pressing. Moisture content in crushed of the kernel, pomace and vegetable oil was determined by arbitrage method, dried at the 105 °C in a drying oven to permanent weight. In the received vegetable oil we measured the content of bioactive matters spectrophotometrically at absorbance 10 % hexane solutions at wavelength of 450 nm for carotenes and 295 nm for tocopherols. As solution of comparison a clean solvent - hexane was used.

Results and Discussion

The research results of moisture-temperature influence treatment are presented in table 1.

Pressed vegetable oil, received at the temperature of pressing 110–120°C and from fiber with less content of free moisture, has greater content of bioactive matters. Obviously, concomitant triacylglycerids connections showing difil properties remain in pomace in the state related to moisture. Moisture in its turn is sorbed by squirrel and cellulose and remains in pomace, promoting its humidity. Probability of chemical transformations of lipids and concomitant matters is great at the high temperature conditions of material treatment and its pressing. Therefore free fat acids and free radicals of the received vegetable oil were measured using standard titrimetric methods. Data are presented in table 2.

Table 1. Influence of the modes of moisture-temperature treatment on high-quality content of bioactive connections in sunflower-seed oil

Material	Table of moisture contents, %	Absorbancy		Material	Table of moisture contents, %	Absorbancy	
		295 nm	450 nm			295 nm	450 nm
Pressing temperature 90-100 °C				Pressing temperature 110-120 °C			
without moistening				without moistening			
Crushed of the kernel	3,07±0,67			Crushed of the kernel	3,07±0,67		
Pomace	5,92±0,67			Pomace	5,47±0,67		
Oil	0,04±0,01	0,248	0,129	Oil	0,04±0,01	0,391	0,182
with moistening				with moistening			
Crushed of the kernel	7,19±0,67			Crushed of the kernel	7,19±0,67		
Pomace	10,36±0,67			Pomace	9,84±0,67		
Oil	0,12±0,01	0,115	0,103	Oil	0,11±0,01	0,193	0,120

Table 2. Content of free fat acids and free radicals in the got sunflower-seed oil

Name	Acid number, mg KOH/ g of oil	Peroxid number, mmol O ₂ / kg of oil
Oil, received without moistening at 90–100 °C	0,24±0,06	0,09±0,02
Oil, received without moistening at 110–120 °C	0,22±0,06	0,12±0,02
Oil, received with moistening at 90–100 °C	0,33±0,06	0,09±0,02
Oil, received with moistening at 110–120 °C	0,26±0,06	0,10±0,02

By high-quality indexes vegetable oil substantially does not differ inter se and corresponds the indices of normative documents for unrefined sunflower-seed oil that testifies the unimportant in these terms flowing of hydrolysis and oxidizing processes.

Conclusions

Researches showed that higher temperature of fiber and its lower humidity is the higher content of bioactive matters in vegetable oil, in particular carotenes and tocopherols. Technology of vegetable oil with high content of bioactive matters obtaining includes the hard modes of wet-thermal treatment of fiber: pressing temperature 110–120 °C and minimum content of moisture in material.

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AROMATIC COMPOUNDS OF WINE OBTAINED FROM THE NEW VARIETY OF DOMESTIC SELECTION VIORICA

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Abstract: The aromatic compounds of the wine obtained from the new variety of domestic selection Viorica have been subjected to researches. The analysis was carried out on gas chromatograph with mass spectrometer Clarus 600 T. About 100 compounds have been identified. The identification of chromatographic peaks (drops) has been conducted under the general library of NIST mass spectrometer. The researches have shown that the composition of aroma (flavor) of Viorica wine is very complex and consists of compounds of different classes: aldehydes, ketones, higher alcohols, esters, terpenes, organic acids, lactones etc. The main compounds of aroma are the higher alcohols: isoamyl and 2-phenylethanol, the percentage share of which is 46%. These compounds form the basis (core) of wine flavor, which is completed by other aromatic compounds such as esters, terpenes, acetals etc.

Key words: aroma complex, aldehydes, ketones, chromatograph, high alcohols.

The aromatic substances have a special role to form the wine quality. It is known the fact that [Țirdea C., 2007; Rusu E., 2011] the wine aroma consists of compounds (aromatic (flavored) substances) which derive from grapes (primary aromas (flavors), arisen during the alcoholic fermentation (secondary aromas (flavors) and during the period of maturation-storage of wine (tertiary aromas (flavors)).

In turn, the aromatic complex of wines is very rich and consists of substances that are part of different classes. So far there have been identified more than 500 odorous substances which, individually or in combination within a great diversity and complexity of mixtures, are able to give about 10.000 fragrant scents to wine [Cotea, V. D. and col., 2009].

On the other hand, the established range of varieties is always subject to the review and completion with new varieties that are of interest. In recent times, on a worldwide scale, more and more countries outline some varieties, so-called “national”, on account of which the wine brand is being created. Most of these are local varieties, less known to wine consumers. At this compartment the Republic of Moldova also has some new varieties of domestic selection, which are invaluable (Țuțuc V. and col., 1998; Apruda P., Berezicov M., 2002).

Unlike wines made from classic varieties, the aromatic complex of those derived from new varieties of selection has not been studied at the fair value thereof. In this context, there shall be mentioned the study of aromatic compounds of Viorica wine conducted by Ajoghina B.A. and Agheeva N.M. (1998). There have been identified 29 compounds belonging to different classes.

Unlike the methods of analysis of aromatic compounds from the 90's, the methods used today are much more sophisticated, especially when coupling the mass spectrometer to the chromatograph. In this context, we consider it appropriate to conduct a study on the aromatic composition of the Viorica wine using such modern devices.

Materials and methods

The object of researches was the sample of wine derived from the new variety of selection Viorica during the wine campaign in 2010.

For a depth research of the aromatic complex, the obtained wine was passed through a cartridge type DIAPAC which consists of extra-reticulated polystyrene of hydrophobic nature, activated pursuant to a special treatment scheme. The elution of aromatic substances adsorbed on the surface of cartridge was performed with a mixture of acetated ethyl and pentane in a 1:1 ratio. The analysis of eluent was made by means of the gas chromatograph with the mass spectrometer Clarus GooT, column PE-WAX ETER with a length of 50 m and inner diameter of 0.32 mm. Analysis conditions: bearer of gas-helium, evaporation room temperature – 220°C, column temperature – 75°C, heating range – 4°C/min up to the temperature of 225°C. The identification of chromatographic peaks (drops) was made according to the general library of NIST mass spectrometer.

The researches have been carried out in the laboratory of Oenology and Wines with Origin Name of the Practical Scientific Institute of Horticulture and Food Technologies and the Practical Scientific Institute of Fruit Growing (Pomiculture) and Viticulture of Northern Caucasia of the Agricultural Academy of Russia, Russian Federation.

Results and discussions

The wine obtained from Viorica variety, from olfactory point of view, is characterized by a very fine aroma (flavor), with intense scents of savory (thyme) in combination with the balsamic ones.

The composition of aromatic compounds examined shows that the highest percentage share is reached by the higher alcohols, and namely the isoamyl alcohol – 31,853%, 2-phenylethanol – 15,773% and isobutanol – 1,143%. But the olfactory characteristics of these alcohols are not far balsamic ones. The isoamyl alcohol is distinguished by its fruitiness, the 2-phenylethanol has a general pleasant aroma (flavor), and the isobutanol has no flavor. As mentioned by Cotea V. D. and col. (2009) the 2-phenylethanol plays an essential role in the formation of wine aroma (flavor), but without giving a certain characteristic feature. From the total number of identified compounds, 27 belong to esters, those with a higher proportion being the mono-Ethyl succinate, ethyl lactate, ethyl propanoate, isoamyl acetate, diethyl succinate, ethyl-4-hydroxy butanoate, 1,4-butanediol-diacetate. From these esters, the mono-Ethyl succinate has a great contribution to the formation of aroma (flavor), the ethyl acetate has a pleasant and fine flavor, the isoamyl acetate – flavor of banana, pear, wild strawberries, diethyl succinate – bunch of maturation, diethyl malate – pleasant odor.

About half of the percentage proportion of compounds identified in the aromatic extract of Viorica wine constitutes the higher alcohols. Of these compounds, three compounds outline to have the large proportion: isoamylol (31,253%), 2-phenylethanol (15,773%) and isobutanol (1,143%). The proportion of the other higher alcohols is under 1%. Given the large share of higher alcohols we suppose that they have an important role in creating the olfactory peculiarities of wine. In this context there shall be noted the influence of some higher alcohols on aroma (flavor), such as: 2-phenylethanol which has a pleasant characteristic odor, the phenylethanol with a pleasant aroma, heptane-2-ol,-1,7,7-trimethyl – canfor aroma, the isoamylol which has a pleasant characteristic (fruitful) odor.

The results obtained as regards the proportion of some higher alcohols in the aroma (flavor) composition are confirmed and supported by data from literature. Thus, Ajoghina

B.A. and col. (1995) confirms that, from the summary quantity of those 29 compounds determined in Viorica wine, 54% fall on those three higher alcohols (isoamyllic, 2-phenylethanol, isobutanol).

A key contribution on the aromatic character belongs to the terpenic compounds, although the content thereof is low. The following terpenes have been identified in the aroma of Viorica wine: β -mircene, limonene, cimene, cis-Linalool oxide, linalool, hotrienol, 2,6-dimethyl-3,7-octadien-2,6-diol, geraniol, 3,7-dimethyl-1,7-octadien-3,6-diol, α -Terpineol. These compounds have pleasant aromas (flavors) of Muscat, coriander, lilac, rose and positively influence upon wine flavor.

The ketones identified in the aromatic extract are: α -butanone, 3-methyl-2-butanone, 2-pentanone, 2-methyl-3-pentanone, 3-methyl-2-pentanone, 3-etoxy-2-butanone, 2,2-dimethyl-4-hydroxy-3-hexanone, 2-heptanone, 1-methoxy-1-octen-4-one. Despite the fact that the ketones are in small quantities, they give to wine a pleasant flavor, dominated by scents of fruits, conveyed by 2-heptanone.

As concerns the aldehydes, there shall be outlined that only four substances have been found in the aromatic extract, and namely the nonanal, possessing an odor of rose, flags (irises) and mandarins, the furfuralul with a pleasant fragrant of fresh bread, benzaldehyde – bitter almonds and 2-phenylacetaldehyde with odor of Zambia.

Among the organic acids the percentage proportion falls on the acetic acid – 9,290%. The presence of this acid in a relatively large quantity is benefic for wine aroma (flavor), because, in the opinion of Cotea V. D. and col. (2009), it is a good solvent for oils, amplifying the olfactory sense. An another organic acid which shall be mentioned is the hexanoic acid with a pineapple flavor, the propanoic acid with a very characteristic flavor, n-butyric acid – fragrant flavor, 3-methylbutyric acid – valerian scent. The other saturated and unsaturated acids have a lower influence on the composition of aroma (flavor).

There have been identified two compounds from the category of lactones: butyrolactone, which has a complex, wine-like odor, and (S)-(+)-2,3-dideoxy ribonolactone, which gives a pleasant flavor to the wine. The percentage share of the last lactones is relatively high and is 0,929%.

Table. Volatile compounds determined in the aromatic extract of Viorica wine

No.	Aromatic compound	Share	Category
1	2	3	4
1	Acetone	0,043	Ketones
2	1,1-dietoxymetan	0,029	Acetals
3	2-butanone	2,361	Ketones
4	3-methyl-2-butanone	0,449	Ketones
5	2,4,5-trimethyl-1,3-dioxolane	0,003	Odor oxides
6	Ethyl propionate	0,801	Esters
7	Propanoic acid	0,008	Organic acids
8	Propyl acetate	0,011	Esters
9	2-butyl acetate	0,016	Esters
10	2-pentanone	0,019	Ketones
11	2-methyl-3-pentanone	0,014	Ketones
12	Isobutyl acetate	0,008	Esters
13	2-butanol	0,123	Higher alcohols
14	2-methyl-3-buten-2-ol	0,010	Higher alcohols
15	3-methyl-2-pentanone	0,006	Ketones

1	2	3	4
16	Propanol	0,082	Higher alcohols
17	1,1-detoxybutan	0,004	Acetals
18	Ethyl butyrate	0,056	Esters
19	Butyl acetate	0,018	Esters
20	Isobutanol	1,143	Higher esters
21	3-etoxy-2-butanone	0,012	Ketones
22	Isoamyl acetate	0,403	Esters
23	Butanol	0,057	Higher alcohols
24	2,2-dimethyl-4-hydroxy-3-hexanone	0,009	Ketones
25	β -mircen	0,021	Terpenes
26	2-heptanone	0,022	Ketones
27	Isoamylol	31,853	Higher alcohols
28	Limonene	0,022	Terpenes
29	Ethylhexanoate	0,122	Esters
30	Pentanol	0,023	Higher alcohols
31	3-Methyl-3-buten-1-ol	0,003	Higher alcohols
32	Hexyl Acetate	0,006	Esters
33	Ethyl pyruvate	0,011	Esters
34	Cimene	0,001	Terpenes
35	Cyclohexane	0,009	Hydrocarbons
36	2-Heptanol (standard sol.)	12,187	-
37	3-Methyl-1-pentanol	0,024	Higher alcohols
38	Hexanol	0,317	Higher alcohols
39	Ethyl lactate	1,819	Esters
40	trans-3-hexenol	0,051	Higher alcohols
41	3-etoxypropanol-1	0,013	Higher alcohols
42	cis-3-Hexen-1-ol	0,014	Higher alcohols
43	1-methoxy-1-octen-4-one	0,013	Ketones
44	Nonanal	0,012	Pelargonic aldehyde
45	Ethyl Octanoate	0,081	Esters
46	cis-Linalool-oxide	0,019	Acyclic terpenes
47	Acetic acid	9,290	Volatile acids
48	Furfural	0,001	Aldehydes
49	Ethyl-3-hydroxibutanoate	0,027	Esters
50	Linalool	0,173	Terpenes
51	2,3-Butanediol	0,048	Polyhydroxilic alcohols
52	Ethyl-2-hydroxycaproate	0,014	Esters
53	1-Octanol	0,007	Higher alcohols
54	Benzaldehyde	0,002	Aldehydes
55	2-methyltetrahydroxytiopen-3-ol	0,014	Sulfur compounds
56	Acetoin	0,017	Saturated ketones
57	Isobutyric acid	0,094	Organic acids
58	Hotrienol	0,184	Acyclic terpenes
59	1-Methoxy-2-Butanol	0,005	Higher alcohols
60	Ethyl decanoate	0,022	Esters
61	Heptan-2-ol, 1,7,7-trimethyl	0,006	Higher alcohols
62	Ethyl methyl succinate	0,004	Esters
63	N-butyric acid	0,270	Organic acids
64	Butyrolactone	0,040	Lactones

1	2	3	4
65	Diethyl succinate	0,383	Esters
66	3-methyl butyric acid	0,205	Monocarboxylic acids
67	2,6-dimethyl-3,7-octadien-2,6-diol	0,089	Terpenic alcohols
68	α -Terpineol	0,166	Terpenic alcohols
69	Methyl-3-thio-propanol	0,012	Thioether + alcohol
70	1,3-propanediol diacetate	0,124	Esters
71	Citronellol	0,010	Terpenic alcohols
72	2,7-dimethyl-4,5-octandiol	0,011	Polyhydroxylic alcohols
73	Diethyl glutarate	0,003	Esters
74	i-butyric acid	0,066	Saturated fatty acids
75	Ethyl-4-hydroxybutanoate	0,128	Esters
76	Methyl-2-hydroxybenzoate	0,008	Esters
77	Ethyl-2-fenilacetate	0,040	Esters
78	Geraniol	0,233	Terpenes
79	Hexanoic acid	1,952	Unsaturated mono-carboxylic acids
80	N-methyl butyl acetamide	0,082	Amides
81	1,4-butanediol diacetate	0,142	Esters
82	Phenyl-methanol	0,048	Higher alcohols
83	2-phenylethanol	15,773	Higher alcohols
84	2,6-dimethyl-7-octen-2,6-diol	0,126	Unsaturated diols
85	S-N-(1-cyclohexanethyl)-acetamide	0,008	Amides
86	Diethyl malate	0,997	Esters
87	Octanoic acid	2,501	Unsaturated acids
88	3,7-dimethyl-1,7-octadien-3,6-diol	0,034	Polyhydroxylic terpinolene
89	Diethyl-2-hydroxypentanedionate	0,527	Esters
90	2-methoxy-4-vinylphenol	0,356	Volatile phenols
91	(S)-(+)-2,3-dideoxy ribonolactone	0,929	Lactones
92	Decanoic acid	0,588	Unsaturated acids
93	Ethyl-2-hydroxy-3-phenylpropanoate	0,007	Esters
94	2-phenylethanal (2-Phenylacetaldehyde)	0,047	Aldehydes
95	trans-Geranic acid	1,072	Monocarboxylic acids
96	Mono-ethyl succinate	7,968	Esters
97	2,3-Dihydrobenzofuran	1,815	Furans
98	Benzoic acid	0,156	Organic acids
99	N-(2-Phenylethyl)-acetamide	0,017	Amides
100	Ethyl-5-oxo-2-pirolidin Carboxylate	0,835	Amine + ester

The aromatic extract of Viorica wine also consists of other compounds, such as 2,3-dihydrobenzofuran, which is the derivative of furan, having also a relatively large percentage share – 1,815%, methyl-3-thiopropanol, which is part of the category of thioethers and which has an odor of mown hay, 2,6-dimethyl-7-octen-2,6-diol, representative of diols and which is characterized by a terpenic character and 2-methoxy-4-vinylphenol, which is part of the volatile phenols.

Conclusions

The study carried out has shown that the composition of aroma of Viorica wine is very complex and consists of compounds of different categories: aldehydes, acetones, higher alcohols, esters, terpenes, organic acids, lactones etc. The main compounds of aroma are the higher alcohols, isoamyllic, 2-phenylethanol and isobutanol, which share percentage is 48,769%. The nominated compounds form the basis (core) of wine aroma, which is completed by other aromatic compounds. It was found that a numeric and quantitative representative class of wine aroma is the esters. Although the share percentage of terpenes in the aromatic composition is low, their contribution to the formation of aroma character of Viorica variety shall not be neglected. We assume that the balsamic character with scents of thyme is determined more by the interaction of aromatic compounds, in a large number, on the ground of main constituents – isoamyllic, 2-phenylethanol and isobutanol acids.

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PROTEIN SUPPLEMENTS BASED ON SOY, FLAX SEEDS AND DRY MILK SERUM

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Abstract: The shortage of digestive protein – is an urgent problem not only in animal agriculture, but also in formula feed and grain-processing industry, which due to the protein shortage can't produce feeding-stuff that is well-balanced by the amino acid content.

In the article an actuality of flax seeds, soy and milk serum usage by the production of the protein supplement for different age groups of animals is observed. The technological scheme of protein supplement production is given.

Key words: protein supplements, flax seeds, dry milk serum

Nowadays the biotechnological direction for the agroindustrial complex especially for the feed stuff production is developing very intensive. Multicomponent protein supplements for the feed stuff enrichment have spread very much. In Ukraine the production of such products is almost out. The market is filled with imported and expensive premixes and additives.

The problem analysis has shown that the demand of such products is high enough and it will be increasing according to the increasing of the animal and bird livestock. The major problems of the breeding are: value increase of the feed stuff and embedded component for the animal management, a poor state support of the agrarian brunch. The only way out for the provision income in farm business – is an integrated production system that would help to reduce production costs and to enable a possibility of a quality improvement.

Ukraine has an actual problem – an animal industry developing, as the animal industry is not produced by the industrial way, and it loses an opportunity of successful using the scientific achievements and new feeding technologies. Under such conditions a marginal cost and a low quality of production are observed.

A modern fodder production is based on the outdated technologies or on the imported purchasing. In developed countries the fodder production is based on high technologies with the using different knowledge gathering of the whole world. Feed stuffs, which are produced on the base of high-level processing of various materials, have an especial actuality. These materials have vegetable, animal, microbiological origin, in other words they are technologically cultivated multicomponent mixtures, which are brought to necessary fineness depending on age and physiological status of animals.

The purpose of every husbandry is the maximum profit by optimal productivity. The part of outgoings for the food stuff and veterinary preparations in general structure of all financial placements in swine rearing works out 70-80%. The feed ration must be well-balanced to achieve animal culture productiveness, to improve their development, to optimize their reproductive functions. The well-balanced feed stuff must accord with all needs of age groups and meet requirements in feedstuffs and biologically active substances according to their physiological state.

ITTF NANU of Ukraine has an experience in technologies and equipment development for farming industry, which includes also fodder production.

We have proposed and explained the usage of soy, flax seeds and dry ilk serum for getting the therapeutic food products, which would be added to the feed stuff. The balanced formulation with fat, protein, carbohydrates and vitamin- mineral constituent content was developed. Such components compose the protein supplement: milk serum, soybeans, flax seeds. The usage of milk serum enables the providing of the product with all valuable components: serum proteins (90% from general protein quantity), carbohydrates, milk fat, vitamin- mineral constituents, and it also fulfils the function of dispersing environment for the solution of dry components in the paste-like additives production or in the production of calf milk replacer.

In the protein supplement production the dry milk serum is used. Serum proteins (milk albumin and lactoglobulin) stimulate the fermentative function of the stomach, help on vegetational protein fraction fixing and they are immune properties carriers.

The leading suppliers of fat, protein and vitamin- mineral constituents are soybeans and flax seeds, therewith all these products confer the feed stuffs medical and preventive properties, the chemical compound of which is given in the schedule 1.

Table 1 Average Weighted, Chemical Compound of the Components

Markers	Soybeans	Flax seed
Proteins, %	36,5	23,0
Fats, %	20,0	35,0
Carbohydrates, %	30,2	35,0
Fibre, %	3,2	9,0
Water, %	8,5	8,0
Vegetable Food Fibers, %	-	28,0
Ash, %	1,7	3,0
Assimilation, %	90,5	91,6
Protein Efficiency Ratio, %	2,32	1,76
Protein Score, %	47,0	56,5

Flax seeds have been at all times used as a source of OFF (oil for food) for the purpose of medicated products. However, the last year examinations have found out the range of properties of the flax seeds, that largely determine its application area as a nutraceutical. The main components, which determine the biological reactivity of the flax seeds, are: fatty oil, proteic substances, vitamins, ferments, mucus, carbohydrates, carboxylic acids, microelements. The flax oil has the lowest content of the unwanted for the ration saturated fats. The unicity of the flax seeds consists in very high (up to 57%) contest of polyunsaturated alfa-linoleic acid (ALA), the indispensable fatty acid in the animal organism, that as a hormone-like preparation supports the carrying-out of the important biological functions in the organism. That is why the flax seed and an exuded from it flax oil have not only technical but also therapeutic value.

Flax seeds should be used in the feed production, as the defecation of the intestinal tract by the means of flax seed can be called not a defecation but an intestinal tract regeneration.

The flax seed consists of a plenty of necessary substances, which excrete, soften and coat the alimentary organs, relief and exert an antiphlogistic effect. Therewith the flax seed

digests very slowly and that supports the mechanical intestine distraction and so guaranties an intensive contraction and an excretion of a food mass.

The improvement of the secretory activity of the gastrointestinal tract is realized by means of alkaloid and linamarin, which are placed in the glume. The mucus, that appears by the grain handling, provides the defence from different excitors.

The feed supplements, which consist of flax seed have a special stuff – lignin, thanks to which the elimination urine system, the probability of nephritis decreases, the liver functioning is being supported. That is why the usage of the flax seeds in the protein supplements production not only enriches the feed stuff with the native protein, fat and microelements but also renders medical and preventive properties.

In ITTF NANU the technology and special equipment for getting the granulated multicomponent mixtures on the base of extruded handled soy were worked out, which can be widely used in the feed stuff production. The mixtures can be used as a self-consisted feed stuff for the young stock so also as a an additive to the feed stuffs for different kinds of animals and birds.

The modernity of the technology lies on the fact that the effect of soy seeds plasticization, dispersed in high pressured extruder, direct in output of the drawing block. On the last stage of extrusion the soy seeds heat up immediately to high temperature (120-160°C), herewith the carbohydrates melting, fats outcome and inhibitor inactivation is performed.

In such a condition “the melt” is placed in the flame source less than 0,1-0,5 sec., after that a carbohydrates recrystallization with the formation of loose product structure takes place. The effect of the molten state is used for the protein-vitamin components introduction into dispersed soy. It provides a deep interreacting of the components, their equal distribution and ordered structuring.

The introduction of protein components and oil cakes or dispersed flax seeds is being realized due to the special developed equipment, made in the Technical Thermophysics Institute HAH of Ukraine .

The production line for the production of granular mixtures enables the production in a high range of concentration and qualities of the cooperating components of the mixture with the enough protein, vitamins minerals and remedial agents.

Flax seeds are prepared as follows: the flax oil is being partially deleted and oil cakes are being produced, or flax seed are being dispersed in the rotor-impulse device with the fluid addition.

There is a technological scheme of a protein supplement production for different animals' age groups (fig.1).

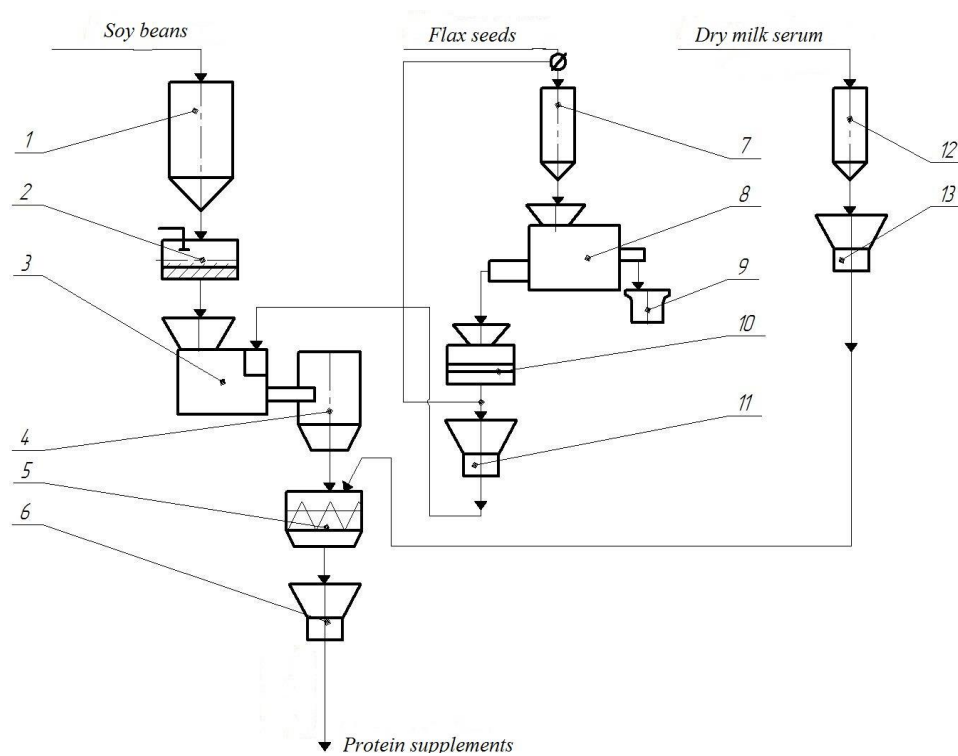


Fig. 1 Technological scheme of protein supplement production

1 – soy beans tanker; 2 – humidifier; 3 – extrusion device; 4 – mixer container; 5 – mixer; 6 – bagging turntable; 7 – flax seeds tanker; 8 – mill; 9 – flax oil container; 10 – oil cakes processing; 11 – bagging turntable; 12 – serum container; 13 – bagging turntable

Thus the developed technological scheme will enable the production of competitive multicomponent protein supplements for the mash enrichment in the base of extruded soy, flax seeds and dry milk serum, which will not bate by their chemical composition the imported high-priced supplements and premixes.

FUNCTIONAL FOODS AND HEALTH CLAIMS IN THE CONTEXT OF EUROPEAN LEGISLATION

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Abstract: functional foods and dietary supplements have undergone recent years austere rules at European level regarding health claims. Countries began to adopt these laws at national level, which will affect food producers. We will present the causes of issue of these regulations, their main characteristics, compliance with these conditions and how to obtain approvals for the use of new health claims.

Key words: functional foods, health claims, legislation, pharmaceutical industry.

Introduction

Functional foods subject is of general interest because it has vast implications. Consumers want to have better health and are looking for healthy foods. Industrial producers want to make a profit from the sale of these products. Scientists seek to broaden the knowledge and research, exploring the topic from various perspectives.

Both pharmaceutical and functional food industry addresses a common market segment: sick people or healthy people who are concerned about health maintenance, disease prevention and longevity. The two industries have a different approach on the subject of health. First seeks the solution in pharmaceutical laboratories, where they synthesize various chemical drugs. The second industry, based on the Hippocratic concept that our food should be our medicine, seek the same solutions as can be found in nature, which is compatible with the human body.

Definitions

The term "functional foods" was first introduced in Japan in the years 1987-1991, as Foods for Specified Health Use (FOSHU). However, the notion of food that has a positive impact on human health has been known since thousands of years ago (traditional Chinese medicine - over 1000 years BC, Hippocrates – IVth century BC).

There have been several attempts to introduce a definition for functional foods, but so far no agreement has been reached and there is no generally accepted definition. But we will present some definitions, as were set by various educational institutions, government and food authorities. Thus, a functional food is:

- A food that provides a health benefit as well as nutrients. The term can refer to whole foods, to fortified, enriched or enhanced foods, and dietary supplements that have the potential to improve mental and physical well being and reduce the risk of diseases. The ingredients responsible for this benefit can be naturally present or may have been added during processing. The levels of nutrients in foods can be increased beyond their natural levels to create an enriched product. Fortified products contain nutrients or ingredients that were not present in the original food (1).
- Natural or processed food that contains known or unknown biologically-active compounds; which in defined quantitative and qualitative amounts, provide a clinically

proven and documented health benefit, and thus are important sources in the prevention, management and treatment of chronic diseases in the modern age (2).

- A functional food is similar in appearance to, or may be, a conventional food, is consumed as part of a usual diet, and is demonstrated to have physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions (3).

- Foods containing ingredient with functions for health and officially approved to claim its physiological effects on the human body. FOSHU is intended to be consumed for the maintenance / promotion of health or special health uses by people who wish to control health conditions, including blood pressure or blood cholesterol (4).

- A modified food that claims to improve health or well-being by providing benefit beyond that of the traditional nutrients it contains. Functional foods may include such items as cereals, breads, beverages that are fortified with vitamins, some herbs, and nutraceuticals (5).

When mentioning "bioactive compounds" or "compounds with functional role," it covers food chemical compounds that may or may not play an energetic role (calories provider), but necessarily has a beneficial role in certain biological processes and good (or better) functioning of the human body.

Approval of drugs and health claims for foods

Both drugs and functional foods must be proved scientifically and clinically for beneficial effects, which are intended to be mentioned on the label or in its advertising.

For drugs are used most often synthetic chemicals that are tested first on cell cultures and animals to determine its lethal doses: DL0, DML and LD50, meaning respectively a single oral dose at which no animal dies in the test group, single oral dose at which the first animal dies and single oral dose at which 50% of the animals die. Afterwards it may be done a test of chronic administration of a substance to see its effects for several months. The recordings are made, attempting to identify at least one effect that could reduce symptoms of some diseases. Sometimes the tests are repeated for sick animal, which are taking the studied substance to verify its intended effect. The same tests can be done on humans. If the effect is confirmed, for this substance will be submitted for drug approbation a file. Other effects of the substance on the body are passed as "side effects" (6).

Currently there are approximately 6,700 approved drugs worldwide (7).

In the case of functional foods, appears the first major legislative distinction from drugs: whatever their effects on health may be, the law does not allow the use of functional foods for disease treatment. The law does not allow neither labeling these products as beneficial for diseases treatment. The only thing that is approved on label or advertisement is the effect of reducing the risk of illness or certain benefits for child health and development. All these expressions specified on the label (or other promotional messages) are defined in the legislation as "health claims" (8). To obtain permission to use a specific new health claim, a file should be submitted to the European Food Safety Authority (EFSA) (9). The file must include a detailed description of product characteristics and clinical trials carried out on it related to the claim which is meant to be used. Dossier evaluation is very restrictive, so in the more than 5,000 cases filed in 2008-2011 were considered for evaluation about 3000, and of these only 7 were approved health claims for reduced risk of a specific disease, 11 health claims on development and health benefits for children, a specific health claim, totaling 19 approved health claims. If the report is made to the 5000

cases filed, the percentage of approved is 0.38% - a very low figure. If it is considered that files typically provide more than one health claim for approval, than the percentage of approved ones must be lowered even more (10).

The EFSA reports for few approved files show an impressive number of clinical trials conducted by different research teams, which have demonstrated the veracity of health claims on both animals and humans.

Health claims are usually restricted to a particular product for which investigations were made. For example, it was approved the beneficial effect of plants sterols and stanols to reduce blood cholesterol (which reduces the risk of cardiovascular disease). But it was approved only for the following categories of products: yellow fat spreads, dairy products, mayonnaise and salad dressings. If we have another food with the same amount of sterols and / or stanols, it's not allowed to use the claim (11).

By a simple numerical comparison of the 6700 drugs with the 19 approved health claims, the difference is about 350 times less for health claims. This major difference is one of the visible evidence of competition that exists between pharmaceutical industry and functional foods industry.

The law that has imposed such restrictive conditions for food in Europe is the Regulation (EC) No 1924/2006 issued on December 20, 2006 (12). Before this law similar guidelines were issued by the Codex Alimentarius, but never were mandatory and with so strongly impact in the EU Member States as last EC Regulation. This has sparked numerous complaints from functional food producers, scientists and the public. It was noted for example, that a part of EFSA staff members, dealing with the approval of health claims are subjected to conflict of interest due to their work in industrial companies (agro-food and pharmaceutical) (13).

The main reason claimed by this law is to remove from the market the products which falsely assume a certain beneficial effect that they do not possess. This may seem to be a benefit of the law, but compared to the disadvantages it brings, this becomes insignificant. Tradition for thousands of years of multiple disease treatment identified natural remedies that can be used successfully for treatment, prevention and amelioration of disease (where functional foods play an important role). Many of these are used successfully until today, but the EU regulation forbids practically their advertising and official use in treatment, so only the pharmaceutical industry remains on the market as absolute monopoly.

There are many scientists who sounded the alarm on the conflict of interest between the pharmaceutical industry and public health, as reflected in recent times also by the Regulation 1924/2006. For example, Prof. Dr. George Mencinicopschi, director of the Institute of Food Research from Bucharest filed a complaint with the European Commission in regard to this Regulation.

Nobel laureate, Joseph Stiglitz had addressed similar topics in his books and articles such as "Globalization and Its Discontents", "Prizes, Not Patents" etc. Other doctors, researchers and politicians who have distinguished themselves in this regard are: Louis de Brouwer, John Virapen, Michael Klaper, Rima Laibow, Colin Campbell, Matthias Rath, Charlotte Gerson, Claire Severac, Jeffrey Smith, Wolfgang Wodarg, Nigel Farage, etc.

Healing axiom

Medical system had established an axiom, which seeks to be imposed worldwide as fundamental truth: the disease can be cured only by medicines. According to this axiom, food supplements or functional foods don't have this quality (hence the restrictions of Regulation EC no. 1924/2006). The reason for establishing this axiom is not stated and can not be proven. But the economic advantages are obvious for the pharmaceutical industry and its partners (including health facilities). It removes all competitors in the medical field. Only the pharmaceutical industry retains the official right to treat patients with its products.

There are many medical authorities that recognize that drugs deal only with symptoms, not the disease itself. The drug information leaflet recognizes this, specifying that it is symptomatic treatment. Dr. John Virapen, with 35 years experience in the pharmaceutical industry, explains this principle in his book and public presentations (14).

The medical health system does not intend necessarily to understand and explain the causes of certain symptoms, but their focus resides in its elimination or mitigation. People, who use drugs and noted that some symptoms had decreased or disappeared develop trust in the effectiveness of drugs, but remain with the same disease. However their situation is worse to the time before the consumption of drugs: the issue is unresolved, but had disappeared or decreased the indicator of organism, which alert people about the problem (the symptoms).

The biggest problem is represented by the adverse effects of drugs, because they are not natural substances and are completely foreign to human body. They affect different organs or systems of the body, creating biochemical imbalances and accumulation of indigestible toxins in the tissues. These are sources of a wide range of chronic and acute diseases, cancer and even death.

Functional foods and health

Some of the famous advocates of functional foods and natural methods of maintaining health in Romania are the following: professor and academician Ovidiu Bujor, who wrote several books on this topic, doctor Virgiliu Stroescu, entitled by the media as apostle of vegetarianism, doctor Emil Radulescu, who wrote a lot of wide spread books on health and nutrition, doctor Margineanu Calin, who are renown for his public presentations and cancer healing protocols, Prof. Dr. Brad Segal, who promoted functional foods through multiple articles and books, among which is the collection of brochures "Vital". Prof. Dr. Segal Rodica, Prof. Dr. Miron Costin and other academic staff from the University of Galati continued in this direction by their works, such as "Functional foods – foods and health" (15) etc.

One of the doctors who have made a global impact through his discoveries was Max Gerson, who around 1920 has discovered the role of healthy foods in the treatment of the most dangerous diseases such as tuberculosis, diabetes, cancer and others. He applied successfully his treatment and improved it over 30-40 years, curing thousands of patients. In 1946 he was invited to the United States Senate to present the results of his treatment. The presentation had an impressive impact. Over the U.S. was publicly announced that it was discovered the cure for cancer. But doctor's therapy foresees no use of the drugs, the techniques of radio and chemotherapy or surgery. It applies primarily the adoption of a diet based on organic fruits and vegetables, juices derived there from, cereals and nuts in combination with certain natural procedures. If this therapy was widely adopted, the pharmaceutical industry would considerably reduce their profitable business of selling

drugs. For this reason doctor's therapy was denigrated by all possible means. Nevertheless Dr. Max Gerson's work was continued by his daughter Charlotte Gerson, who founded the Gerson Institute in California in 1977 and two clinics of treatment (*Clinica Nutrición y Vida* in Mexico and *The Gerson Health Centre* in Hungary). These clinics operate successfully until now (16).

There are numerous international research projects, which confirmed the role of healthy eating in preventing and treating chronic diseases and cancer. Among them: FP6 project HELENA - Healthy Lifestyle in Europe by Nutrition in adolescence (17), FUFOS: Functional Food Science in Europe (18), China study - the most comprehensive study on nutrition (19), FLORA: Flavonoids and related phenolics for healthy Living using Orally (20), etc.

The World Health Organization has issued an action plan to prevent so-called non-communicable diseases (21), which include cardiovascular disease, diabetes, cancer and chronic respiratory diseases. Among the four factors of prevention is listed diet, with emphasis on eating fruits and vegetables.

Conclusion

Agricultural, food and medical modern systems have many operational deficiencies. Governing laws are often in favor to large agro-food or pharmaceutical companies and in detriment to science and consumers. We have to analyze critically the situation in these fields and take the documented and impartial attitudes and actions, which will promote the scientific truth, the quality of food products and the health of population.

As is repeatedly stated by WHO, EFSA and other food or health organization, we must consider that not only food determines the health, but also other lifestyle factors (22) such as physical exercises, clean air, drinking water, rest, balanced work, emotional and spiritual equilibrium (23).

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GLYCAEMIC INDEX OF SORIZ (SORGHUM ORYZOIDUM) GRAINS AND GROATS

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Abstract: The glycemic index (GI) of foods rich in carbohydrates characterize on a scale from 0 to 100 so far as they raise blood glucose levels after their consumption. Foods with high glycemic index (> 70) are digested and easily absorbed into the body, causing a sudden increase and high blood glucose levels. Frequent states of hyperglycemia can lead to metabolic disorders, diabetes and obesity. The paper presents results of experimental determinations of glycemic index of boiled soriz grains and groats compared with glucose.

It was found that the samples investigated aren't aliments with high glycemic index. Obtained data complete bibliographic information available with new varieties of cereal products and their glycemic index to be useful and necessary in developing food rations for different population groups.

Keywords: boiled beans and hulled soriz, glycemic index, glycaemia, glucose.

Introduction

It's known that the human body uses the carbohydrates in the form of glucose as an energy source easily available. For a normal vital activity of human body is indispensable permanently maintaining of the level of blood glucose in physiological limits of 70-100 mg/dl or 3.9-5.6 mmol/l [1; 2]. The accessible glucose use by cells may be food or can be readily available from the reserves of glycogen.

The glycemic index of foods rich in carbohydrates is an important parameter to define the energetic availability of carbohydrates and characterized on a scale from 0 to 100 which raises the level of glucose in the blood after their consumption [3]. With the help of this index are classified as foods containing carbohydrates according to the impact that they have on blood glucose. Foods with high glycemic index (> 70) digest and quickly absorbed in the human body, generating a sudden rise and high glucose levels in the blood. The frequent states of hyperglycemia can lead to metabolic disorders, diabetes mellitus, obesity [4].

Foods with a low glycemic index (<50) leads to gradually increasing blood sugar and insulin levels because they are difficult to digest and absorb the blood. They are also effective in maintaining body weight as controls appetite and offers a feeling of satiety.

Knowing GI for each food separately allows us to choose the ones that cause a moderate increase in blood glucose level, which is the guarantee correct and long vascular functionality [5].

Sorghum Oryzoidum or soriz is an indigenous perspective autochthonous cereal culture whose IG has not been determined so far. The results obtained will complete the existing bibliographic information with new varieties of cereal products and their glycemic index, which will be useful and necessary in the preparation of food rations for different categories of population.

Materials and methods

As research materials were used:

- Soriz grains "Alimentar" boiled (about 120 min);
- Soriz groats (obtained by grinding during 3 minutes) boiled (about 40 min);
- Glucose - GOST 975-88;

The glycemic index of samples tested was determined in vivo by monitoring the level of glucose in the blood of the participants at the experiment before and after the consumption of researched food products, according to ISO 26642: 2010. Glycemic response after the consumption of each product was compared with the stimulated glucose consumption as reference substance [6].

Data obtained were used to build curves glycemic response of participants after drinking samples tested. Surface area under the curve was determined by mathematical method using AutoCAD through the program "Inquiry" that calculates the exact surface area. Finally glycemic index was calculated as:

$$IG = \frac{S_a}{S_g} 100; \quad [1]$$

where:

GI-glycemic index of the food analyzed;

AS - surface area under the glycemic curve of studied food;

GS - surface area under the glycemic curve of glucose;

Capillary blood sugar of the subjects experiment was determined by method glycosidase -final point to biochemical analyzer "STAT-FAX 1904" [7].

Principle of the method: Glucose, under the action of glycosidase, turns into gluconic acid. H₂O₂ resulting will be decomposed by peroxidase, resulting the reaction involving and pointer Trinder (phenol and 4- aminoantipyrine), resulting in a product of condensation in the red coloring with the absorption maximum at $\lambda = 505$ nm. Extinction is directly proportional to the concentration of glucose [8].

Results and discussions

The average glycemic response of the participants at the experiment before and after the consumption of equivalent amounts of carbohydrates (50 g) with glucose, cooked soriz grains or groats are presented in Table 1.

Table 1 Evolution of glycemia after glucose, soriz grains and groats consumption

	Time, min							
	0	15	30	45	60	90	120	180
Sample	Glicemia, mmol/l							
Glucose	3,8±0,8	5,6±1,1	6,8±1,2	5,6±0,9	4,5±0,7	4,9±0,9	4,4±0,6	3,7±0,4
Soriz grains	3,8±0,6	4,8±0,6	5,9±0,9	5,0±0,4	4,6±0,4	4,4±0,4	4,1±0,6	3,9±0,6
Soriz groats	3,8±0,6	4,9±0,8	6,5±0,9	5,5±1,0	4,5±0,5	4,3±0,5	4,2±0,5	4,0±0,5

The average pre-prandial glycemia of participants in the experiment was in the optimal range of 3.8 ± 0.8 mmol/l. After consuming the samples examined maximum glycemia was reached over 30 minutes. In relation to glucose, cooked soriz groats have determined a higher glycemic response (6.5 ± 0.9 mmol/l.) than boiled soriz grains (5.9 ± 0.9 mmol/l). In the following period was ascertained a slower decrease of blood glucose level participants after eating cooked soriz groats compared with that of the grains. This can be explained by the presence in higher quantities of fibres in integral soriz grains compared with groats, due to digestion and absorption of carbohydrates in blood occurs more slowly [9; 10]. Over 3 hours after consuming the samples studied blood glucose values have come down approximately at the level of initial values.

The glycemic index of cooked soriz grains and groats in relation to glucose is presented in Figure 1.

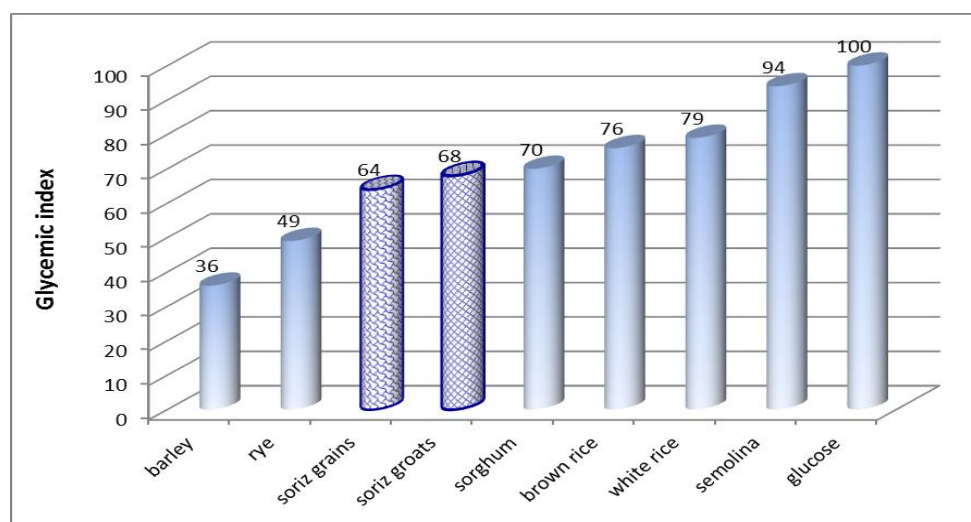


Fig. 1 GI values of sorize grains and groats compared to other cereals

Thus, the integral soriz, being a hybrid of sorghum, had it close values of IG- respectively 64 and 70. The glycemic index of cooked grains and soriz groats was lower than of cereals as: brown rice (IG = 76), white rice (IG = 79), semolina (IG = 94), belonging to the category of foods with high glycemic index, but higher than that of barley (36) and rye (49), which belong to the group of foods with low GI [11, 12]. Theoretically this can be explained by the different composition of the carbohydrate complexes of cereals, including starches (the degree of gelatinization, the ratio of amylopectin and amylose content etc.), that influences their speed of digestion and absorption [13].

Conclusions

This study for the first time relates the information about glycemic index of soriz grains and groats (*Sorghum Oryzoidum*) and glycemic response after their consumption. The experimental results have shown that the boiled integral soriz caused a slower dynamics of blood glucose in the human body in relation to groats, which allows them to be more effective in reducing the risk of diabetes and cardio-vascular diseases.

Also, the determined values of the glycemic index of cooked soriz grains (64) and groats (68) in relation to glucose demonstrates their class membership food with moderate GI (55-70). Those, even in moderation, are recommended by nutritionists in a healthy diet, unlike foods with high glycemic index ($GI > 70$) [14].

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BIOLOGICALLY ACTIVE SUBSTANCES IN RED WINES MOLDOVAN

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Abstract: In red wines from grapes grown in Moldova, polyphenols, which have a pronounced antioxidant activity are represented mainly monomeric anthocyanins and their derivatives (cyanidina, delphinidin, peonidina, petunidina), flavonoids (quercetin and rutin) and stilbenes (resveratrol). Using high performance liquid chromatography (HPLC) determines differently principal monomeric polyphenols content, resveratrol, quercetin and routine dry red wine grapes. Separation schemes developed can be successfully used to study phenolic compounds in wine during various investigations.

Were studied stilbene compounds in red wine grape grown in Moldova, the different years of harvest, from different manufacturers. It was found that the content of these compounds in wines analitizate are: quercetin (0.4 to 107) mg/dm³, routine - (0.7 to 14.6) mg/dm³, rezveratrol - (1.2 to 9.5) mg/dm³. Concentrations, these substances contained in red wine Moldovan allow sufficient to assume their biological activity because, according to the statements of many scholars, namely the group of polyphenols act as antioxidants and antimutagenic human body.

Keywords: red wines, polyphenols, biologically active.

Introduction

Red wine contain a significant amount of natural extractives, with special attention deserve polyphenols.

In recent years, in Italy, France, Portugal, China and other countries [1,2] conducted research related to the study of polyphenols with antioxidant properties, in red wines from both the classic red grape varieties of Cabernet, Merlot, Pinot Franc, and from local zoning classes.

Most often for the quantitative determination of antioxidants and bioactive polyphenols used spectrophotometric, electrochemical methods, and techniques of high performance liquid chromatography [5,7].

Most red grapes zoned in Moldova, contain significant amounts of phenolic compounds. A number of them, such as quercetin, rutin, resveratrol, anthocyanins and other exhibit antioxidant properties, scientists. The first publication of the content of resveratrol in the Moldovan wines appeared in 1995 year, then - in 2007 [8]. However, due to the lack of methodology and related equipment in Moldova conducted systematic research of other active compounds of red wines. Therefore, we are interested in the study of their content in red wines produced from both the classical grape varieties and other varieties grown in the country.

The total content of phenolic compounds in red wine, according to some researchers [6,7], does not always correlate with biologically activity. Study of individual phenolic compounds in red wines can indirectly assess the biological activity wine and, therefore, its biological value [3].

Differential determination of phenolic compounds - a complex procedure, for which the necessary modern equipment and highly qualified specialists.

Materials and methods

Determination of monomeric polyphenols were determined by high performance liquid chromatography (HPLC), which allows to separate the phenolic compounds in a special chromatography column, then define them quantitatively. We picked up the optimum conditions for separating a mixture of monomeric polyphenols and used the technique developed for the quantitative determination of the above differentiated compounds in red wines from grapes zoned in Moldova: Cabernet Sauvignon, Feteasca Neagra, Rara Neagra, Kodrinsky.

Mastering mode determination of phenolic compounds by HPLC and further studies are carried out on a liquid chromatograph LC-20A Prominence, Shimadzu column CC 125/4 Nucleosil 100-5c 18 Nautilus. Detector SPD-20AV UV/VIS, which is equipped with this liquid chromatograph includes deuterium and tungsten halogen lamps, as a result of expanded analytical capabilities to the visible range.

Eluted in gradient mode with a feed rate of the mobile phase 0.75 ml / min. Solution A: methanol solution B: bidistilled water

Detection was performed at wavelengths of 305 nm (resveratrol), 363 nm (rutin, quercetin).

This mode is used to determine the routine, quercetin and resveratrol.

For anthocyanins and their derivatives were used the following solutions: Solution A: orthophosphoric acid to 10% solution B: acetone + orthophosphoric acid (1:1). Detection was performed at a wavelength of 520 nm

Results and discussion

Monomers anthocyanin groups creating a characteristic ruby color, found in the dry red grape wines are presented in *Table 1*.

Table 1. Monomeric anthocyanins and their derivatives in red grape wines in Moldova.

Anthocyanin	% of the total anthocyanin							
	vintage 2009				vintage 2010			
	Feteasca Neagra	Rara Neagra	Kodrinsky	Cabernet-Sauvignon	Feteasca Neagra	Rara Neagra	Kodrinsky	Cabernet-Sauvignon
Delphindin-3-glucoside	3,7	0,7	2,9	1,4	3,2	2,1	7,6	5,6
Cyanidin-3-glucoside	0,6	-	0,8	1,0	0,4	-	1,0	1,0
Diglucoside malvidol	0,5	0,2	1,0	0,8	0,4	0,3	0,5	0,2
Petunidin-3-glucoside	6,3	3,2	3,6	1,4	6,7	4,7	8,7	6,2
Peonidin-3-glucoside	2,4	2,3	2,2	1,1	3,0	2,9	4,0	3,0
Malvidin3-glucoside	30,1	45,0	23,7	15,6	53,8	45,3	38,3	33,2
Σ associated anthocyanin	56,5	48,6	65,8	79,0	32,4	44,6	40,0	50,7

Analyzing the data, we can say that the red wine produced from local grapes, bringing on the content of anthocyanins in wines from European varieties. This low percentage concentration diglucoside malvidola - 0.2-1.0% of the total amount of anthocyanins and quite high - malvidin-3-glucoside – 15,6-53,8%.

According to the literature, malvidin-3-glucoside is the major anthocyanin coloring in red wines from European grape varieties. Moldovan grape wines examined in this study are highlighted in large content malvidin-3-glucoside, especially Feteasca Neagra (53.8%) and Rara Neagra (45.3%).

Of the other monomeric flavonoids, normally found in the skin of the grape berries, and then in red wine, along with anthocyanins were found quercetin and rutin. Their contents are shown in *Table 2*, and the chromatographic profile - in *Figure 1*.

Table 2. Monomeric flavonoids in red grape wines in Moldova

Red wines	Quercetin, mg/dm ³		Rutin, mg/dm ³	
	vintage 2009	vintage 2010	vintage 2009	vintage 2010
Feteasca Neagra	0,57	0,97	5,45	4,09
RaraNeagra	0,63	0,43	4,09	2,9
Kodrinsky	4,57	3,65	10,46	12,67
Cabernet-Sauvignon	3,08	9,17	9,20	8,92
Merlot	8,90	10,72	13,1	14,6

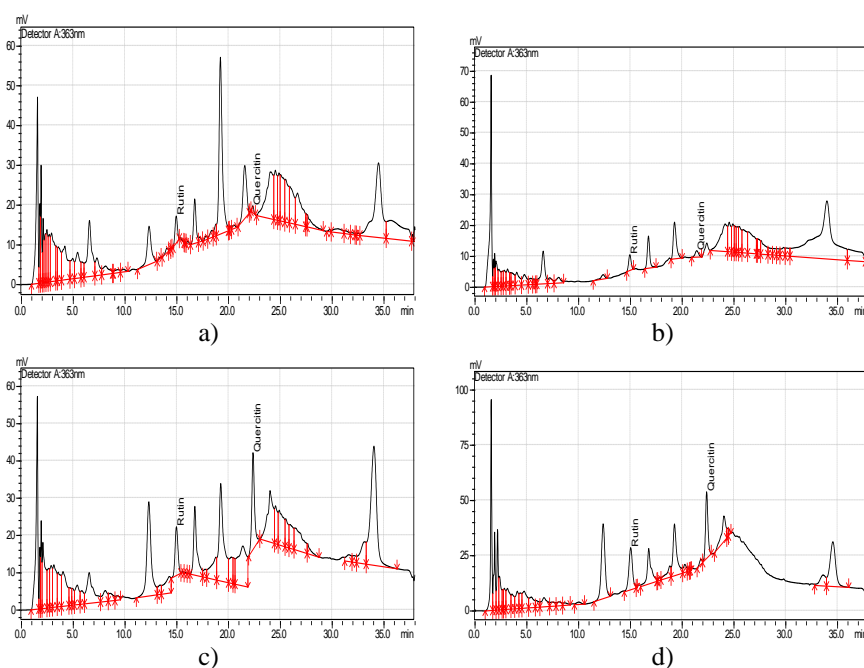


Fig. 1. Chromatogram quercetin and rutin in red wines: a) Feteasca Neagra b) Rara Neagra c) Kodrinsky d) Cabernet Sauvignon.

According to the results presented in *Table 2* and *Figure 1*, the wine from local grapes and Feteasca Neagra Rara Neagra the content of quercetin and rutin were markedly

different from the classic Cabernet Sauvignon and Merlot, these substances in the past it was much more. While wine varieties Kodrinsky the content of quercetin - 4.57 and routine - 12.67 mg/dm³ consistent Cabernet Sauvignon and Merlot.

Of monomeric polyphenols neflavonoidnyh grapes we have identified resveratrol, shown in Table 3 and Figure 2.

Table 3. Stilbene compounds in red grape wines in Moldova

Red wines	Resveratrol, mg/dm ³	
	vintage 2009	vintage 2010
Feteasca Neagra	7,36	9,47
RaraNeagra	1,21	4,04
Kodrinsky	5,86	4,66
Cabernet-Sauvignon	3,20	4,25
Merlot	3,80	4,15

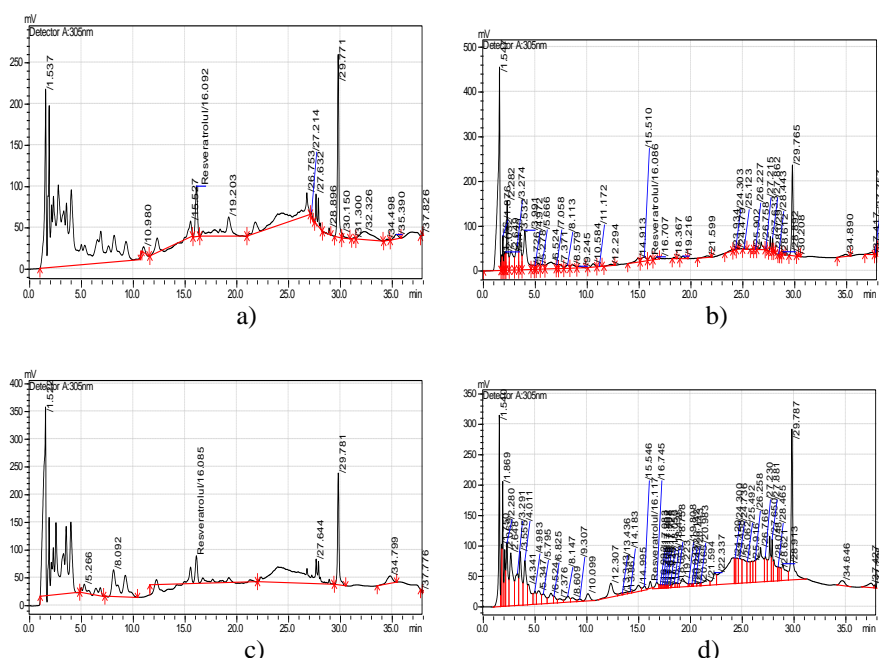


Fig. 2. Chromatogram of the resveratrol in red wine: a) Feteasca Neagra b) Rara Neagra c) Kodrinsky d) Cabernet Sauvignon.

According to the content of resveratrol Moldovan grape wines Feteasca Neagra (9.47 mg/dm³) and Kodrinsky (5.86 mg/dm³) can compete with European varieties. This suggests that these varieties are worthy of further study.

Conclusion

We found that the polyphenols in grapes, which have antioxidant activity in red Moldovan wines are mostly monomeric anthocyanins and their derivatives (cyanidin, delphinidin, peonidin, petunidin), flavonoids (quercetin and rutin) and stilbene compounds (resveratrol).

Concentration, in which these substances are contained in the studied wines can judge their biological activity or sufficient value because, in the opinion of many scientists, these groups of polyphenols act as antioxidants and antimutagenic in humans [4,9].

Studies on the identification of polyphenols have biological activity, it is appropriate to continue, given the wide range of red grapes grown in Moldova, and the objective justification of new technology making red wines, fortified by these substances

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BACTERIAL AND YEAST STRAIN SELECTION FOR TREATMENT OF GOOSE DOWN WASHING WASTEWATER

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Abstract: 15 strains bacteria and 3 strains yeasts are investigated for their ability to treat goose down washing wastewater. Bacterial strains 2, 3.1LBG, 3.2 LBG, 3.3LBG, 3.4LBG, 3.1.MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS, 4, 5, 7, 8 and yeast strain Y are isolated from soil, strain *Micrococcus* 2 – from raw dried meat products. Yeast strains C1 and C2 are provided from yeast strains' collection. Bacterial strains 2, 3.4LBG and 3.7LBG are non-spore forming long rods, while strains 3.1LBG, 3.2 LBG, 3.3LBG, 3.1.MRS, 3.2MRS, 3.3MRS, 3.5MRS, 4, 5, 7, 8 - non-spore forming short rods. Analyses are carried out with preliminary flocculated and filtered goose down washing wastewater. It was found that yeast strains show higher purification degree of goose down washing wastewater than bacterial strains. Short rods treat wastewater with 27, 24 % more effectively than long rods. From yeast strains C1 has highest purification degree – 28,53 %, and from bacterial strains – MRS 3.3 – 25,58%.

Keywords: bacteria, yeasts, washing wastewater, purification degree

1. Introduction

During washing poultry feather highly contaminated with organic substances wastewater, which involve the risk of environmental contamination, is received. It contains large amounts of fats and proteins [1], which are presented in form of finely dispersed difficult to separate emulsion. Their removal is possible if wastewater is subjected to prior treatment with flocculants and subsequent flotation process [2-3]. These two processes act predominantly on suspended pollutants, but do not affect the solutes [4]. Removing the last ones is possible using biological treatment methods. Most of them are based on the biochemical degradation of pollutants by the action of specially adapted to the particular wastewater bacterial and yeast strains [5-7].

The aim of this work is the selection of bacterial and yeast strains for the treatment of preliminary flocculated and filtered goose down washing wastewater.

2. Materials and methods

2.1. Microorganisms

In this work are used 15 strains of bacteria and three strains of yeasts. Bacterial strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS, 4, 5, 7, 8 and yeast strain Y are isolated from soil. Bacterial strain *Micrococcus* 2 is isolated from raw-dried meat products. Yeast strains C1 and C2 are provided by the microorganisms' collection of the Department "Microbiology" at University of Food Technology, Plovdiv, Bulgaria. Through microscopic observations it is found that bacterial strains 2, 3.4 LBG and 3.7MRS are non-sporeforming long rods with rounded ends, while strains 3.1LBG, 3.2LBG, 3.3LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 4, 5, 7 and 8 are non-sporeforming short rods with rounded ends.

2.2. Nutrient mediums

2.2.1. Luria – Bertany glucose medium (LBG) with composition (g/dm³): triptone (Difco) – 10 g, yeast extract – 5 g, NaCl – 10 g, glucose (Scharlau) – 10 g. pH=7,5. The medium is sterilized for 25 minutes at 121° C.

2.2.2. Luria – Bertany glucose agar medium (LBG agar) with composition (g/dm³): triptone (Difco) – 10 g, yeast extract – 5 g, NaCl – 10 g, glucose (Scharlau) – 10 g, agar – 20 g. pH=7,5. The medium is sterilized for 25 minutes at 121° C.

2.2.3. Malt extract medium (ME) with composition: malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol). pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121° C.

2.2.4. Malt agar medium (MA) with composition: malt extract (Kamenitza, Bulgaria) in 1:1 ratio with tap water (vol/vol), agar – 20 g. pH=6,5 – 7,0. The medium is sterilized for 25 minutes at 121° C.

2.2.5. Lactobacillus medium on the formulation of de Man, Rogosa and Sharpe (MRS) with composition (g/dm³): peptone (Scharlau) – 10 g, meat extract (Scharlau) – 5 g, yeast extract (Scharlau) – 5 g, glucose (Scharlau) – 20 g, K₂HPO₄ – 2 g, diammonium hydrogen citrate – 2 g, NaOOCCH₃ – 5 g, MgSO₄ – 0,1 g, MnSO₄ – 0,05 g. pH 6,5 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.2.6. Lactobacillus agar medium on the formulation of de Man, Rogosa and Sharpe (MRS agar) with composition (g/dm³): peptone (Scharlau) – 10 g, meat extract (Scharlau) – 5 g, yeast extract (Scharlau) – 5 g, glucose (Scharlau) – 20 g, K₂HPO₄ – 2 g, diammonium hydrogen citrate – 2 g, NaOOCCH₃ – 5 g, MgSO₄ – 0,1 g, MnSO₄ – 0,05 g, agar – 20 g. pH 6,5 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.2.7. Soya – caseine broth medium (SCB) with composition (g/dm³): triptone (Difco) – 17 g, soya peptone (Scharlau) – 3 g, NaCl – 5 g, K₂HPO₄ – 2,5 g, glucose (Scharlau) – 2,5 g. pH=7,3 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.2.8. Soya – caseine agar medium (SCA) with composition (g/dm³): triptone (Difco) – 17 g, soya peptone (Scharlau) – 3 g, NaCl – 5 g, K₂HPO₄ – 2,5 g, glucose (Scharlau) – 2,5 g, agar – 20 g. pH=7,3 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.2.9. Medium for Micrococcus varians and Staphylococcus saprophyticus (SMS) with composition (g/dm³): yeast extract (Scharlau) – 5 g, meat extract (Scharlau) – 1 g, peptone (Scharlau) – 10 g, NaCl – 5 g, glucose (Scharlau) – 10 g, K₂HPO₄ – 0,5 g. pH 6,8 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.2.10. Agar medium for Micrococcus varians and Staphylococcus saprophyticus (SMS agar) with composition (g/dm³): yeast extract (Scharlau) – 5 g, meat extract (Scharlau) – 1 g, peptone (Scharlau) – 10 g, NaCl – 5 g, glucose (Scharlau) – 10 g, K₂HPO₄ – 0,5 g, agar – 20 g. pH 6,8 ± 0,2. The medium is sterilized for 25 minutes at 121° C.

2.3. Wastewater:

In the experiments goose down washing wastewater to slaughterhouse is used. Wastewater is treated prior to addition of 15 cm³ 0,1% solution of flocculant HENGFLC 82 412 (Beijing Hengju)/100 cm³ wastewater. The composition is stirred and allowed to stand for 20 min. After the reaction time wastewater is centrifuged at 3000 min⁻¹ for 10 min and filtered through a glass filter to separate the flocculated impurities. The resulting clear filtrate of the effluent is used for further analysis.

2.4. Cultivation and storage of microorganisms.

Bacterial and yeast strains are grown on different media, respectively for bacterial strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG – LBG; for bacterial strains 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS – MRS; for bacterial strains 4, 5, 7 and 8 – SCB; for bacterial strain *Micrococcus* 2 – SMS; for yeast strains Y, C1 и C2 – ME at $30^{\circ} \pm 2^{\circ}$ C in thermostat for 48 h and are stored in refrigerator at $4^{\circ} \pm 2^{\circ}$ C for 3 weeks.

2.5. Analytical methods.

2.5.1. Development of isolated bacterial and yeast strains in pre-treated with flocculants and filtered wastewater.

2.5.2. With biomass from developed on mediums LBG agar, MRS agar, SMS agar, SCA at $30^{\circ} \pm 2^{\circ}$ C for 48 h strains of bacteria and yeasts developed on medium MA 5 cm³ wastewater is inoculated. Wastewater samples with investigated cultures are cultivated for 72 h at $30^{\circ} \pm 2^{\circ}$ C.

2.5.3. Purification degree wastewater determination using the permanganate oxidizability method.

Developed for 72 h at $30^{\circ} \pm 2^{\circ}$ C in wastewater cultures are centrifuged at 3000 min⁻¹ for 10 min. The resulting supernatant is analyzed for permanganate oxidizability in accordance with standard BS 17.1.4.16-79.

100 cm³ sample or lower volume brought to 100 cm³ with distilled water, is placed in a 250-300 cm³ flask, which has added pumice. 5 cm³ H₂SO₄ (1 +2) and 20 cm³ 0,01 N KMnO₄ solution are added. The composition is heated so as to boil for no more than 5 min and boiling for 10 min. To the hot solution 20 cm³ 0,01 N solution of HOOC-COOH are added. Hot discoloured solution is titrated with 0,01 N KMnO₄ solution. The temperature of the solution during titration should not be below 80° C. If the boiling solution is discolored or brown precipitates are formed, the determination is repeated with a smaller sample size.

During titration should not spend more than 12 cm³, and not less than 4 cm³ of KMnO₄ solution for diluted sample. Similarly, a blank is done with wastewater.

Oxidizability (X) in mg/dm³ oxygen is given by the formula:

$$X = \frac{(a - b) \cdot N \cdot 8000}{V}, \text{ where}$$

a – V_{KMnO₄}, spent for titration of the sample, cm³;

b – V_{KMnO₄}, spent for titration of blank, cm³;

N – exact normality of KMnO₄ solution;

V – volume of sample taken for analysis, cm³.

Similarly oxidizability of a control sample of analyzed wastewater at the corresponding dilution of 1:1, 1:5 and 1:10 is determined.

Purification degree PD, in %, is determined using the formula:

$$PD = \frac{X_c - X_s}{X_s} \cdot 100, \%, \text{ where}$$

X_c – oxidizability of control sample, mg/dm³ oxygen;

X_s – oxidizability of sample, mg/dm³ oxygen.

3. Results and discussion.

In a set of experiments the ability of bacteria strains 2, 3.1LBG, 3.2LBG, 3.3LBG, 3.4LBG, 3.1MRS, 3.2MRS, 3.3MRS, 3.5MRS, 3.7MRS, 4, 5, 7, 8 and *Micrococcus* 2 and yeast strains Y D, C1 and C2 to break down pollutants in pre-flocculated and filtered wastewater are investigated. Summarized results are presented in Figure 1 and Figure 2. Experimental data in Figure 1 show an average level of treatment for all strains of bacteria and yeasts, while Figure 2 – average purification degree of bacterial strains.

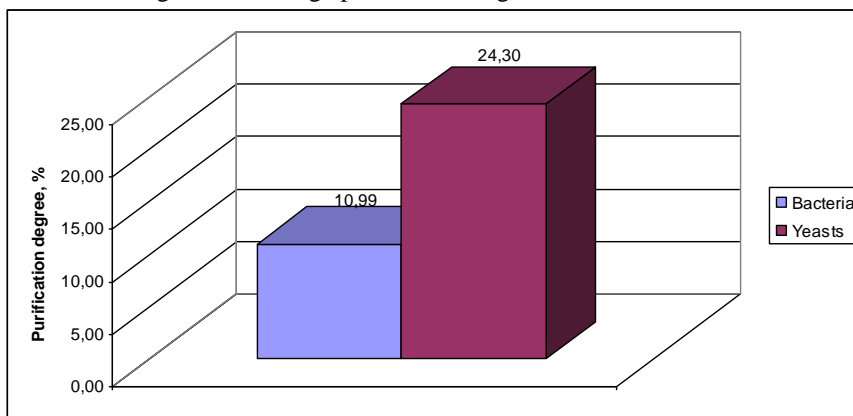


Fig. 1. Average purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial and yeast strains.

On Figure 1 is clearly shown that yeast strains exhibit higher purification degree of pre-flocculated and filtered goose down washing wastewater with 13.31% in comparison with bacterial strains.

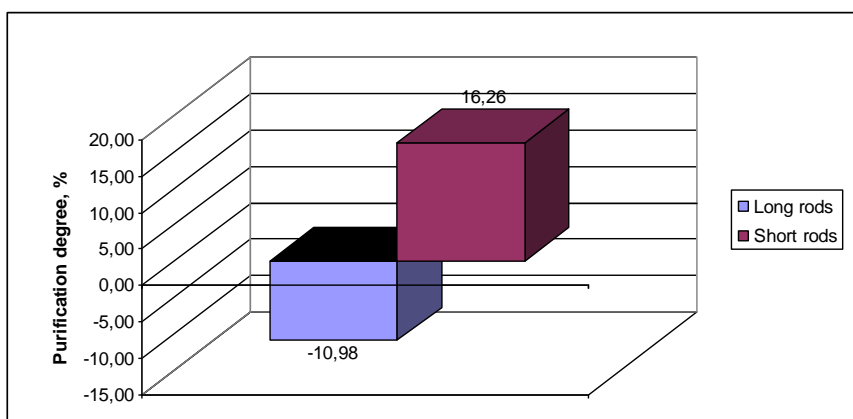


Fig. 2. Average purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial strains long rods and short rods.

In comparison of bacterial strains short rods treat better preliminary flocculated and filtered goose down washing wastewater better than the long rods (Figure 2) with 27,24%.

Comparative characteristics between bacterial and yeast strains for the purification degree of preliminary flocculated and filtered goose down washing wastewater. Results from these investigations are presented in Figure 3.

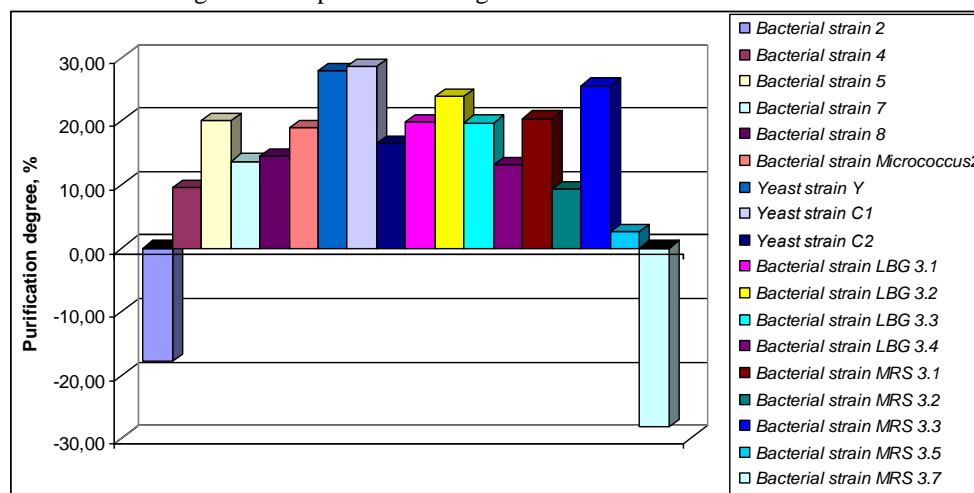


Fig. 3. Purification degree of preliminary flocculated and filtered goose down washing wastewater with bacterial and yeast strains.

The experimental data shown in Figure 3 shows that with purification degree of preliminary flocculated and filtered goose down washing wastewater over 20% are yeast strains - Y and C1 and strains of bacteria - 5, 3.2LBG, 3.1MRS and 3.3MRS. Yeast strain with the highest purification degree is strain C1 with purification degree – 28,53%, followed by yeast strain Y – 27,89%. At bacterial strains the highest purification degree has bacterial strain 3.3MRS – 25,58%, followed by 3.2LBG – 23,86% and 3.1MRS – 20,42%. With the lowest purification degree is bacterial strain 3.7MRS, followed by bacterial strain 2, which give negative values.

4. Conclusion.

As a result of investigated analyses on preliminary flocculated and filtered goose down washing wastewater treatment the following more important conclusions can be made:

1. Investigated yeast strains exhibit higher purification degree of wastewater compared to the strains of bacteria;
2. Bacterial strains short rods treat more effectively effluent compared to the strains of bacteria long rods with 27.24%;
3. The highest purification degree is determined in yeast strains C1 and Y 28,53 % and 27,89 %, respectively. Bacterial strains with the highest purification degree is strain 3.3MRS – 25,58 %, followed by 3.2LBG – 23,86 % and 3.1MRS – 20,42 %.

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A STUDY ON THE CHANGE OF TRANSPORT AND SELECTIVE PROPERTIES OF ULTRAFILTRATION POLYMER MEMBRANES AFTER VACUUM METALLIZATION WITH IRON-CHROMIUM-NICKEL ALLOY

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Abstract: The possibility for vacuum metallization of ultrafiltration polymer membranes with iron-chromium-nickel alloy was studied after preliminary modification with Sn^{2+} in acid medium. The change of transport and selective characteristics of the vacuum metal coated membranes was studied, as well as the durability of the vacuum obtained coating during membrane performance.

Key words: ultrafiltration polymer membranes, vacuum metallization, iron-chromium-nickel alloy

Introduction

As polymer systems, the membrane structures can be metallized using either chemical or physical methods. The process of metallization provides possibilities to change membrane transport and selective properties thus making them applicable in various fields of science and technology. The preparation of the metal coatings depends on polymer characteristics, metallization methods and the technological parameters of the process. Two types of polyacrylonitrile membranes with differing characteristics and structure were studied to find the effect of exposition on the vacuum metallization, the possibilities for preliminary chemical modification of membrane surface. The structure and the basic transport and selective characteristics of the membranes were studied.

Experimental

The metal coated membranes are polymer membranes covered with thin metal layer. Metal can be deposited on membrane surface by several methods: magnetron sputtering, chemically – in a solution, vacuum coating methods, including ion bombardment and thermal evaporation [1, 2]. The process of metal coating of membranes has a number of specific characteristics related to the type of polymer material and the method of metallization. The membranes used for the present studies were prepared under laboratory conditions by the phase inversion method, known also as the method of Loeb. The first membrane (PAN I) had the following composition: PAN – 16.25 mass%, PMMA – 0.25 mass%, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ – 2.5 g/l. The other membrane (PAN II) had the following polymer composition: PAN – 14.25 mass%, PMMA – 2.25 mass%. The use of PMMA of modifying polymer gives membranes of higher efficiency. The change of transport characteristics of these membranes were due to the incompatibility of the two polymers. The addition of PMMA in the polymer solution during the phase inversion leads to formation of membranes of higher porosity which stipulates the lower selectivity of the membranes. This, in turn, determines the necessity for additional modification which can be

accomplished either in the polymer solutions or on the working surface of the membranes obtained.

The metal coating was prepared in vacuum installation BUP-5 (Russia) by sputtering flat target of iron-chromium-nickel alloy (H18N9T) with size $\varnothing 100 \times 100$ mm, at distance target/membrane $L_{M-N} = 180$ mm and specific sputtering power $N_p = 5,4 \text{ W/cm}^2$.

Magnetron deposition of iron-chromium-nickel alloy was carried out at initial vacuum in the operation chamber $P_i = 1.10^{-3} \text{ Pa}$, medium Ar with purity of 99,99% and working pressure in the chamber $P_w = 4.10^{-2} \text{ Pa}$. Five deposition times were used: 5, 10, 15, 20 and 25 s. At the end of the process of deposition, the membranes were cooled to room temperature in the Ar medium and then they were taken out in air. The power of the direct current source of the magnetron was 8 kW with smooth current control $0 \div 10 \text{ A}$.

The performance characteristics of the metallized membranes - permeation flux (J) and retention (R) were determined on a laboratory apparatus "Sartorius" SM-165 (England) by the following equation:

$$J = \frac{V(t)}{Sbt} (m^3 / m^2 h) \quad R = \frac{C_0 - C_i}{C_0} \cdot 100(\%) \quad \text{Eq. (A.1)}$$

where $V(t)$ denotes the volume of passed liquid (m^3); Sb – effective area of the membrane (m^2); t – time (h); C_0 - initial concentration (kg / m^3); C_i - concentration of the filtrate (kg / m^3).

Membrane selectivity was measured using the calibrant "Albumin" – human serum $M_w = 67000$ (Fluka) with initial solution concentration 1 g/l. The separation ability of the membrane compared to the calibrant was determined spectrophotometrically at wave length $\lambda = 280 \text{ nm}$ on a UV/VIS spectrophotometer "Unikam"-8625- France.

The micrographs have been taken on a scanning Electron Microscope JEOL JSM-5510. Prior to viewing the membrane sample was fractured in liquide nitrogen gold coated.

Discussion

Knowing and controlling the process of interaction between the metal layer and the polymer membrane is very important for the vacuum metallization, since it determines the type and quality of the metal coating [3, 4]. The factors determining the process are the material of substrate (membrane) and coating, the preliminary treatment of the membranes and the technological and conditions and parameters [5]. One of the conditions for better metallization (better adhesion between the polymer and the metal coating) is the preliminary treatment of the polymer surface. It ensures activation of the polymer surface to facilitate the formation of nuclei for the following vacuum metallization [6]. For this purpose, the opportunities for modification of the polymer structure with various chemical components were preliminarily studied [7, 8]. In this case, the most suitable turned out to be the aqueous system containing 50 g/l $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, 100g/l HCl – referred to further as "the modifying system". The preliminary chemical modification of both types of membranes with the aqueous system selected appeared to be good enough which can be seen in Fig.1 where the efficiencies of modified and unmodified membranes are shown for comparison (Fig. 1).

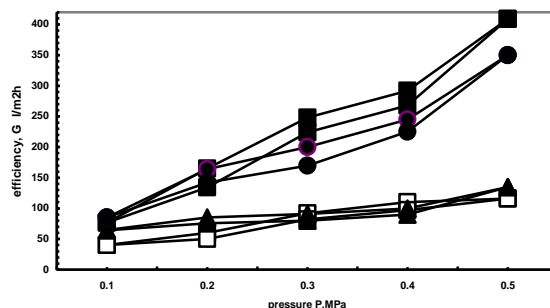
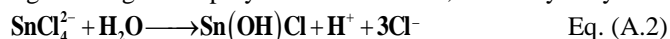


Fig. 1. Hysteresis curves of modified and unmodified ultrafiltration membranes □-PAN I ▲- PAN I mod ■-PAN II ●- PAN II mod.

The preliminary chemical modification of the membranes improves the adhesion between the polymer and the metal coating which is determined by the processes taking place within the system. The treatment with the aqueous solution activated the polymer surface, so the deposition of Sn(II) onto polymer surface did not occur in the sensibilizing solution but during the following washing of the polymer surface when, due to hydrolysis:



the scarcely soluble product $\text{Sn}(\text{OH})_{1.5} \cdot \text{Cl}_{0.5}$ is formed. It remained on membrane surface due to laminar coagulation and can form a layer of tens to thousand Å.

The modified membranes were vacuum metallized under the conditions described in the Experimental section and their basic characteristics were studied.

As can be seen from Fig.2, the water efficiency of chemically modified and vacuum metallized membranes decreased from 90 l/m²h to 40 l/m²h with the increase of exposition from 5 to 25 s (Fig. 2)

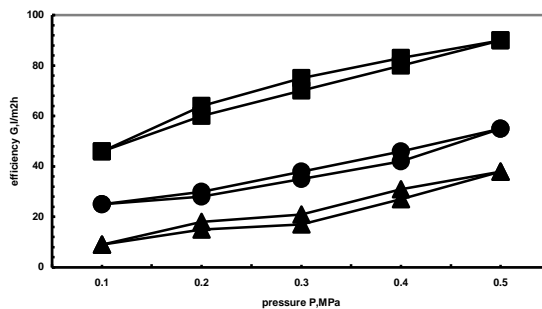


Fig. 2. Hysteresis of membranes PAN-I chemically treated with the modifying system and vacuum metallized with H18N9T alloy ■-5sec ●- 15sec ▲-25sec

During the process of metallization, membranes efficiency significantly decreased compared to the chemically modified ones. Thus, this process is used to correct the selective layer of the membrane.

A comparison of the efficiencies of the initial non-treated and non-metallized membrane (116 l/m²h) and chemically modified non-metallized one (135 l/m²h) with the efficiency of a membrane coated with X18H9T, it can be seen that the latter had lower efficiencies for water regardless of the sputtering time (Fig.2).

The coating deposited in vacuum is not strong enough mechanically, as studied by the method of replication. The adhesion between the polymer and the metal layer depends on the surface energy of the polymer. The adhesion was measured by the method of sticking tape and the strength of the adhesion was calculated by the relationship [9]:

$$A = \frac{S}{S_0} \quad \text{q. (A.3)}$$

where:

S – area of the remaining coating

S₀ – total area of the glued tape.

The method of “sticking tape” was used to study the adhesion between polymer and nickel deposited electrolytically without palladium catalyst. The results obtained from these experiments proved the stability of the metal coatings during their performance. Some additional studied showed also that the adhesion metal-polymer can be strengthened by preliminary deposition of a sub-layer of oxides of the metals used for the coating. Thus, carrying out chemical modification, followed by deposition of metal oxide sub-layer and main coating, better mechanical strength of the modified membrane structure can be obtained. The basic characteristics of such membranes are presented in Fig.3.

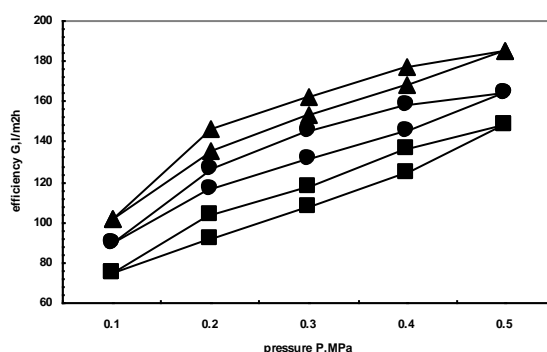


Fig. 3. Hysteresis curves of PAN-I membranes chemically modified and vacuum metallized with H18N9T, with additional sub layer of the oxides of the alloy metals. ■-5sec ●- 15sec ▲-25sec

It can be seen from the membranes' hysteresis curves that their water permeability increased from 148 l/m²h to 185 l/m²h with the increase of sputtering time from 5 to 25 s. This tendency was contrary to the one discussed above for membranes of the same polymer composition but having only main coating. This was probably due to the additional sub-layer of the oxides of the metals of the sputtered alloy and the possibility for formation of a uniform metal surface on the membranes.

The second type of membranes used for the present studies (PAN-II) have much higher water efficiency compared to the PAN-I membranes, measured as initial structures. The higher efficiency leads to lower selectivity of these membranes (compared to PAN-I),

as determined by the experimental data. It was interesting to find how this higher efficiency would be affected by the vacuum metallization. (*Fig.4*)

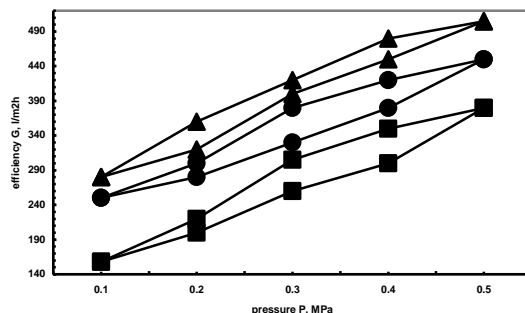


Fig. 4. Hysteresis curves of PAN-II membranes chemically modified and vacuum metallized with H18N9T alloy with sub layer of the oxides of alloy metals. ■-5sec ●- 15sec ▲-25sec

With the increase of coating time from 5 to 25 s, their efficiency increased from 380 to 505 l/m²h (*Fig.5*). The value of the efficiency at exposition time of 25 s (185 l/m²h) was close to these of chemically non-treated and non-metallized one (87 l/m²h) and chemically treated non-metallized one (350 l/m²h) membranes.

Similar to the other type of membranes studied, the small exposition times have higher effect on the surface in the presence of an sub-layer of metal oxides. The deposition of an additional oxide sub-layer gives a system where it is possible to obtain denser structure through the interaction between the metal oxides and small number of atoms, which could lead to destructive processes in the selective layer of the membrane under longer exposition. When only metal coating is deposited, membrane characteristics depend simply on the thickness of the metal coating.

The studies of membrane selectivity towards calibrant Albumin showed a dependence proportional to their efficiency. The structures of the initial membranes determine their selectivities while the presence of an oxide sub-layer changes it within certain interval. These changes, however, are small. For the membranes with polymer composition PAN-II modified chemically with the solutions described above and vacuum coated with X18H9T alloy, the selectivity towards Albumin increased from 46% (5 s exposition) to 52% (25 s) which is close to the values for the initial non-coated membranes. The membranes with the same polymer composition, same chemical modification and vacuum coated with X18H9T alloy but with additional sub-layer of metal oxides, the selectivity decreased from 40% (5 s exposition) to 26% (25 s).

For the chemically modified and vacuum metallized membranes with composition PAN-I, the selectivity towards Albumin increased with exposition time from 92 to 94 % which is the same tendency observed for the other type of membranes studied. The selectivities of the membranes of the same polymer composition but with additional sub-layer of metal oxides remained the same (67%) regardless of the sputtering time.

To establish the stability of the metal coating of the membranes, their selectivity was studied at different intervals of flushing the porous structure of the membranes.

Table 1. Flushing was carried out in regime of ultrafiltration

Membrane type	200 ml	500 ml	1000 ml	1500
PAN-II without sub-layer	55	54	55	54
PAN-II with sub-layer	40	42	42	40
PAN-I without sub-layer	93	92	92	93
PAN-I with sub-layer	67	68	67	68

The flushing was carried out in regime of ultrafiltration with water volumes from 200 to 1500 ml (Table 1). The change of the selectivities of the membranes studied was found to be insignificant and within the preciseness of the experimental method used.

The following dependence was observed for the membranes having an additional sub-layer of metal oxides: the values of the selectivity remained almost unchanged which means that the additional sub-layer improves the adhesion between the main metal coating and the polymer substrate.

The present studies showed that the method of vacuum metallization can be used for modification of polymer membranes to obtain different structures and properties required by certain processes.

The overall process depends on the initial structure of the membranes, the type of the polymer, the method of preliminary treatment and the sputtering time.

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COMPARATIVE EVALUATION OF VACUUM AND AIR OVEN MOISTURE CONTENT DETERMINATION METHODS FOR SOME VARIETIES OF DRIED FRUITS AND VEGETABLES

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Abstract: Исследовалась кинетика высушивания в вакууме (температура 60 и 70 °C) образцов сушеных фруктов и овощей с различным исходным уровнем влажности в пределах коммерческого диапазона: яблок (4 %-28 %), вишни (18 %-32 %), моркови и тыквы (8 %-14%), томатов (17 %). В процессе высушивания вишни (60 и 70 °C), моркови и томатов (70 °C) наблюдалось непрерывное снижение массы испытуемого образца, что указывает на протекание значительных процессов деградации и искажение результатов определения содержания воды в этих продуктах. В работе представлена также кинетика высушивания исследуемых образцов при атмосферном давлении и температуре 98 °C для фруктов и 88 °C для овощей и обсуждены условия применения исследованных методов.

Key words: vacuum and air moisture determination methods; dried fruits; dried vegetables

Introduction

Drying methods are the most widely used techniques. Drying techniques with convective heating principle comprise ordinary oven drying and vacuum oven drying. It is important to be aware that drying techniques do not measure the water content as such. The result is a mass loss under the conditions applied or “moisture” is often used instead. These conditions can principally be freely chosen and the results are, consequently, variable. Drying to a constant mass is often required, but a real constancy is only achieved in rare cases. The mass loss is not only caused by water but by all the substances volatile under the drying conditions, either already contained in the original sample or produced by the heating process. The application of low pressure in vacuum ovens reduces the danger of producing volatile decomposition compounds but does not allow a distinction to be made between water and other volatile substances already present in the product. To shorten the long determination times, more intensive heating is used. The results can vary in a very broad range depending on the drying parameters applied. Mass-loss results can, however, be matched with the results of another method, particularly a reference method, by adjusting the parameters in an appropriate way. In these cases the most suitable calibration must be established for every type of product in a specific way [1].

The aim of this work was to evaluate two methods of oven moisture determination: a) under reduced pressure, and b) at atmospheric pressure, applied to traditional (apples, sour cherries, carrots) and lesser-common (sweet cherries, pumpkin and tomatoes) species of dried fruits and vegetables.

Materials and methods

The study methodology consisted of simultaneous moisture content analysis of test samples with using vacuum and air oven, evaluation of accuracy and comparison of obtained results.

In preparing the dried samples, fresh apples, cored and sliced; cherries stoned; carrots, washed, skinned, diced, and steam blanched; muscat pumpkin, peeled, de-seeded, diced, and water blanched; red tomatoes, quarters were dried in a laboratory tunnel air drier at the temperature of 60 - 65 °C and 2,5- 3,0 m/s air velocity.

Seven samples of dried apples with various moisture in a range from 4,2% to 28,0%, six samples of dried sour cherry with moisture in a range from 18,0% to 32,0%, and one sample of dried tomatoes with moisture about 17 % were prepared. Two samples of dried carrots and in two samples from each of the two batches (I and II) of dried pumpkins, that differed in the texture (brittle and hard or poorly elastic) were also obtained. The moisture levels met commercial moisture range for each test product. Test preparation and laboratory equipment, the procedure of analysis were in accordance with GOST 28561-90 with the following clarification: the test portion placed in a drying dish did not exceed 0.2 g at cm².

In preparing the tests for the analysis, dried fruits and tomatoes were cut with scissors onto 1-1.5 mm size bits; carrots and pumpkin samples from batch I, both with brittle and hard or elastic texture, were ground in the 160 W electric coffee mill, avoiding to be heated and turned into fine powder. The sieve analysis of the pumpkin I test, prepared from the sample with brittle and hard texture, showed that the oversize and undersize of 1 mm sieve was, respectively, 3% and 97%, including the 79% passage through the

sieve of 0.5 mm. The test prepared from the sample with elastic texture passed through the 3 mm sieve, including the 47% passage through the 2 mm sieve.

The pumpkin samples from batch II were ground in the 90 W electric coffee mill and the corresponding tests with visually bigger particle size were obtained.

The prepared tests were packed and tightly closed.

Vacuum oven drying: A vacuum oven with automatic temperature regulation with accuracy ± 2 °C was used. Drying parameters: the temperature 70 or 60 °C, the pressure of 10 mm Hg. (1,33 kPa).

Air oven drying: An air oven with natural ventilation and automatic temperature regulation with accuracy ± 2 °C was used. The drying temperature: 98 °C or 88 °C.

We investigated two methods of moisture: 1) drying for a specified time at a specified temperature, and 2) drying to a constant mass.

With method 2), drying tests were kept in the heated oven for a time equal to approximately 70 % of the total drying time, established a priori, and then weighed. The drying was continued until two consecutive weighings do not differed by more than 0.40 mg per g of test taken, the second weighing following an additional drying time (about 10 % of the total drying time).

Mathematical treatment: The mathematical treatment of the experimental results was carried out according to [2] and included: a) calculating the average values of parallel measurements, precision estimations, and confidence intervals, using t-test; b) checking by the Fisher – test the hypothesis of the mean values equality c) regression analysis and checking linear hypothesis using F- test, d) correlation analysis and calculated regression line by using the method of least squares.

Results and discussion

Moisture variations in the samples, depending on vacuum and air oven drying time, were presented and analyzed in a graphic form.

The test samples of apples lost the bulk of the moisture during the first 2-hours and reached constant mass for 6-12 hours of vacuum drying at 70 °C. The mean value of moisture reduction for the drying period from 6 h until reaching a constant mass was ranged from 0 to 0.34 abs.%, average 0.20 ± 0.10 abs.%.

None of test sample of sour cherries attained a constant mass during the vacuum drying for 83 hours at 60 °C and for 30 hours at 70 °C.

The samples of dried carrots, regardless of their initial moisture content, reached a constant mass after 48 hours of vacuum drying at 60 °C; at the same time, the mass decreasing in both of the samples took place at 70 °C throughout the 48 hours measurement period. 8.3 and 14.6 hours of vacuum drying at 70 °C resulted in the moisture values equal to those obtained at 60 °C when constant mass was attained, respectively for the brittle (hard) and elastic samples.

Vacuum drying kinetics, obtained for dried pumpkin at 70 °C, showed that all the studied samples reached a constant mass during the test. Both brittle samples with a moisture of about 8 %, from the batch (I) and (II), differed in bits' size, and the sample with elastic texture and moisture content of about 14% from the batch (I) were completely dried for 18 hours. At the same time it took 23 hours to achieve a constant mass in the second elastic sample (batch II). Such increasing in the drying time may be the result of the larger sample particles, obtained with low-powered mill, in combination with the higher sample moisture (about 16 %).

Constant mass of dried tomatoes was not reached after 24 hours of vacuum drying at 70 °C; continuous rising of moisture during all measurement time was observed by the relevant graphic.

The results were evaluated with the following: 1) an application of drying to a constant mass indicates no significant sample degradation, and means that the applied drying conditions allows you to get the result what meet the water content, and 2) a continuous decreasing test sample mass during drying indicates significant degradation and the possible distortion measurement results.

In the entire investigated moisture range (4%...28%), the results obtained for apple drying confirm the admissibility of the following vacuum drying parameters: 6 h, 70 °C. These parameters correspond to the official laboratory method used in [3].

Cherries and tomatoes lose mass continuously, even in mild drying conditions which were used in the experiments; it indicates a significant degradation of these materials. The reduction in mass, observed during the cherry samples drying, may result from the anthocyanin destruction [4]. Tomatoes are extremely sensitive to drying temperature, and degradation reaction proceeds via browning. At temperatures of 50-60 °C browning reactions occur uniformly throughout the process of tomatoes drying [5] and, therefore, a resulting loss in the sample mass can take place even when the temperature of moisture determination will be reduce below 70 °C. Because drying to constant mass was unacceptable for dried cherries and tomatoes, it seems reasonable to determine the moisture by drying for specified time.

As follows from the results, the method of vacuum drying to constant mass at 70 °C is suitable for dried pumpkin analysis, but not dried carrots. The processes occurring during carrot drying and affecting the change in material mass are reflected in the scientific

literature [6]. There are fat, carotene, tocopherol oxidation, native volatile loss, and deamination of amino acids. Good results were obtained for dried carrots at

60 °C. At this temperature, a constant mass was reached for the same time, regardless of the initial moisture content of the samples. Thung S. [7] concluded that the temperature below 70 °C in determining the moisture content in the dehydrated vegetables by vacuum drying should be used to avoid the effects of thermal decomposition. 60 °C compared to 70 °C may be advantageous also for the pumpkins, because of a possible drying time leveling for the samples with different initial moisture content; so the method of specified time can be used.

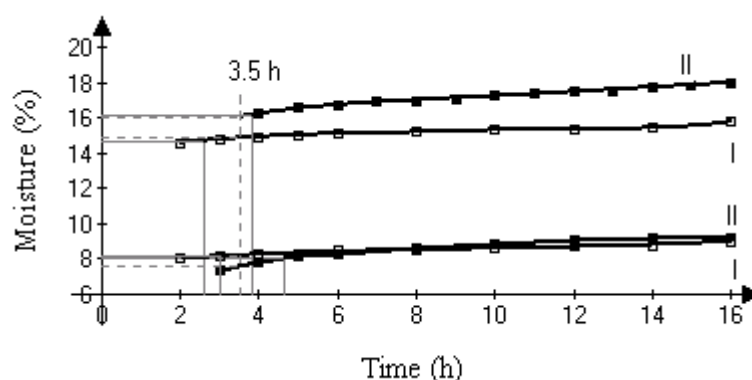


Fig. 1. Moisture measured for dried pumpkin samples from batch I and II vs. there air drying time at 88 °C

The measured moisture values for dried pumpkin samples vs. there air drying time at 88 °C are shown in Figure 1. The samples tested within 16 hours of drying have not reached a constant mass, regardless of their initial moisture content. These samples were used for the moisture content parallel determinations by vacuum method using the parameters: 70 °C, 6 h. Moisture values obtained in a vacuum oven, are plotted as horizontal lines. The drying time in an air oven, at which moisture measured by the two methods are the same, are determined by the intersection point of the horizontal and the corresponding curve. The time range is from 2,6 to 4,6 hours, the average – 3,5 h. Moisture values referring to 3,5 hours of air drying were determined for each of the tested samples and significant differences (at the confidence level $P=0.95$) between the values obtained by the two methods were found. The differences range from minus 0,425 abs. % to 0,14 abs. %.

The mass of tomato samples decreases continuously during 16 hours of air drying at 88 °C. 2,5 hours of the air drying ($W=17,37\pm0,34$ %; $s=0,2276$; $n=3$) corresponds to 24 h of vacuum drying at 70 °C ($W=17,405\pm0,394$ %; $s=0,2350$; $n=4$). Unfortunately, the moisture value referring to 6 h vacuum drying ($W=15,16\pm0,34$ %; $s=0,2159$; $n=4$) is out of the range of air drying results.

A comparison of the moisture content measurement results in dried cherries, obtained by air oven drying method (98 °C, 3 h) and vacuum oven drying method 70 °C, 6 h), was studied. Non-linear regression relationships between the values of moisture determined by the two methods were presented in graphical form. Using the method of least

squares closest correlations of the following types were established for dried sour cherries (1) and dried sweet cherries varieties Rossoşanscaia zolotaia (2) and Tehlovan (3):

$$W_v = (5.26784 \times 10^{-4}) \times W_a^3 - 0.019562 \times W_a^2 + 1.19910 \times W_a - 0.0021339 \quad (1)$$

$$R^2 = 0.99999, S = 0.0296421;$$

$$W_v = -(4.03946 \times 10^{-4}) \times W_a^3 + 0.022432 \times W_a^2 + 0.729918 \times W_a - 0.00168 \quad (2)$$

$$R^2 = 0.9998, S = 0.176087;$$

$$W_v = (6.79154 \times 10^{-4}) \times W_a^3 - 0.032741 \times W_a^2 + 1.30891 \times W_a + 0.00199081 \quad (3)$$

$$R^2 = 0.9993, S = 0.2585;$$

Evidently, the obtained correlations or graphs should be used to better match methods. It should be noted that the investigated varieties of cherries have a significantly different quality: Rossoşanscaia zolotaia belongs to the light-colored fruits (yellow), with a gentle pulp, Tehlovan - to a group of dark-red fruit with firm, crisp pulp [8]. Additional studies may be needed to confirm that the established differences in the moisture determination are not random.

Conclusions

Different kinds of dried fruits and vegetables require an individual approach to choose the behavior for moisture content determining, with using both vacuum and atmospheric oven.

Method of drying to constant mass under reduced pressure can be applied to dried apples (70 °C), carrots (60 °C), pumpkins (70 °C). It is not suitable for the dried sour cherries and tomatoes analysis at temperatures both 60 °C and 70 °C, due to significant degradation of their chemical composition under the influence of prolonged exposure to temperature. Method of drying to constant mass at temperature of 88 °C and atmospheric pressure also can not be used for dried pumpkin and tomatoes analysis.

3,5 and 2,5 hours, respectively, were found as specified drying time.

Some differences in the results of moisture measurement in cherries, using vacuum (70 °C, 6 h) and air (98 °C, 3 h) drying methods, were noticed, and the empirical relationships between the moisture values obtained by the two methods were established.

Nomenclature

W umiditate, %

n number of parallel measurements

s standard deviation estimation

R^2 coefficient of determination

Symbols used as subscripts: v-vacuum; a – aer.

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THE INFRARED ANALYZER MA35 SARTORIUS APPLICATION FOR MOISTURE DETERMINATION IN DRIED APPLES AND CARROTS

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Abstract: A comparison of the moisture content measurement results in dried apples with moisture from 2.5% to 28% (wet base) and dried carrots with moisture from 4% to 15% (wet base) obtained by infrared drying rapid method and classical vacuum oven method is presented. It was shown that IRD results depend both on the drying temperature and on the moisture level in the tested samples. We determined optimal drying conditions/ temperature for analyzer application being of 82 °C for dried carrots, and 82 °C or 98 °C for the dried apples with a brittle (and hard) or elastic texture, respectively. The systematic measurement errors that appear for dried samples with different moisture content at the same IRD temperature have been corrected due to reference values data using appropriate graphs and empirical equations.

Keywords: moisture determination; dried apples and carrots; infrared drying method; Sartorius Analyzer application.

Introduction

Rapid methods for determining the moisture are needed for a proper technological process management and to obtain the dried product of a standard quality. The Sartorius (Germany) moisture analyzer is widely used in analyzing the moisture content of chemical products, pharmaceutical, paper, cosmetic, textile, food and animal feed industry. In moisture determination the Sartorius application data for dried fruit and vegetables is very limited.

This is a thermogravimetric IR analyzer. The results obtained by infrared drying can be reproduced if the drying parameters are carefully chosen, as already reported in the literature [1, 2]. When the infrared dryer/analyzer is properly calibrated, it is well suited for application in the food manufacturing process [3]. The calibration is product-specific [4].

The aim of this work was to find out infrared drying conditions/temperatures to match the mass loss results with the moisture content of dried apples and carrots determined by classical vacuum oven method, used as a reference method.

Materials and methods

Samples preparation. Fresh apples (*Malus domestica Borkh*) of two varieties, Golden Delicious and Prima, from local market, 14-15° Brix, were used in this study. The apples, each variety separately, cleaned, sliced and mixed, were divided randomly into 7 portions. They were processed to obtain commercial quality dried apples samples in the range from 2.4% to 28% (wet base) moisture content, with 4% increments.

Each portion was dried in a pilot-plant tunnel air drier at the temperature of 62 ± 2 °C and 2.5 m/s air velocity. Moisture loss during the drying was measured by weighting the

sample at the beginning and periodically during the drying process, till the required mass and, therefore, the corresponding moisture content was attained.

Fresh carrot roots (*Daucus carota* L.), from the local market, having the average dry matter content of 14.7 %, were used in this study. The cleaned roots were cut into long thin strips and steam blanched at 100 °C, atmospheric pressure, for 5 minutes. They were cooled and drained on a sieve for a couple of minutes, mixed and divided randomly into several portions. They were processed to obtain commercial quality dried product in 4% to 14% (wet base) range. They were dried under the same process parameters as the apples.

Both apples and carrots portions were broken up to 2 – 4 mm uniform size bits, ground in an electric coffee mill, avoiding to be turned into fine powder. The ground samples were packed and tightly closed.

Reference method (RM) for moisture determination

The test portions of 2.5 ± 0.1 g for brittle and hard product and 3.5 to 4.0 g for elastic texture were placed in non-corrosive metal containers, provided with well-fitting lid, about 45 mm of diameter and dried in an electric vacuum oven under the pressure of

10 mm Hg (1.33 kPa). Cyclic weighings at approximately 10% of the total drying time were performed. The drying was carried out at constant temperature of $70 \pm 1^\circ\text{C}$ for apples and $60 \pm 1^\circ\text{C}$ for carrots to the constant mass point (At this point, the reduction in weight is less than 0.0010g in 2 hours of drying for apples and 6 hours of drying for carrots.).

The moisture content is defined as the mass loss between before and after drying measurements expressed as percentage of mass. Five parallel determinations for each analysis were carried out on the same test sample. Their average arithmetic value was taken as a final result.

Rapid method for moisture determination by IRD

A Sartorius MA 35 analyzer was used for infrared drying (IRD) rapid method. For sample heating, the MA35 is equipped with two 360 W metal tubular heating elements. These elements give more of an oven effect, providing intense heat throughout the whole heating chamber, and allowing for better drying of all sides of the sample. A built in balance with 1mg resolution do the test portions weighting.

The device offers two modes of operation, automatic and time mode. In the automatic mode the cyclic moisture content measurements are carried on till no change in mass is registered during a certain short time interval. In the time mode the operator is choosing the drying time from the beginning. He results will depend on the experience and reproducibility of drying curves. In both modes the drying temperature is to be chosen.

When decomposition reactions occur, resulting in values too high for the mass loss, a lower temperature has to be applied.

The preliminary experiments revealed that the determination of moisture, using the time mode led to unsatisfactory results. We used the automatic mode in our experiments. The first measurement results were excluded from each row of data, as reflecting an operating transitory state that appears at the beginning of the experiment [3]. Two-minute intervals between parallel measurements were set [1].

Two series of experiments were performed in this work. In the first series we varied the temperature and the sample moisture. Each analysis was carried out at least three times. The arithmetic average value was taken as a final result.

In the second series, the selected temperature was kept constant for each of three groups of the samples tested: the samples of dried apples with a brittle or hard texture and the samples

of dried carrots were measured at the temperature of 82 °C; the samples of dried apples with an elastic texture were analyzed at the temperature of 98°C. Each analysis was carried out at 5-9 times. All tests were performed by the same operator. *Statistical analysis*

There were six procedures for statistical analysis of IRD experimental data in this study:

- calculating the average values (\bar{y}_j) and precision estimations (s_j) of the measurement results (y_{jk}) in the conditions of repeatability, for each level j of moisture (ISO 5725 -2);
- measurement results examination for consistency and outliers, using the Grubbs` tests, described in ISO 5725 -2, with the recalculation the statistics if data are rejected;
- investigation whether s_j depend on the average \bar{y}_j , and if so, the functional relationships determination, using the methods described in ISO 5725 -2;
- checking by the Fisher - test (with 95% confidence) whether the dispersions (s_j^2) calculated for various moisture levels are similar or dissimilar. If the null hypothesis (no difference) is accepted, the weighted average value of dispersions ($s_{n,q}^2$) and corresponding precision ($s_{n,q}$) are determined [5];
- repeatability limit (r) value determination (with 95% confidence) by ISO 5725-6;
- measurement results bias estimating ($\hat{\Delta}_j$) at each moisture level is expressed as an absolute value by ISO 5725 - 4:

$$\hat{\Delta}_j = \bar{y}_j - \mu_j, \quad (2)$$

where μ_j is an accepted moisture reference value (%), wet base;

The 95% confidence interval for the systematic error ($\hat{\Delta}_j$) of measurements is approximating calculated as follows:

$$\hat{\Delta}_j - A s_j \leq \hat{\Delta}_j \leq \hat{\Delta}_j + A s_j \quad (3) \text{ or } \hat{\Delta}_j - A s_{n,q} \leq \hat{\Delta}_j \leq \hat{\Delta}_j + A s_{n,q} \quad (4)$$

Here, A is a factor used for calculating the bias estimate uncertainty:

$$A = 1.96/\sqrt{n} \quad \text{or} \quad A = 1.96/\sqrt{N_{n,q}} \quad (5)$$

If the confidence interval includes zero, then the systematic error is not significant (α -level =0.05), otherwise it should be considered significant.

Results and discussion

Moisture determination. The objective of the first series of experiments was to find out a proper working value for the drying temperature.

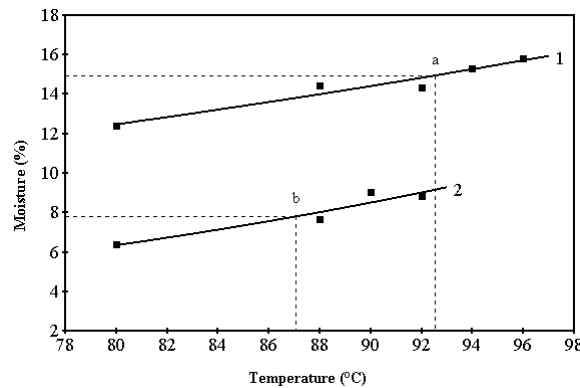


Fig. 1. Influence of IRD temperature on measured moisture value (\bar{y}_j) for different carrot moisture level (%): 1– 14.96, 2 – 7.82

We have presented the rapid method measured moisture results as a function of IRD temperatures for seven portions of dried apples; the most suitable temperature parameters have been found as the 82 °C and 98 °C for testing dried apples below and above 7.5 % moisture content, respectively [6]. Figure 1 displays the rapid method measured moisture results as a function of IRD temperatures for two portions of dried carrots. We determined two values for most suitable temperatures of 87 and 93 °C for dried carrots with the true moisture content of 7.82% and 14.96%, respectively. We expect lower temperatures required for dried carrots samples with moisture below 7%. Based on the dried apples experiment results [6], temperatures around 80°C would be good for this case. As the carotenoids in carrots are unstable to heat, to avoid thermal degradation resulting in distortions of IRD measurement results, an 82 °C temperature for the whole range of sample moisture was applied in all our experiments with dried carrots.

Statistical analysis. The statistical analysis of measurement results, for the first and second series in common, for three groups of products: dried apples with a brittle or hard texture, dried apples with an elastic texture, and dried carrots showed that (Table 1):

- no functional noteworthy relationship between values s_j and \bar{y}_j or μ_j was detected for any of the tested groups of products;
- no significant differences were found between the values of s_j within the same group of samples, which allowed us to unite in one batch all statistics results obtained for the same group of product and to calculate weighted average of standard deviation and repeatability limit (Table 2);
- the measurement bias at 82 °C for four levels of brittle or hard dried apples were found to be insignificant values in two of the levels and significant values for the other two. The measurement bias for 98 °C – dried apples and at 82° C – dried carrots were considered significant values for all of the indicated levels with one single exception. A systematic error in IRD moisture determination for all three groups of tested dried samples appeared. Based on this fact, correlations of \bar{y}_j with μ_j was studied using linear and non-linear least square regression analysis. The correlations were good:
for dried apples

$Y(x) = 2.1507e^{0.1660x}$, $R^2=0.995$, $s=0.1638$ in the 2.5% – 7.5% moisture range;
 $Y(x) = 1.5115 + 0.9380x$, $R^2=0.9976$, $s=0.3274$ in the 7.5%–28% moisture range;
 for dried carrots
 $Y(x) = 0.6258 + 0.8450x$, $R^2=0.975$, $s=0.5187$ in the 4.1%–15% moisture range.

Table 1. Statistical estimation of IRD moisture values

Statistical indicators								
j	n_j	s_j	\bar{y}_j	μ_j	$\hat{\Delta}$	The cnfd. interval (4)		Δ sgn
						$\hat{\Delta} -$	$\hat{\Delta} +$	
obtained at 82 °C for brittle or hard samples of dried apples								
1	3	0.0611	4.20	4.24	0.04	- 0.06	0.14	no
2	3	0.1305	7.57	7.67	0.01	- 0.09	0.11	no
3	5	0.1297	7.56	7.41	0.15	0.05	0.25	yes
4	5	0.2969	3.35	2.54	0.81	0.16	0.36	yes
obtained at 98 °C for samples of dried apples with elastic texture								
1	3	0.31953	8.46	7.67	0.79	0.7	0.88	yes
2	3	0.26514	14.17	13.29	0.88	0.79	0.97	yes
3	3	0.40004	18.92	18.06	0.86	0.77	0.95	yes
4	3	0.22605	23.49	23.73	0.24	0.15	0.33	yes
5	3	0.03214	25.03	25.47	0.44	0.35	0.53	yes
6	3	0.63540	28.01	28.02	0.01	- 0.08	0.10	no
7	8	0.14870	9.30	8.70	0.60	0.51	0.69	yes
8	8	0.43398	15.60	14.75	0.85	0.76	0.94	yes
9	7	0.41457	19.26	18.49	0.77	0.68	0.86	yes
10	7	0.20254	23.82	23.70	0.12	0.03	0.21	yes
11	6	0.14774	25.46	25.90	0.44	0.35	0.53	yes
obtained at 82 °C for dried carrots								
1	8	0.3731	4.36	4.09	- 0.27	0.11	0.43	yes
2	4	0.4361	6.72	7.82	- 1.10	0.94	1.26	yes
3	7	0.5438	9.14	9.49	- 0.35	0.19	0.51	yes
4	9	0.4656	9.36	9.61	- 0.25	0.09	0.41	yes
5	4	0.4053	12.89	14.96	- 2.07	1.91	2.23	yes

The IRD measurements performed at different times could be subjected to environmental changes influence. It follows that if s_j is a precision estimate in terms of repeatability, then $s_{n,q}$, according to ISO 5725-3, may be regarded as the standard deviation of the intermediate precision with differences in the "time" factor, i.e. $s_{I(T)}$. Therefore, the absolute value of the difference between the results of two measurements obtained by IRD in the conditions of intermediate repeatability (at the differences in the factor "time"), should not exceed, with 95% confidence or probability, the value of $r_{n,q}$ specified in Table 2. There are relatively low. This is concluded by comparing them with ones standardized for oven drying method (the r values of 1% when samples are drying together and 3% when samples are drying at different times or in different laboratories are included in the national standard of the Republic of Moldova).

Table 2. Statistical indicators for the united batch of various types of product

Sample				
Dried apples with brittle and hard texture	16	12	0.2099	0.58
Dried apples with elastic texture	54	43	0.3212	0.89
Dried carrots	32	27	0.4532	1.25

Conclusions

Moisture content data obtained by infrared drying rapid method and classical vacuum oven method for dried apples with 2.5% to 28% moisture and dried carrots with 4% to 15% moisture were studied. It was shown that IRD method results depend on the drying temperature as well as on the level of moisture in the tested samples.

In analyzing the dried apples IRD method results we used different temperature behaviour approach for different dried apple textures: 7.5% to 28% moisture dried apples have an elastic texture, below 7.5% moisture have a hard to brittle texture.

The systematic measurement errors that appear for dried samples with different moisture content at the same IRD temperature have been corrected due to reference values data using appropriate graphs and empirical equations.

The statistic studies confirm that the infrared drying rapid method can be used to measure moisture content in dried fruit/vegetables industrial and commercial control units under strict conditions. For valid results, the moisture level of tested product should be taken into consideration.

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AUTENTIFICATION OF GEOGRAFICAL ORIGIN OF WINES BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY (ICP-AES)

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It is known that macro- and trace element composition of plants and living organisms depends on the elemental composition of the environment. European researchers found that even 10 years after bottling, the wine continues to bear the "chemical signature" of the vineyard and forest, from which the barrel was made in which the wine is matured. Naturally, this fact can be used to confirm the origin of wines with geographical appellation, as well as to detect wine falsification [1,2].

The identification of the product at the place of origin means that it has some special features related to this area because of the unique natural and climatic conditions and existing traditions. Also, the geographical appellation bears the information that there is some control over this production. Starting 2011, OIV (International Organization of Wine and Wine) first introduced in the book of recommended methods of analysis for wine, must and grape juice the multi-elemental analysis - OIV-Oeno 344-2010 [3], which can be carried out by atomic emission spectrometry with inductively bound argon plasma (ICP-AES or ICP-AES) and mass spectrometry with inductively coupled argon plasma (ICP-MS or ICP-MS).

This paper presents the results of tests carried out in order to validate trace elements analysis of wines by ICP-AES. Studies were carried out by atomic emission spectrometry with inductively bound argon plasma at ICPE-9000 instrument (ICP-AES, Shimadzu Co., Japan). We used standard for ICP, Fluka TraceCERT-ISO/IEC 17027, ISO Guide 34 (Sigma-Aldrich GmbH). For preparation of calibration solutions, water and nitric acid class TraceSELECT Ultra were used. For the multi-elemental analysis of wine two sets of standards were used: series (1), including trace elements: zinc, copper, arsenic, lead, cadmium, iron, aluminum, etc., and series (2), including macro-elements sodium, potassium, calcium, magnesium, lithium. For the first series, the multi-elemental standard is prepared with a 5% solution of ethanol, and for the second series (2) the influence of alcohol is not taken into account, since a significant dilution of the samples (25-100 times) negates its effect.

It is established that the content of trace elements depends not only on the composition of the soil, but also on applied technological operations. The content of micro and macro-elements also depends on the crop year. In particular, relatively low levels of elements such as copper and iron indicates the absence of exogenous metals, of agronomic or technological origin. Relatively low levels of potassium suggests that technological operations such as sulfitation with potassium metabisulfite, demetallization (potassium ferrocyanide), clarification with alginate potassium or caseinate, etc. were not used. The multi-elemental analysis performed by ICP-AES method is of considerable interest to confirm the geographical origin of the product, as well as an informative source on applied

technological methods. In fact, such an analysis is primary for wines of designated origin (VDO). Also, the method can be used to identify counterfeit products.

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PRINCIPLES AND MODALITIES TO DIMINISH THE INFLUENCE OF THE CORK TAINT ON WINE QUALITY

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Abstract: The cork taint is a major problem of the winemaking, with the significant economic damages, in which the main responsible is considered the cork. Essential component of this defect is considered the compound 2,4,6-trichloroanisole (TCA) and its bromo/chloro derivatives that affects aromatic profile of the wine. The development of some methods rapid and efficient technologies led to the reduction of TCA in wine (INNOCORK process, Procork membranes, innovative filters, etc.) and the sterilization of corks with ionizing radiation, sulphur dioxide solutions (3-5% SO₂). The systems described do not change the aromatic character, the composition of wine and presents an efficiency of 90-95%.

Key words: wine, cork taint, 2,4,6-Trichloroanisole

1. Introduction

The wine industry is facing a multitude of problems of toxicological, with possible impact on the quality of wine, including cork taint (english – "Cork Taint"; french "Goût de Bouchon". According to the profile literature, a wine with a broken cork has a range of smell: mould, drugs, ground, cardboard or wet paper and cellar. Responsible for these anomalous smells is considered the cork, however, there were found wines with similar defects without being in contact with the cork and other products in the field of bottling so there are other sources of contamination of wine with this defect.

The cork taint is responsible for significant losses in the wine industry. Several researchers (Alvarez, Amon, Bertrand, Chatonnet, Margot, etc.), associate the cork taint with the presence in wine of the following compounds: geosmina, gaïacol, 1-octen-3-one, 1-octen-3-ol, metilisoborneol, 2,4,6-trichloroanisole (TCA) and its bromo/chloro derivatives: 2,3,4,6-tetrachloroanisole (TeCA), 2,3,4,5,6-pentachloroanisole (PCA) and 2,4,6-tribromoanisole (TBA) (Teixeira M., Tanner H., and others, 2006). In most cases, 70-80%, the cork taint is attributed to the presence of TCA (Chatonnet P.; Dubourdieu D.). In smaller quantities, the presence of TCA in wine masks its aromatic intensity.

According to the statistics, the quantities of wine affected by trichloroanisoli vary from one source to another. About 4-15% of the bottled wine is contaminated presenting cork taint. Several reasons may explain this variation number. One of them is the direct contamination of wine by trichloroanisoli. Having a threshold of perception in the range of 1,5 to 4 ng/L, trichloroanisoles give the wine an easily detectable smell of mould. As a rule, the wine consumers refuse the wine which contains an amount about 10 ng/L of TCA.

Analysis of the presence of TCA in wine, origin and its effects on human health have become recently targets of research, many aspects of these are still being studied. The necessity of researches result from a number of reasons, one being the economic. It is obviously, that the withdrawal from a contaminated consignment circuit creates a series of entrepreneurial problem, diminishing in a clearly way its effectiveness and efficiency. As

far as the consumer, he must be supplied with the harmless products, balanced, with high biological value and pleasant sensory properties.

2. Contamination of wines with chloroanisoles

Chloroanisoles can be synthesized by moulds based on chlorophenols derivatives which are used extensively as insecticides for wood treatment. Volatile enough, they may be spread in the cellar or in other places, condensed into the places where moulds are present. This defect is the result of enzymatic activity of methylation of chlorophenols, of some filamentous species. The transformation in chloroanisoles is performed in the chloroanisoles through a detoxification mechanism of species of moulds, having the basic process their methylation chloroanisoles correspondents. Genesis simplified scheme of chloroanisole compounds is:

Phenolic substances + source of chlorine = 2, 4,6, trichlorophenol

2,4,6, trichlorophenol + mould (Aspergillus, Penicillium)= 2,4,6- trichloroanisole

But not only fungicidal products are responsible for this cork taint. Chlorophenols can have other origins. Chlorine enter into the composition of many washing and disinfecting agents, used in wine-making, being present in different chemical forms, depending on the pH: Cl^- , HClO and ClO^- , but also in the form of free radicals, which can interact with phenols to form polychlorophenols.

Some fungal species of the genus *Penicillium* are able to synthesize the 2,4,6-trichlorophenol (TCP) on the shikimic acid way in the presence of free chlorine or embedded in methionine. Different filamentous fungi belonging to the different cork microflora: *Aspergillus sp.*, *Penicillium sp.*, *Actinomyces sp.*, *Botrytis sp.*, *Cinerea sp.*, *Neurospora sp.*, *Mucor sp.*, *Phyobium sp.* and *Streptomyces sp.* are able to form numerous intermediate, volatile and soluble molecules in the water-alcoholic medium of wine. Under the influence of the O-methyltransferase on trichlorophenol takes place the formation of the compound 2,4,6-trichloroanisole (TCA). The conversion of other derivatives of anisoles occurs similarly.

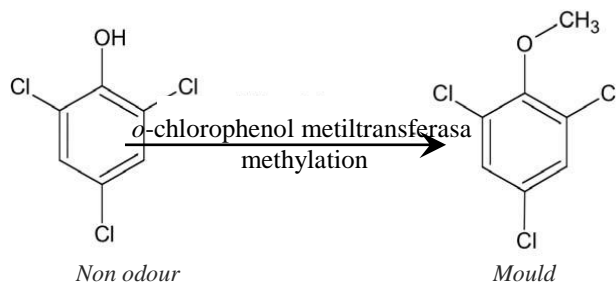


Fig. 1. The mechanism of TCA formation by micro-organisms (Alvarez -Rodriguez M. 2002 and Rubio-Coque J. J. 2005)

3. Methods for the determination of TCA in wine

Determination of TCA in wine has a relatively complex method due to the extremely low level of sensory perception of it. The content of TCA in wine and its derivatives can be determined only by means of GC/MS, with headspace solid-phase microextraction (HS-SPME), method which possesses a great sensitivity and selectivity and is fully automated (Wang H. and others, 2010).

The extraction of 2,4,6-TCA from corks stoppers is accomplished with a solution of hydro-alcoholic and its adsorption on a fiber covered with a film of polydimethylsiloxane. It follows desorption of trichloroanisole in the chromatographic column and the detection by mass spectrometer (Figure 2).

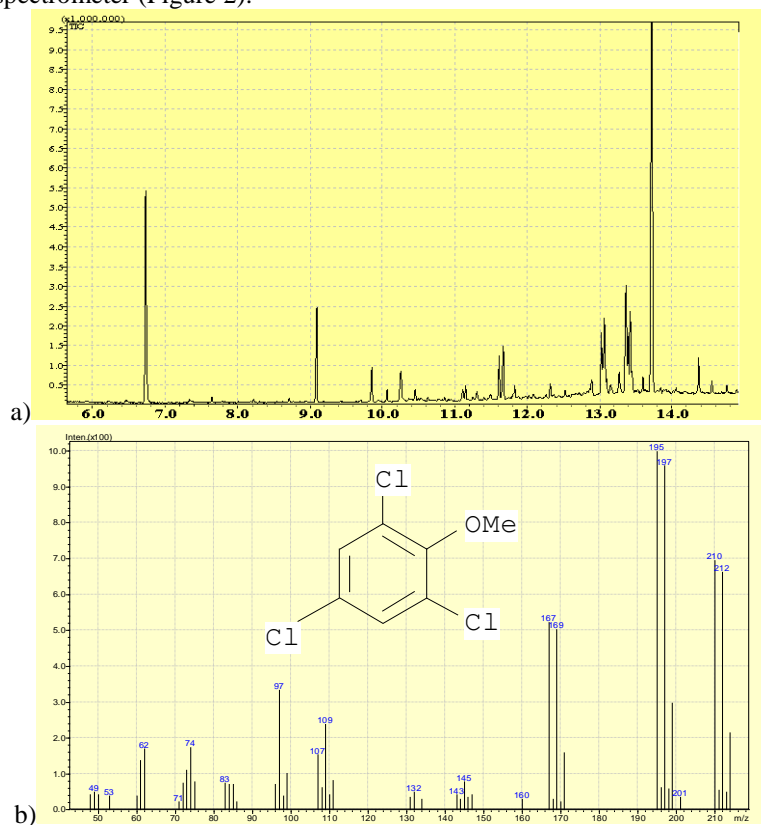


Fig. 2. a) HS/SPME GC chromatogram of a red wine with cork taint ($t_R(\text{TCA})=10,06$ min. (SPB5 25 m, 0,25 mm, 0,25 μm ; 40 $^{\circ}\text{C}$ 2 min, 15 $^{\circ}\text{C}/\text{min}$ 250 $^{\circ}\text{C}$ 10 min; He 49,2 cm/s); b) the detection mass-spectrometric of the TCA (NIST-5)

4. Methods for reducing the risk of contamination of wine with TCA

The need to detect and to prevent the risk of contamination of the wine with TCA is required both economically and hygienic way. There are several possibilities for contamination of the cork stopper with TCA, which cause also the further contamination of wine during the storage of wine in bottles:

- 1) the contaminated cork - initial contamination, is certified a content of TCA to 1500 ng/g of cork;
- 2) the infested cork which contaminates other corks – subsequent contamination during storage of wine in bottles;
- 3) the infested cork during storage - external contamination, the content of TCA lower than the TeCA and PCA.

The content of TeCP, TeCA, PCP and PCA may increase by the winery operations - decanting, wine bottling, etc. In humid and unsanitary conditions; treatment of wood, with the pallets of the derivative sodium of 2,3,4,5,6 pentachlorinated phenol; the use of insecticides and pesticides in wine storage aries; bentonite used in the treatment of wine, stored in unsuitable conditions, all of these wine/corks contaminations with polychlorinated derivatives of phenol generate in bottled wine a content of TeCP, PCP, TeCA and PCA major (Bertrand A., Barrios M., 1994, Chatonnet P. 1995).

To avoid the present of these compounds in wine is recommended:

- avoiding the excess of moisture and the temperature increased during all stages of wine processing, as it facilitates the development of moulds;
- exclusion of insecticides, fungicides and all the compounds of treatment and disinfection containing chlorine (sanitary-hygienic maintenance of premises, and in particular the cork processing industry)
- surface treatment corks with polymethylsiloxane instead of compounds with nutritional character for moulds (fatty acids, wax, glue, etc.);
- the use of effective methods of cork treatment both on the surface and in the depth and even the removal of TCA from corks through modern methods (Innocork process);
- the atmospheric conditioning and hygienic control strictly in the production and storage aries of auxiliary products;
- the use of plastic and metal pallets for keeping bottles and corks.

An alternate solution would be the endowment of Corks, on the surface which is in the contact with wine, the silicon disc (Figure 3). That being neutral reduces the risk of a subsequent infection of wine.

This procedure not change the aromatic character, the composition of wine and presents an efficiency of 90-95% of reducing the content of 2,4,6-TCA in wine.



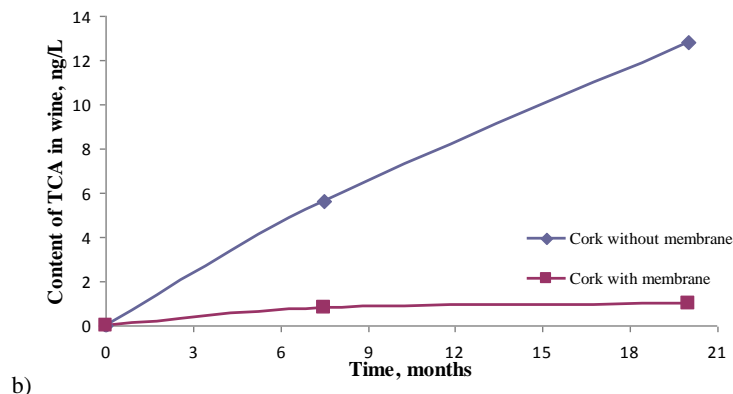


Fig. 3. a) photo presentation of ProCork membrane type B (Procork Project); b) the evolution diagram of TCA in wine during the period of contact (20 months).

Conclusions

Considering the potential contaminant of TCA in wine by both cork and through the wood of oak used for ageing, it is imperative to implement a new approach to detect their presence in cork and oak barrels before the technological use of their duration.

It is necessary to develop rapid and efficient methods and technologies to monitor primarily four groups of pollutants, namely: derivatives of benzene solvents, haloanisoles, halophenols and aromatic hydrocarbons. To ensure the full cycle traceability with risk measurement of wine pollution in general and especially with TCA, which is a problem not only theoretical but also practical matter.

Exclusion of sanitation compounds containing chlorine and the processes of preparation of corks and oak wood would effectively prevent the contamination with TCA. Corks endowment in contact with the wine surface of silicon discs (membrane Procork) it would reduce essentially the risk of contamination.

However, the most effective method of prevention would be sterilization by ionizing radiation of Co^{60} and with the solutions of sulphur dioxide (SO_2 3-5%). This method ensures the complete inactivation of fungi growth able to contaminate the cork and excludes the formation of TCA and other derivatives.

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POLYMERS FROM RENEWABLE RESOURCES FOR FOOD PACKAGING

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Abstract: Bio-based food packaging materials (FPMs) are packaging materials obtained from renewable agricultural resources. There are some aspects of great importance that are to be considered in connection with bio-based FPMs, namely: degradation rate under specific conditions, mechanical properties variation during storage, microbial growth, release of toxic by-products, etc. This paper reviews these issues in order to estimate the suitability of polymers from renewable resources as FPMs.

Keywords: food packaging, bio-based polymers, renewable resources.

1. Introduction

Published statistic data indicate that food packaging accounts for almost two-thirds of total packaging waste by volume and approximately 50% (by weight) of total packaging sales [1]. Disposal of food packaging materials (FPMs) is already an environmental problem, not only in terms of efficiency, but also in terms of by-products toxicity (as result of composting, combustion, landfilling) (Fig. 1).

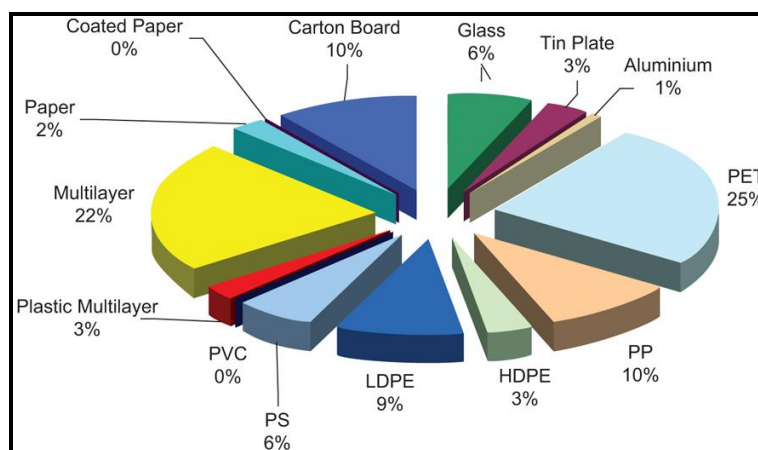
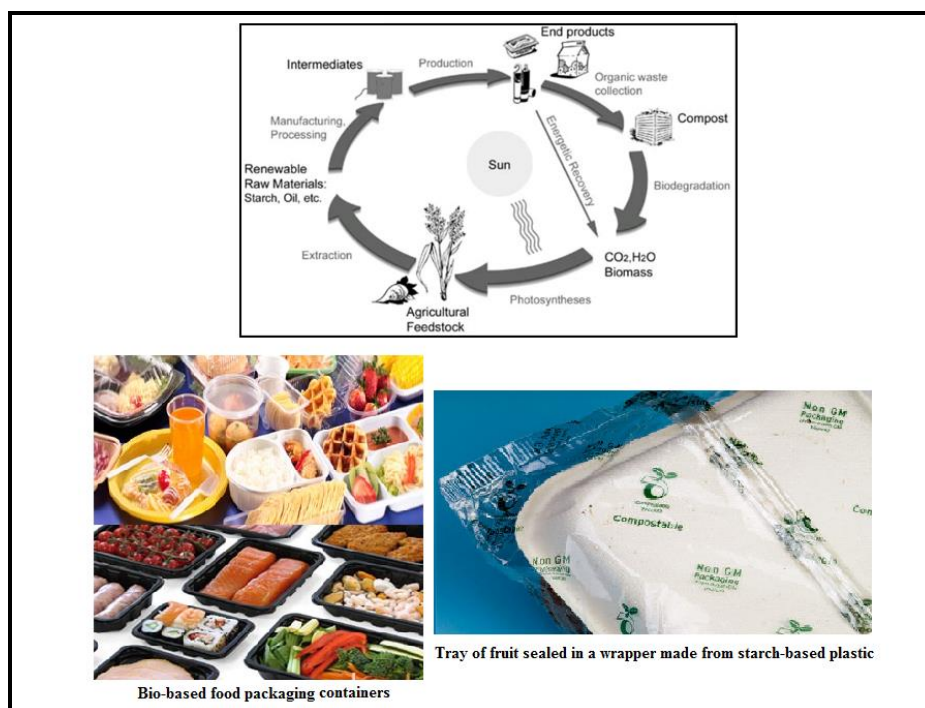


Fig. 1. Materials consumption for food packaging as estimated by the European Food Safety Authority (EFSA, available at <http://www.efsa.europa.eu/>)

Therefore, biodegradability must be one of the most important characteristics of new food packaging materials in order to provide alimentary industry with environmentally friendly FPMs able to ensure food quality preservation, as well as effective non-toxic disposal (by-products as carbon dioxide, water, good quality compost). This goal can be achieved in different ways. During the last decade, due to joint efforts made by R&D community along with packaging and food industries, a real progress was recorded, mainly in the field of bio-based food packaging materials (Fig. 2).



The roles of food packaging are to protect food products from outside influences and damage, to contain the food and to provide consumers with ingredient and nutritional information. Traceability, convenience and tamper indication are secondary functions of increasing importance. But the main goal of food packaging is to contain food in a cost-effective way that satisfies industry requirements and consumer desires, to maintain food safety and minimize the environmental impact after disposal. A wide variety of materials are used for food packaging: glass, metals (aluminum, tin), plastics (polyolefins: polyethylene and polypropylene; polyesters: PET, polycarbonates, etc; polyvinyl chloride; polystyrene, polyamides), paper and paperboard. Regulations on solid waste management indicated that source reduction, recycling, composting, combustion and landfilling are agreed as methods for an integrated approach.

Disposal of FPMs is already an environmental problem, not only in terms of efficiency, but also in terms of by-products toxicity (as result of composting, combustion, landfilling). Therefore, biodegradability must be one of the most important characteristics of new food packaging materials in order to provide alimentary industry with environmentally friendly FPMs able to ensure food quality preservation, as well as effective non-toxic disposal (by-products as carbon dioxide, water, good quality compost). Bio-based biodegradable packaging has commanded great attention and numerous research projects are under way in this field. One important reason for this attention is the marketing of environmentally friendly packaging materials. Furthermore, use of biodegradable packaging materials has the greatest potential in countries where landfill is the main waste management tool. Bio-based FPMs are materials obtained from renewable agricultural

resources and include both edible films and edible coatings, along with primary and secondary packaging materials [2]. Such FPMs can be used for almost all types of food: fresh and cured meat, sea food, eggs, dairy products, fruits and vegetables, butter, fats and oils, as well as dry products (such as: bakery products, cakes and cookies, pastas, chocolate), beverages and frozen food.

2. Bio-based food packaging materials (FPMs)

Bio-based FPMs are the present and future generation of FPMs due to the fact that they are derived from renewable resources and are biodegradable (although, some natural polymeric materials vary in their rate of degradation in the environment and some proteins, for example, cannot presently be classified as biodegradable). Polymers derived from renewable resources are broadly classified according to method of production, which yields in the following three main groups:

1. polymers obtained directly from natural materials (mainly plants): polysaccharides (such as: starch, cellulose) and proteins (casein, wheat gluten);
2. polymers produced by classic chemical synthesis from bio-derived monomers obtained from renewable raw materials: polylactate, a biopolyester from lactic acid monomers produced by fermentation of carbohydrate feedstock [3];
3. polymers produced by microorganisms or genetically transformed bacteria: polyhydroxyalkanoates, mainly polyhydroxybutyrates and copolymers of hydroxybutyrate (HB) and hydroxyvalerate (HV) [4].

FPMs made of or based on polymers from all these categories are either already used for packaging or have considerable potential in this field. However, the food packaging is still dominated by oil-derived polymers, despite global concerns about the environment, indicating that problems remain associated with the use of these renewable materials. A notable exception is cellulose and cellulose-based FPMs, which in the form of paper and cardboards/cartons enjoy wide usage as exterior packaging. Although highly biodegradable, paper is fibrous and opaque, with a poor barrier and moisture resistance properties, therefore, its role will remain limited to exterior packaging of foods, except in very specific cases (dry products).

Problems associated with renewable FPMs are threefold: performance, processing, costs. These issues are interrelated in all cases, but problems due to processing and level of performance are more complicated when it comes to polymers obtained directly from biomass (cellulose, starch, proteins). Polymers from categories 2 and 3, generally behave very well and are easily processed into films by standard plastics techniques, but they are expensive compared with synthetic analogues. Most commonly available natural polymers (category 1) are extracted from agricultural or forest resources (ligno-cellulosic polymers, starch, pectins and vegetal proteins). They form cell wall, plant storage structures or are structural polymers; by nature, they are hydrophilic and crystalline (in various degrees); all these are yielding in processing and performance problems [5].

2.1. Polysaccharides

2.1.1. Cellulose (Fig. 3) is the most abundant natural polymer in nature, is a cheap raw material (0.5-1 euro/kg, prior to derivatization) and, due to its chemical structure, it is highly crystalline, fibrous and insoluble. Hence, for cellophane film production, cellulose is dissolved in an aggressive, toxic mixture of sodium hydroxide and carbon disulphide and then recast into sulphuric acid. It has good mechanical properties, is hydrophilic and,

consequently, sensitive to moisture. Cellophane is not thermoplastic and, therefore, not heat-sealable. Because of its poor moisture barrier properties (at relative low humidity, cellophane is a good gas barrier, but barrier properties are reduced at higher humidity), it is often coated with nitrocellulose wax (NC-W) or polyvinylidene chloride (PVDC) to minimize side effects, but this procedure inevitably increases production cost.

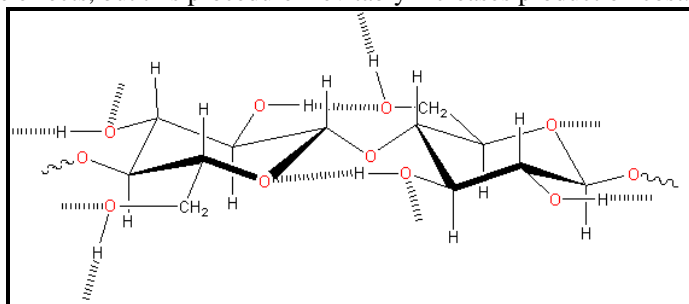


Fig. 3. Cellulose structure: β -glucose units tridimensionally connected by hydrogen bonds

Cellulose may be functionalized from the solvated state, by esterification or etherification of free hydroxyl groups, and derivatives commercially available are cellulose acetate, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose. These processes are expensive (the cellulose diacetate is 3-4 euro/kg) and for further thermoplastic processing an addition of up to 25% plasticizer is required. The gas and moisture barrier properties of cellulose acetate are not optimal with respect to food packaging. Some grades of cellulose acetate (degree of substitution < 1.7) are biodegradable, although at a slower rate than cellophane [2].

2.1.2. Starch is another widely abundant polysaccharide obtained as granules from corn, cereal grain, rice and potatoes; basically, it is a mixture of amylose (a linear polymer made of α -glucose molecules bound in position 1-4) and amylopectin (a highly branched polymer made of α -glucose units linearly linked by 1-4 [glycosidic bonds](#), while branching takes place through 1-6 bonds occurring every 24-30 glucose units, resulting in a soluble molecule that can be quickly degraded as it has many ends for enzymes to attach onto) (Fig. 4). The ratio of the two components varies according to starch types used.

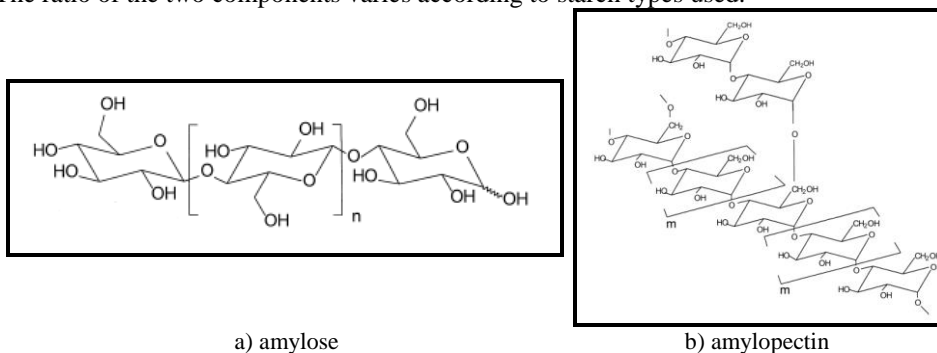


Fig. 4. Structure of starch components: a) amylose; b) amylopectin

Starch is widely used in several non-food sectors, most notably in sizing and coating paper, as adhesive, a thickener and as additive to simple composite materials such as briquettes [6]. As FPM, starch has received great attention only recently [7]. It is highly biodegradable, partially crystalline and quite inexpensive (0.5-1.5 euro/kg), but is also very hydrophilic, therefore poor moisture barrier, and shows moderate gas barrier properties. Films based on starch have moderate mechanical properties, generally inferior to synthetic polymers. When a plasticizer is added, such as water, starches exhibit thermoplastic behaviour [2].

Due to all these factors, starch requires substantial processing in order to obtain stable films for FPMs. This goal is achieved either by destructure and plasticization in an extruder, or by chemical modification. Blending with various amounts of synthetic polymers (as polyvinyl alcohol, PVA, or poly- ϵ -caprolactone, PCL) is also used to produce transparent films [8]. The starch component of the film is biodegradable, while the others degrade during composting. Starch-PVA materials are very sensitive to moisture, therefore improving hydrophobic characteristics will result in higher costs.

An alternate way is starch direct chemical modification. Industry already produces modified starch for a various applications, but these are surface-modified starch granules (i. e., cationic starches for paper treatment) [9]. Only few chemically modified starches are now available on the market because this method is expensive, despite the fact that starch is more chemically accessible and has lower crystallinity than cellulose. In addition, starch is more sensitive to degradation during chemical treatment than cellulose, so mild conditions are required in order to prevent extensive depolymerization and loss of properties.

Intensive research is still needed in this field for using starches as FPMs to their full potential. Despite all issues above mentioned, starch remains the one of the most promising of the bio-based available polysaccharides for food packaging, as it is easier to process than cellulose, at low cost, and highly biodegradable. The challenge is, now, to design strategies to improve the moisture stability of starch films without losing beneficial characteristics.

2.2. Proteins

Proteins from renewable resources (as casein, whey, soy, corn, zein, collagen, wheat gluten, keratin, egg albumen) have attracted renewed attention as biodegradable polymers for FPMs. They are used in adhesives, as edible and non-edible films and coatings, and have considerable potential as slowly degrading packaging. Proteins possess multiple chemical functionalities and molecules with a wide range of properties are available in nature (i.e., extrusion applications are possible with respect to plant proteins such as wheat glutens and seed proteins). Crosslinked protein films are often more stable than their polysaccharide-based counterparts and have a longer lifetime. Animal proteins (casein, keratin, collagen) are also available, although proteins derived from animal tissue, like collagen, are not so attractive from the consumer point of view, due to the adverse publicity on the BSE (bovine spongiform encephalopathy) syndrome.

Technically, protein films have good gas barrier properties and many of them are water resistant, though not entirely hydrophobic [2]. A drawback to the mass production of plant proteins as FPMs is the lack of some specific knowledge concerning tertiary and quaternary structures of complex materials (such as gluten, a [protein](#) composite made of a [gliadin](#) and a [glutelin](#), found in foods processed from [wheat](#) and related grain species). Protein-based FPMs are not expensive (1-10 euro/kg), but their mechanical properties still need improvement. Nanotechnology is a viable route to improve both tensile and barrier

properties (i.e., addition of montmorillonite nanoparticles led to increased tensile strength and elastic modulus [10]). Nanoparticles and variations at the nanometer scale affect electronic and atomic interactions without changing the chemistry.

2.3. Polyesters

2.3.1. Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are a family of polyhydroxyesters (Fig. 5) and, as attractive biopolymers with a wide range of applications, have been extensively studied.

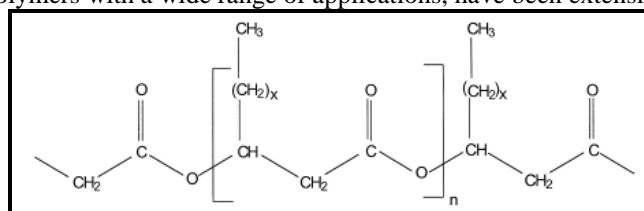


Fig. 5. PHAs structure

Besides studies on bacterial species and transgenic plants [11], many new strategies have been employed to effectively and economically produce PHAs, including the use of renewable materials or industrial waste. PHB and PHBV are representative members of PHAs that are produced at commercial scale. Although PHAs have properties close to classic plastics (polyethylene, PE, polypropylene, PP, or polyethylene terephthalate, PET), are biodegradable in soil and water resistant, can be readily processed using typical plastics technologies, they are much more expensive, mostly due to the separation from the microbiological medium. Properties of the PHBV copolymer films, i. e., may be adjusted by changing the ratio between hydroxyvalerate (HV) and hydroxybutyrate (HB), which can be achieved by manipulation of the growth media. A high content of polyhydroxybutyrate (PHB) gives a strong and stiff material, whereas polyhydroxyvalerate (PHV) improves flexibility and toughness. The PHAs are more hydrophobic than the polysaccharide-based materials, resulting in FPMs with good moisture barrier properties, at satisfactory prices (10-12 euro/kg). Under these circumstances, the potential of these PHAs as FPMs is excellent.

2.3.2. Polylactides (PLAs)

A more immediate option is polylactides (PLAs) (Fig. 6) – a class of [thermoplastic aliphatic polyester](#)s based on lactic acid from [renewable resources](#), such as [corn starch](#) (in the USA), [tapioca](#) products (roots, chips or starch mostly in Asia) or [sugarcane](#)s (in the rest of world).

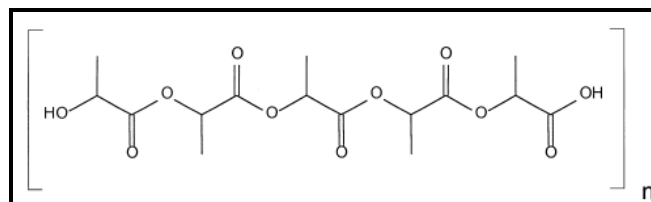


Fig. 6. PLA structure

PLAs display good mechanical properties compared with standard thermoplastics and the moisture barrier is higher than for the starch-based materials, as the production of

flexible, water-resistant PLA films has demonstrated. The final polymer cost strongly depends on the efficiency of the initial fermentation process to produce the lactic acid monomer.

The bio-based FPMs may contain further natural extracts as components, i. e. lignin and waxes which act as preservatives stalling the initial stage of food degradation processes. This still needs thorough testing, as well as the use of natural antioxidants, plasticizers, etc.

3. Economic and technologic considerations

A comparison between properties of bio-based FPMs and two other classic plastics (LDPE and PS) was made [5] and its conclusions are presented herein (Table 1.)

Table 1. Basic properties of some bio-based FPMs and classic plastics

Polymer	Moisture permeability	Oxygen permeability	Mechanical properties
Cellulose/Cellophane	High – medium (low if coated)	High	Good
Cellulose acetate	Moderate	High	Moderate (plasticizers needed)
Starch-PVA	High	Low	Good
Proteins	High - medium	Low	Moderate
PHAs	Low	Low	Good
PLA	Moderate	High - moderate	Good
LDPE	Low	High	Moderate - good
PS	High	High	Good - moderate

In general, thermoplastics commonly used FPMs, such as PE, PS, PET, are made in high volume at large scale production plants and are relatively inexpensive due to their low cost per unit (PE costs 0.7-1 euro/kg, PS costs twice as much). Because of the high amount, continuous production, product quality and performance it is easy to control and adapt parameters such as moisture and gas barrier.

On the other hand, many of the bio-based polymers (cellulose esters and ethers, starch derivatives, etc.) are still produced in batch reactors and are more expensive and subject to quality variations, despite the relatively low cost of the starting materials. A key factor in the spreading of bio-derived polymers in food packaging is, therefore, the development of analogous continuous processes to produce biopolymer films at reduced costs and with designed characteristics.

Another key factor is the production of polymer resins that can be readily processed into films using existing machines, with eventual minor modification. In this respect, PLAs and the PHAs are the polymers with the best prospects for commercial production, if initial fermentation or bio-production costs can be reduced.

In addition, the starch based FPMs which can be continuously extruded are also promising, provided factors such as moisture resistance and moisture barrier can be improved.

One of the challenges facing the bio-based FPMs is to match the durability of the packaging with product shelf-life: they must remain stable without changes of their mechanical and/or barrier properties and must function properly during storage until

disposal. Subsequently, the FPMs should be biodegradable. Environmental conditions that favours the biodegradation must be avoided during storage and the most important parameters for controlling stability of the biologically based FPMs are humidity, pH, nutrients, oxygen, storage time and temperature.

Prior to their use, bio-based FPMs must be tested in terms of interactions with food. For many years, coated cellophane (bakery products, fresh products, processed meat, cheese, candy) and cellulose acetate (bakery products, fresh products) have been used for food packaging. The moisture and gas barrier properties of cellulose acetate are not optimal for food packaging. Still, the film is excellent for high-moisture products as it allows respiration and reduces fogging [12].

Recently, a biodegradable laminate of chitosan-cellulose and polycaprolactone film was developed in order to investigate the possibility to use the laminate for modified atmosphere packaging [5] for fresh vegetables, such as head lettuce, cut broccoli, whole broccoli, tomatoes, sweet corn. The results were promising. The same in the case of a PE film containing 6% corn starch [13]. The type of packaging film seemingly did not affect the evaluated quality parameters, i.e. bread staling, broccoli colour, lipid oxidation of ground beef. However, a significant loss of elongation occurred in corn starch-containing film, which could be due to interactions between the film and free radicals developed during lipid oxidation in ground beef during frozen storage. Therefore, corn starch based PE films are recommended to be used only for packaging of wet and dry low-lipid foods. However, their use for high fat content foods was discouraged due to possible interactions with free radicals derived from lipid oxidation.

Studies on lean beef and bologna established that the microbiological quality of the foods was not affected by the presence of corn starch in PE films [5]; this leads to the conclusion that these FPMs have true potential as primary food containers for selected products.

In Belgium, packaging containing starch is used commercially for fast-food packaging of French fries. Other applications include disposable food service items and paper coatings [2].

Research on polylactate and polyalkanoate FPMs have been intensified during the last decades, so packaging materials are now readily available in dairy products industry (i.e., polylactate based cups for yoghurt). Other potential commercial applications include disposable food service items and bags (as for bakery products). With respect to polyalkanoates, suggested use as FPMs includes beverage bottles, coated paperboard milk cartons, cups, fast food packaging, as well as films [14].

More recently, it was demonstrated that the use of gluten films may actually be advantageous for storage of respiring fresh products [15] because it has suitable O_2 barrier properties, while remaining sufficiently permeable to carbon dioxide. Thus, a modified atmosphere containing 2-3% O_2 and 2-3% CO_2 was developed, which seems to be favourable to the overall quality of mushrooms.

4. Conclusion

Taking into consideration the presented data, it may be assumed that most of the present problems will be solved due to fundamental and applied research efforts. Legislative and consumer pressure are the driving force able to attract more attention on this sensitive field and the rate of development will parallel this enhanced commercial awareness.

Great possibilities exist for bio-based FPMs, aside from the most known products which are high price and niche products (i.e., organic products). However, further research within different areas of bio-based packaging (legislation, processing technology, food-packaging compatibility studies) must be achieved before bio-based FPMs can be used at large scale for primary food packaging.

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COEFFICIENT OF DIFFUSION OF AROMATIC PRODUCTS FROM ANATOLIAN HAWTHORN (*Crataegus Orientalis* Pall. Ex. Bieb.)

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Abstract. The coefficient of diffusion (D) of aromatic products – concrete and resinoid, obtained through extraction of leaves and fruits from hawthorn (*Crataegus orientalis* Pall. ex Bieb.) have been determined. The highest values of D with respect to resinoid extraction from leaves and fruits are calculated at 70 °C ($9,65 \cdot 10^{-9}$ cm²/s and $8,61 \cdot 10^{-7}$ cm²/s), respectively and with respect to concrete extraction from leaves and fruits – at 40 °C ($9,86 \cdot 10^{-9}$ cm²/s and $8,33 \cdot 10^{-7}$ cm²/s).

Keywords: *Crataegus orientalis*, coefficient of diffusion, aromatic products.

Introduction

The genus *Crataegus* from fam. *Rosaceae* consists of nearly 21 species in Turkey, which have many hybrids and populations [12, 15]. It has been reported that the fruits and leaves from the species *C. orientalis* Pall. ex. Bieb. are a rich source of flavonoids, vitamin C, glycoside, saponins, tannins and have found application in traditional and official medicine [5, 13]. The fruits extracts are widely used for the treatment of various cardiovascular diseases [6].

In our previous work we have obtained extracts from fruits and leaves of two species of the hawthorn - *C. monogyna* Jacq. from Bulgaria and *C. orientalis* Pall. ex. Bieb. from Turkey, with the ethanol, aiming at their application in cosmetics [9, 10]. The extracts have been characterized in terms of tannin content and their diffusion have been estimated [4, 11, 14].

There is no data on obtaining of this products from the fruits and leaves from – concrete and resinoid, from hawthorn, as well as on the determination of extracts` molecular diffusion coefficients, which is the aim of the current work.

Experimental

Plant material: Fruits and leaves of hawthorn (*C. orientalis*) from the market from Turkey were used in the investigation. The raw material was characterized in terms of: moisture content by drying it up to constant weight, at 105 °C [3].

Determination of the diffusion coefficients: Extraction was carried out as a batch static process by maceration in the solvent at a ratio of raw material to solvent = 1:10 under the following conditions: for concrete: solvent – petroleum ether; temperature - 20, 30 and 40 °C; for resinoid: solvent – 96 vol. % ethyl alcohol; temperature - 20, 40, 60 and 70 °C. For both aromatic products - size of material particles - 0,04 cm (fruits) and 0,11 cm (leaves); duration of extraction 1 h, with the solvent replaced and analyzed for extracted tannins after each 10 min interval. As a criterion for effectiveness of the process the quantity of concrete and resinoid was determined.

The diffusion coefficients were estimated by Minosian`s equation [1]:

$$D = \frac{l^2 \cdot 2,3 \lg(E_1 - E_2)}{\pi^2 (\tau_1 - \tau_2)} \quad (1)$$

where: l - size of the material, cm;

τ_1, τ_2 - duration of extraction, s;

E_1, E_2 - initial and final concentration of concrete or resinoid, %.

All experiments were carried out in threefold repetition and mean values with the respective error are presented in the figures below.

Data presented on the figures are processed with Microcal Origin Micro Soft tool.

Results and discussion

The analyzed hawthorn leaves and fruits were with 11.1 % and 12.4 % moisture level, respectively.

Figures 1, 2, 3 and 4 present the schemes of the experiments that have been carried out and the obtained results. Data reveal that with the increase in extraction duration the quantity of the obtained aromatic products decreases. Regardless of the plant material – leaves or fruit, maximum amount of concrete is extracted at temperature 40°C, and respectively, maximum amount of resinoid – at temperature 70°C, which finding is attributed to the influence of temperature on the extractive potential of the solvent.

On the basis of the experimental results from figures 1 – 4 the diffusion coefficients of concrete and resinoid are calculated, and their variations are presented on figures 5 - 8. With the increase in the temperature of extraction the values of the diffusion coefficients also increase.

The highest values for resinoid from leaves and fruits are calculated at 70°C ($9,65 \cdot 10^{-9} \text{ cm}^2/\text{s}$ and $8,61 \cdot 10^{-9} \text{ cm}^2/\text{s}$), respectively, and for concrete extraction from leaves and fruits – at 40 °C ($9,86 \cdot 10^{-9} \text{ cm}^2/\text{s}$ and $8,33 \cdot 10^{-9} \text{ cm}^2/\text{s}$), which could be explained by the facilitated diffusion. The differences in the values of the diffusion coefficients for the two studied aromatic products are due to the different structure of the plant organs and the applied solvent.

In our previous work we have obtained the different values of diffusion coefficients for the aromatic products from leaves and fruits from hawthorn (*C. monogyna*), for example resinoid - at 70 °C ($9,02 \cdot 10^{-8} \text{ cm}^2/\text{s}$) and 60 °C ($17,77 \cdot 10^{-8} \text{ cm}^2/\text{s}$), respectively and concrete – at 30 °C ($8,93 \cdot 10^{-8} \text{ cm}^2/\text{s}$) and 40 °C ($9,49 \cdot 10^{-8} \text{ cm}^2/\text{s}$) [11].

Compared to the values of diffusion coefficients cited in the literature for other plant materials, our results appear to be lower by one order, for example – concrete from lavender (*L. angustifolia*) flowers ($42,7 - 82,6 \cdot 10^{-9} \text{ cm}^2/\text{s}$), pine (*P. sylvestris*) needles ($33,9 - 50,8 \cdot 10^{-9} \text{ cm}^2/\text{s}$), etc. [2, 6, 7].

These results are connected to the nature and the size of the raw material, the included aromatic substances and the specific conditions of the extraction process – solvent and temperature.

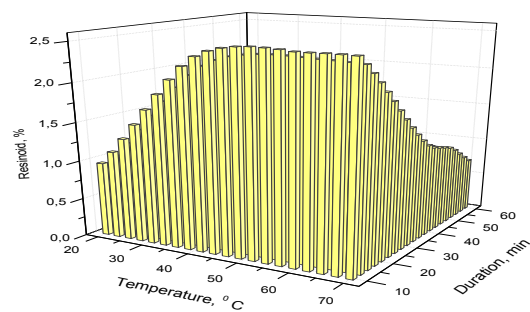


Fig. 1. Content of resinoid from leaves.

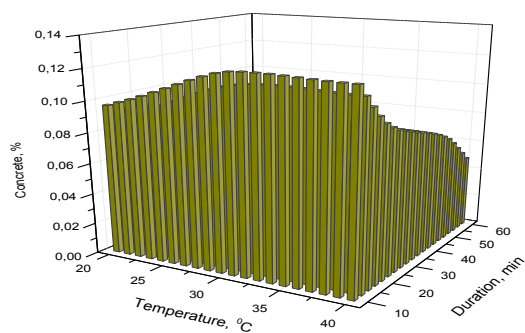


Fig. 2. Content of concrete from leaves

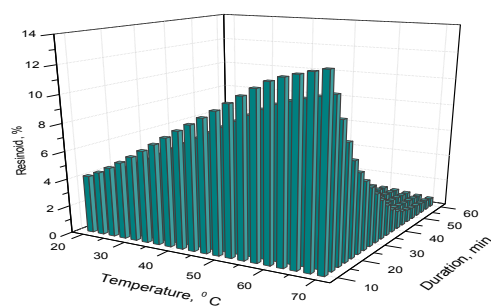


Fig. 3. Content of resinoid from fruits.

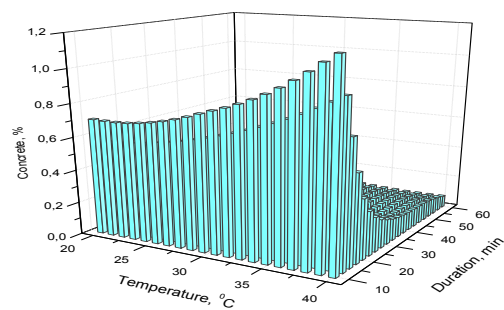


Fig. 4. Content of concrete from fruits.

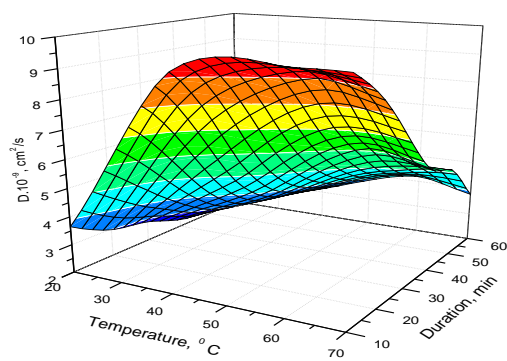


Fig. 5. Diffusion coefficients of resinoid from leaves.

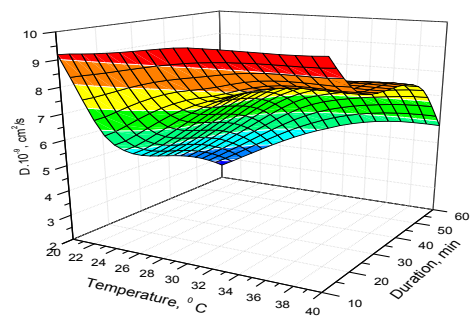


Fig. 6. Diffusion coefficients of concrete from leaves.

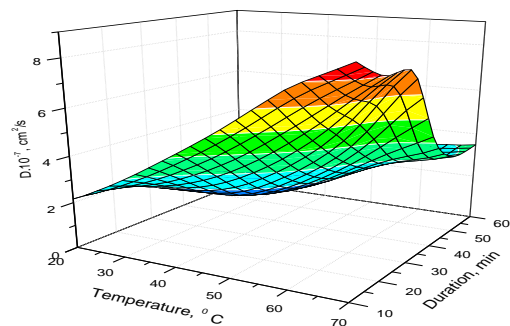


Fig. 7. Diffusion coefficients of resinoid from fruits.

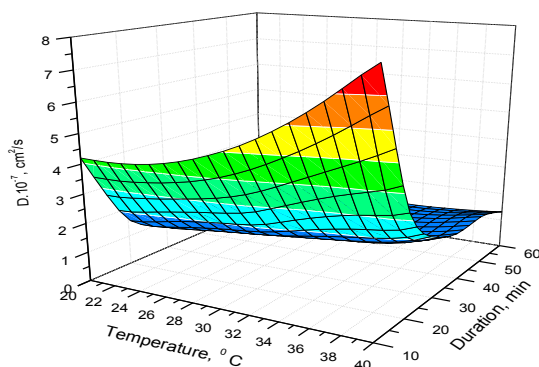


Fig. 8. Diffusion coefficients of concrete from fruits.

Conclusion:

The highest values of coefficient of diffusion with respect to resinoid extraction from leaves and fruits from hawthorn (*C. orientalis*) are calculated at 70°C ($9,65 \cdot 10^{-9} \text{ cm}^2/\text{s}$ and $8,61 \cdot 10^{-7} \text{ cm}^2/\text{s}$), respectively and with respect to concrete extraction from leaves and fruits – at 40 °C ($9,86 \cdot 10^{-9} \text{ cm}^2/\text{s}$ and $8,33 \cdot 10^{-7} \text{ cm}^2/\text{s}$).

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TECHNOLOGY REGYMES OF DISTILLATION PLANTS AS PROCESS CORIANDER

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Abstract: A comparative analysis of the regime parameters used in distilleries processing coriander in Russia and Bulgaria. Calculated specific consumption of steam and cooling water relative to feedstock for periodic equipment used in Bulgaria in a different capacity of the distillation apparatus and to D: H (1:1 and 1:2).

Keywords: distilling oil from fruits of coriander, distillation

INTRODUCTION:

Essential oil plants, and consequently essential oils have long been a man and used for medicinal purposes and as a flavoring [5, 10, 13].

For the production of essential oils are used in Russia approximately 40 species of aromatic materials as one of them it is a leading manufacturer such as coriander oil - 90% and Sage - 75-80% of total oil production [8, 13].

Coriander is an annual herb of the family *Apiaceae*. Essential oil is contained in all parts of the plant, but for industrial processing is obtained by steam distillation of ripe fruits of coriander [5, 6, and 9]. In them it is 0,2 to 2,2% depending on the origin, varieties and cultivars. For example, coriander from Morocco 0,2 to 0,6% essential oil from Argentina from 0.3 to 0.8%, Russia 0.8 to 2.2% [6].

Essential oil is used in perfumery or isolation linalol, which is a raw material for obtaining many flavoring substances. Oil is used in aromatherapy massage, baths and inhalations [6].

In [11] is determined the energy efficiency of distillation processes for color and leaf material processed in Bulgaria. Technological regimes are presented for processing color of roses (water-steam distillation), lavender petals, pine needles and grass, geranium (steam distillation) with a volume of the distillation apparatus 5 m³. For processed commodities is fixed cost of steam and cooling water using 2 and 4 pieces distillery distilling apparatus in one cycle. It is determined the specific heat consumption i.e. proportion of the use of steam compared to the amount of raw material required for the cycle: a pink color – 2, 3; lavender petals – 1,8; pine needles – 1,2 grass and geranium – 2,8. It is estimated proportion of the cost of cooling water used in condenser-cooler to the quantity of processed raw material needed to cycle: a pink – 1,3; lavender petals – 0,795; pine needles – 0,788 and geranium grass – 1,325 [11].

Akteryan [2] is analyzed the energy efficiency of distillery processing of pink, lavender inflorescences and coniferous doing, holding the distillation apparatus 5 m³. The author states: specific fuel glowing vapor (kg steam / kg raw material), heat distillation (MJ heat / kg raw material) and the cost of cooling water in condenser-cooler (kg water / kg raw material).

In the processing of raw materials essential oil in certain technological regimes are differences, due to the type of distillation apparatus - periodic and continuously operating,

the type of distillation (water-steam and steam), but also the technological parameters of the distillation process (speed, temperature distillate and duration of the process).

In [12] is presented the energy efficiency of distilleries processing muskatna Sage in Bulgaria and Russia. It is determined the specific heat consumption and total cost of steam for moving, periodic and continuous operating units. On the base of the comparison made is determined that it is more appropriate use of continuously operating apparatus in comparison with others. But market conditions in Bulgaria it is appropriate to use mobile and stationary devices periodically.

There are no data representing comparisons of different technological regimes used for processing fruit of coriander in Bulgaria and Russia.

THE PURPOSE

The purpose of this paper is to compare the modes of processing of coriander in periodic and continuous operating apparatuses at distillery used in Russia and Bulgaria.

In Bulgaria, the extraction of essential oil used fruit of coriander with the following procedure: distillation is carried out periodically in active devices, such as fruits intact or pre-ground [5]. In practice, with a view to better utilization of raw materials in a market economy is now appropriate to use distillation apparatus with a smaller capacity (1 and 2 m³) than traditional (5 m³).

To extract the essential oil of coriander in Russia in 1952-1953 years was used periodic sets [9]. Such devices are inexpensive performance and had a relatively large volume (2-4 m³ - for devices without stirring the raw material and 6 m³ for apparatus with a stirrer) high cost of steam, water and labor for their operation [1, 8].

Following development Aleksievi eterichnoekstraktionsen plant design is available continuously operating apparatus for processing ITR coriander consisting of a vertical cylindrical tank set (column) with a volume about 10 m³ [9].

Subsequently it was proposed and implemented in industry more advanced continuously operating apparatus for obtaining essential oil from grain raw materials - and Ponomarenko Pokolenko. The unit is a separate column with a height of about 10 m, diameter 2 m, the unit volume is 29 m³, and the working volume 21,3 m³ [8, 9].

For small and medium enterprises with different forms of ownership arises an urgent need to create no less-and emki metoloemki a universal input apparatus productivity 1-2 t / h. To this end, the possibility has been investigated for use in processing of fruits of coriander in one of the basic devices - NDT, which is used primarily for processing of color-grassy material by steam distillation method [7].

In 1968 [3] was proposed a method of processing grain aromatic raw materials, especially coriander in hot steam.

There are no data representing comparisons of different technological regimes used for processing fruit of coriander in Bulgaria and Russia.

MATERIALS AND METHODS:

Periodic distillation apparatus used for processing of fruits of coriander in Bulgaria has the following parameters: 1 m³ in place around 300 kg of ground, speed of 8-10 % distillation, distillate temperature of 37-45 °C. The process for crushed fruit is 2,5-3 h. In the deep intermediate apparatus used grids [10].

For processing are received: - speed of distillation 10 % distillate temperature 41 °C and the duration of the 3 h.

In Russia, is mainly used continuously operating apparatus and Ponomarenko Pokolenko with the follow parameters:

Output of oil – 0,8221 percent; productivity of 1 m³ of working volume of the unit - 480 kg/h; Oil content in distillate output – 0,0111%, electricity consumption per 1 kg of oil - 0,082 kWh; residence time raw material in the apparatus - 28-30 min [9]. During the processing of fruits of coriander in a continuously operating apparatus (NDT) defined the following optimal regimes for the work:-performance - 1,5 t/h; - fuel vapor - 0,55 t/h;-water consumption - 5 m³/h; -Temperature of the distillate - 40 °C [7].

Table 1 Parameters of periodically operating machines for processing of fruits of coriander

Parameters	Periodical operating distillation apparatuses					
	Ratio D:H – 1:1			Ratio D:H – 1: 2		
	1m ³	2m ³ [4]	5 m ³	1m ³	2m ³	5 m ³
	Bulgaria					
Productiveness, kg/h	100	200	500	100	200	500
Temperature of the distillate, °C	41	41	41	41	41	41
Consumption of steam, kg/h	114,1	227,7	567,4	115,0	229,0	570,1
Consumption of cooling water kg/h	24,6	49,2	122,9	24,6	49,2	122,9
Duration of distillation,min.	180	180	180	180	180	180
Specific consumption of cooling water kg water/kg raw material	0,246	0,246	0,246	0,246	0,246	0,246
Specific consumption of steam, kg steam/kg oil	76,1	75,9	75,7	76,7	76,4	76,0

The data presented in Table 1 shows that the specific consumption of steam relative to the resulting oil is slightly higher when using periodic distillation apparatus with respect to D: H = 1:2. This is probably due to the large volume of the distillation apparatus. Impressed significantly lower specific consumption of steam in the apparatus with a larger volume - 5 m³ in comparison with others, the lowest in terms of equipment using D: H = 1:1, i.e. more appropriate to use devices with greater capacity than smaller. Cooling water consumption increased in proportion to the increase of the distillation apparatus, set at the same temperature of the distillate. Specific consumption of cooling water is constant, from which it can judge that it is more appropriate to use the distillation apparatus with a larger volume.

Table 2 Parameters of a continuously operating apparatus for processing fruit of coriander

Indices	Continuously operating apparatus		
	Pomarenko and Pokolenko [8,9]	NDT [7]	Steaming apparatus [3]
Productiveness, kg/h	10 000	1500	57
Temperature of the distillate, °C	41	40	-
Consumption of steam, kg/h	492,61	550	80
Consumption of cooling water kg/h	122,92	500	-
Duration of distillation,min.	28-30	-	-
Specific consumption of heat, kg steam/kg raw material	0,61	0,37	1,4
Specific consumption of steam, kg steam/kg oil	41,5	-	-

Table 2 presents the results from the processing of coriander in continuously operating apparatus (Pomarenko-Pokolenko, NDT and Steaming apparatus), as shown by the data displayed is the lowest specific consumption of heat in NDT apparatus compared with the other two. This apparatus is used primarily for processing of color-grassy material, but attempts have been made for the processing of grain and raw material mainly coriander. In Fig. 1 provides a diagram reflecting the specific heat consumption (kg steam / kg raw material) for periodic distillation apparatus with varying capacity of the unit and to D: H. As seen from the figure the greater the specific consumption of heat for distillation apparatus to D: H = 1:2 compared to typically used in practice apparatus with respect D: H = 1:1, which is due to the larger volume of the apparatus. Impressed that the growth of the distillation apparatus specific steam consumption decreases, therefore, is much more appropriate use of equipment with greater capacity.

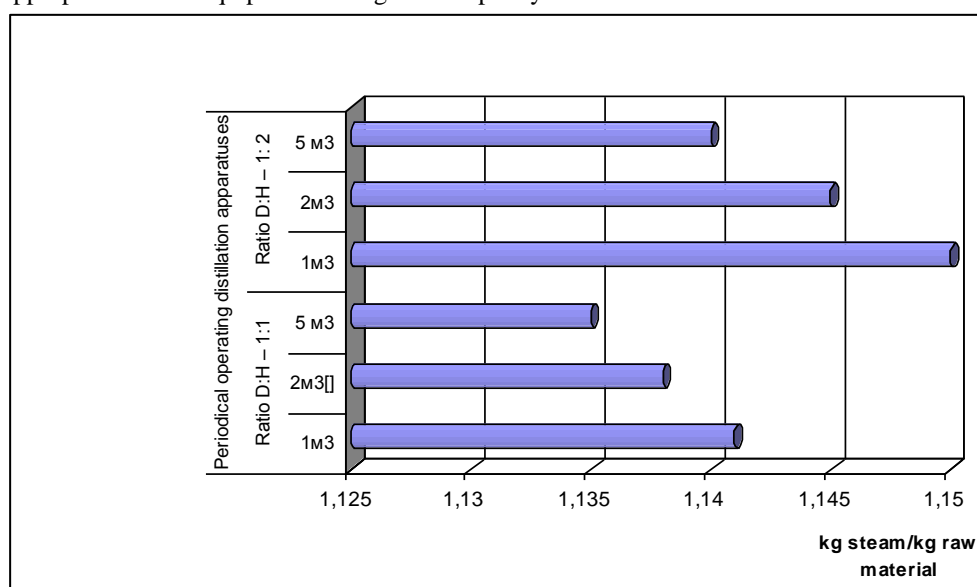


Fig. a Specific consumption of heat in the volume of the distillation apparatus

CONCLUSION:

On the basis of the data for different process modes for processing fruit of coriander can say that it is appropriate use of continuously operating apparatus (the apparatus is performing NDT - primarily used for treatment of grass-colored material) in compared to regular force. If necessary, the use of periodic distillation apparatus is better to use machines with greater volume and with a D: H = 1:1, as practiced now in Bulgaria.

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PHYSICOCHEMICAL CHANGES OF WALNUT OIL (JUGLANS REGIA L.)

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Abstract. Among the representatives of oil crops, fruits of walnut (*Juglans regia* L.) are differ by the richest in lipid content 65...75%, proteins 15...17%, vitamins, minerals, polyphenolic compounds etc. Processing of nuts along with the organization of industrial production of walnut oil is among the most promising directions for the national economy of the Republic of Moldova. Walnut oil is a product of an exceptionally high nutritional value, with high content of biologically active substances, including essential unsaturated fatty acids, the ω -3 and ω -6. During storage, walnut oil is extremely unstable, subject to physical and chemical changes with the accumulation of primary and secondary oxidation compounds.

Keywords: walnut (*Juglans regia* L.), oil, saturated and polyunsaturated fatty acids, oxidation

Walnut fruit (*Juglans regia* L) is one of the most valuable oleiferous raw materials from nutritional and health benefits point of view. Walnut cultivation is possible in geographic regions with specific climatic conditions and soil composition. In Europe walnut cultivation is developed in France, Italy, Spain, Romania, Bulgaria, Hungary and Moldova. The cultivation of walnut is also developed in the U.S.A and China. In the last decade the Republic of Moldova has enlarged the walnut plantations, increased walnuts harvesting and export. Results of study on walnut quality have shown that the nutritional components of this raw material are characterized by significant biological and dietetic value. Walnut kernels contain between 60...75% lipids. Walnuts provide appreciable amounts of proteins 15...18%, carbohydrates 12...16%, fiber 1.5...2.0% and minerals up to 1.8 %. Walnut oil contains approximately 10 % saturated, 18 % monounsaturated and 72 % polyunsaturated fatty acids [7]. Compared with most other nuts, with contain mostly monounsaturated fatty acids (MUFA), walnuts are highly enriched in omega-6 (ω -6) and omega-3 (ω -3) polyunsaturated fatty acids (PUFA), which are essential dietary fatty acids. In fact, MUFA and PUFA composition can influence various physiological and biochemical processes, including blood pressure regulation, glucose metabolism, lipids metabolism, platelet aggregation, and erythrocyte deformability [1, 2].

Taking into account the importance of lipids and their changes on the quality of walnuts and walnut products, the aim of our research was to estimate the oxidative changes of oil extracted from walnuts during storage.

Research started with a comparative analysis of chemical compositions of walnut oils produced in France, Hungary, Italy, Spain and oil from Moldova walnuts.

The data presented in table 1 show that the composition of fatty acids in the oil from walnuts produced in European countries [7] and walnut oil from Moldova according to normative documentation [9] is practically identical. For example, analyzed oils are rich in unsaturated fatty acids, content of linoleic acid (ω -6) equal with 53...70%, oleic acid from group of MUFA acids presents in quantity of 14...30%.

Table 1. Fatty acid composition of walnut oil (percent of total fatty acids)

Symbol	Fatty acid	Walnut oil from countries				
		France	Hungary	Italy	Spain	Moldova
C14:0	Myristic	-	-	-	-	0.1
C16:0	Palmitic	6.5-7.3	5.8 - 7.7	7.3 -8.1	7.1 -7.5	2.9 - 7.0
C16:1	Palmitoleic	0.1	0.1	0.1	0.1	0.1
C17: 0	Margaric	0.1	-	0.1	0.1	-
C18:0	Stearic	1.7 - 2.9	2.1 - 2.2	2.2 - 2.9	1.9 - 2.8	0.9 - 2.5
C18:1	Oleic	15.1- 18.9	17.4 - 22.2	14.5 -15.3	14.3- 19.2	14.0 - 30.0
C18:2	Linoleic	57.4 - 64.3	58.3 - 60.8	60.2 - 63.1	57.6 - 62.5	53.0 - 70.0
C18:3	Linolenic	11.3 -15.4	10.8 - 11.6	11.8 -14.3	12.4 - 13.2	9.8 - 13.0
C20:0	Arachidic	0.1	0.1	0.1	0.1	-
C20:1	Eicosenoic	0.2 - 0.3	0.2	0.2	0.2	1.7
C22:1	Docosenoic	-	-	-	-	3.8

Compared with other edible vegetable oils (olive, sunflowers, soybeans oils), the walnut oil is the only oil containing linolenic acid (ω -3) over 10%. In compared oils the ratio of polyunsaturated to saturated fatty acids varies between 7.2 ... 7.8 (table 2).

Table 2. Ranges of saturated, monounsaturated, and polyunsaturated fatty acids in walnut oil

Fatty acid	Walnut oil from countries				
	France	Hungary	Italy	Spain	Moldova
Saturated	8.5 – 9.7	8.3 – 9.9	10.2 – 10.5	9.5 – 10.2	4.1 - 9.8
Monounsaturated	15.4 – 19.1	17.7 – 22.5	14.8 – 15.5	14.6 – 19.5	14.3-19.2
Polyunsaturated	71.2 – 75.5	69.1 – 72.4	74.3 – 74.9	70.7 – 75.2	62.8 - 75.7
Polyunsaturated / Saturated, ratio	7.8	7.8	7.2	7.5	7.7

Presented data denotes that walnuts oils have a high biological value independently of country of their production due to the PUFA content. However, walnut oil is unstable, and during storage it starts oxidizing in a short time. The PUFA acids are first of all oil components that are exposed to oxidation.

The rate of oxidation of fatty acids increases in relation to their degree of unsaturation. The relative rate of autooxidation of oleate, linoleate, and linolenate is in the order of 1:100–1200:2500 on the basis of oxygen uptake and 1:12:25 on the basis of peroxide formation [3, 5].

According to presented data, the initiation of oxidation reaction of linolenic acid starts after 1.34 hours of oil extraction. The linoleic acid oxidation occurs after 19 hours (table 3). The rate of oil oxidation is accelerating in foods with increased water activity, in

presence of metals of variable valence, under the influence of prooxidants and other factors [4, 8].

Table 3. Induction period and relative rate of oxidation for fatty acids at 25 °C [3]

Fatty acid	Number of allyl groups	Induction period (h)	Oxidation rate (relative)
Stearic, (C ⁰ ₁₈)	0	-	1
Oleic, (C ¹ ₁₈)	1	82	100
Linoleic, (C ² ₁₈)	2	19	1200
Linolenic, (C ³ ₁₈)	3	1,34	2500

In addition, the highly polyunsaturated nature of walnut lipids makes them prone to oxidative instability. Despite the oxidative problem, oil extracted from walnuts is sold and enjoyed as high-quality specialty oil in many parts of the world. The three major components involved in lipid oxidation are unsaturated fatty acids, oxygen and water [4].

Autooxidation reaction of lipids in walnut kernels is initiated by biochemical reactions, via specific actions of the enzymatic system, including with involvement of oxidases. Oxidation of oil extracted from walnuts is carried out by biochemical and pure chemical reactions. During storage the walnut oil is subject to autooxidation by chemical reaction, catalyzed by radiation, metal of variable valence and water present in the oil composition. It is considered that in the beginning stage of autooxidation process the primary compounds of reaction - peroxides and hydroperoxides are formed. Primary compounds are unstable. "Shelf life" of hydroperoxides doesn't exceed 1.0 s.

Rapid changes of hydroperoxides are followed by the extension of autooxidation process which leads to accumulation of secondary compounds of oxidation. These compounds include aldehydes, ketones, alcohols, low-molecular weight fatty acids, volatile compounds and others. The decomposition of hydroperoxides to secondary compounds is shown in figure 1 [6]. The mechanism of process consists in bond cleavage of oxygen molecules in hydroperoxide radical and formation the intermediate free radicals of hydroxyl and alkoxy ($R-O-O-H \rightarrow RO^\bullet + HO^\bullet$). After that these free radicals by chemicals reactions are transformed in secondary compounds and generate some new free radicals of alkyl type R^\bullet .

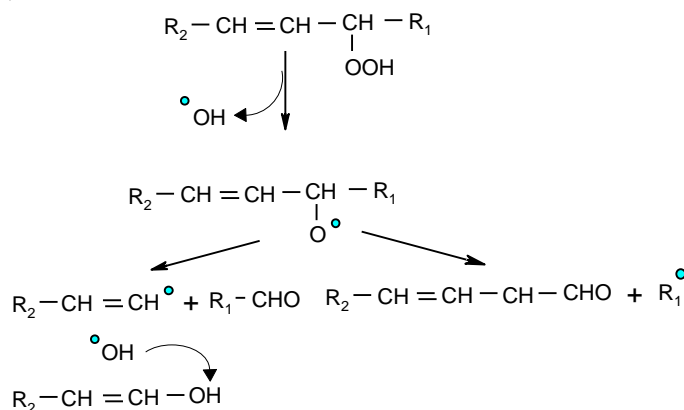


Fig. 1. The scheme of reactions of hydroperoxides decomposition and secondary compounds formation in autooxidation process of lipids

Based on previously mentioned formation of primary and secondary compounds, the autooxidation of lipids in oil represents one integrated process. These chemical reactions are interrelated and reflect the dynamics of the overall process. The generalized scheme of lipid autooxidation process is presented in this paper (fig.2). It is an attempt to explain the dynamics of process of polyunsaturated fatty acids autooxidation in walnut oil.

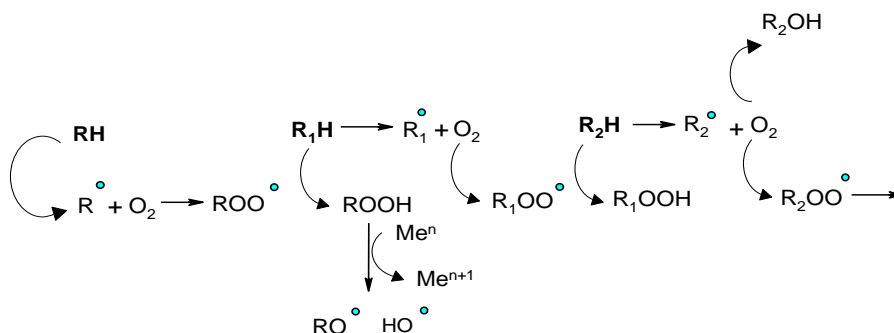


Fig. 2. The scheme of process of polyunsaturated fatty acids autooxidation in walnut oil: RH; R₁H; R₂H - polyunsaturated fatty acids; ROO• - peroxide radical, ROOH - hydroperoxide, R•, R₁•, R₂• - fatty acids radicals, RO•; HO• - alkoxy radical and hydroxyl; Meⁿ; Meⁿ⁺¹ - metals of variable valence.

According to the presented scheme, the PUFA autooxidation is an irreversible process. The reactions of this process occur consecutively. The hydroperoxides are intermediate compounds in the process of fatty acids autooxidation. They execute the function of substrate in subsequent reactions that lead to their transformation into secondary compounds of oxidation process. The acceleration of hydroperoxides decomposition occurs by involving in reactions the metals of variable valence which brings to formation of new radicals such as alkoxy (RO•) and hydroxyl (HO•). The next stage of the process is activated, on the one hand, for involving in reactions of new fatty acids (R₃H) and, on the other hand, accumulation of final secondary compounds. Such oxidative changes of PUFA are continuous [6].

The conformity of the real process of oil oxidation with presented scheme was verified by experimental research. For this purpose the walnuts harvested in 2011 were used. The oil was obtained by pressing the walnut kernel, filtered and placed in sealed bottles for 7 months of storage at + 4...6°C temperature. During storage the dynamics of oil autooxidation process was studied. There were determined modifications of PUFA content in oil, indices of peroxide value (PV), secondary compounds concentration by 2-thiobarbituric acid value or TBA test. The changes of these indices in oil during storage are shown on Figure 3.

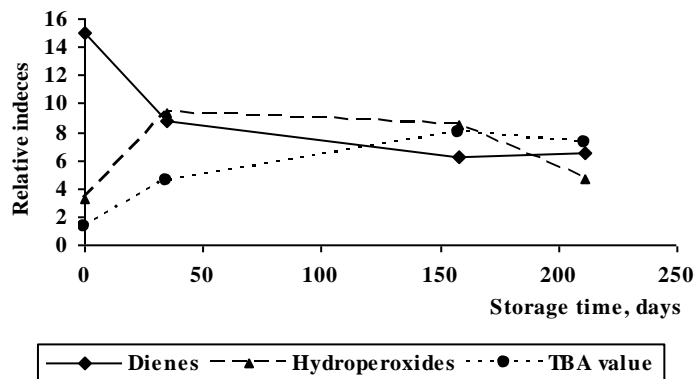


Fig. 3. Dynamics of primary and secondary compounds formation in the autooxidation of linoleic acid in walnut oil during storage

It was found that during storage of oil the PUFA concentration has been permanently decreased at a rapid rate in the first 30 days of storage. The process of hydroperoxides accumulation has been variable. Initial concentration has increased; reached the maximal value and after 150-200 days the hydroperoxides concentration has constantly decreased. Simultaneously, during oil storage the concentration of secondary compounds has been steadily growing.

Character of hydroperoxides concentration changes shows that these compounds are intermediates in PUFA oxidation reaction and represent the substrate of reactions for secondary compounds formation. It was also found that PUFA autooxidation in walnut oil takes place by consecutive reactions in which the hydroperoxides are intermediate compounds. Generally, one of the possible pathways of PUFA oxidation process in oil can be presented as follows:



where: **RH** - polyunsaturated fatty acids,

ROOH - hydroperoxides,

RCHO - aldehydes,

K₁ - rate constant of reaction of hydroperoxides formation, 1/h

K₂ - rate constant of reaction of secondary compounds formation, 1/h

The rate constants of reactions depend on several internal and external factors. Complexity of the oxidation process consist in transformation of isomeric configuration cis/cis of PUFA in configuration cis/trans, trans/cis of hydroperoxides, which generate not only aldehydes and ketones, but alcohols, low molecular weight fatty acids, volatile compounds. Walnut oil contains the natural antioxidant γ -tocopherol in quantities of 250...500 mg/kg. [7]. However, γ -tocopherol in these quantities is not possessed the necessary activity to reduce the oxidative degradation of walnut oil.

In conclusion, walnut (*Juglans regia* L.) is characterized by increased biological and nutritional value. Autooxidation of polyunsaturated fatty acids (PUFA) in walnut oil is an irreversible process. Formation of primary and secondary compounds occurs simultaneously with consecutive reactions in which hydroperoxides are intermediate compounds. Formation of secondary compounds is dependent on the concentration of hydroperoxides.

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TECHNOLOGY OF SEMI-FINISHED PRODUCTS ON THE BASE OF QUINCE FOR WHIPPED SWEET DISHES

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Abstract: Expediency of using fruit of quince in the technology of semi-finished products for sweet whipped dishes with foam structure of high nutritional value was proved in materials of the article. Regularities of the process of acid hydrolysis of cornel semi-finished products in the presence of acidulous puree were determined.

Content of polyphenol composition of different varieties of cornel was learnt, comprehensive quality indexes of semi-finished products were determined.

Key words: functional products, technology, quince, cornel, semi-finished product.

One of the most important conditions for human health, capacity for work, resistance to disease and life expectancy is a well-balanced ultimate nutrition, which includes consumption of biologically active products. Semi-finished products based on fruits and vegetables are the most promising in terms of functional properties and applications in manufacturing foodstuff. Semi-finished products of high readiness are preferable from their diversity, that is purees, pastas, homogenates, juices prefer.

Semi-finished products based on vegetable primary materials are purees of primary materials and they contain a considerable amount of substances useful to a human being, including biologically active ones, in particular vitamins, minerals, dietary fiber, dyes, mono-disaccharides, pectin and inulin –containing substances.

At present level of development of pharmaceutical and food industries, a great importance is given to obtaining natural inulin and oligofructan for its further use as a source of monosaccharides.

One of the sources of plant materials for the production of semi-finished products for the desserts is fruits of quince. Due to the high content of bioactive compounds (polyphenols, vitamins, pectin and mineral substances) quince is recognized as a valuable food of a human being. Among other fruits, quince, first of all, is distinguished with a high content of pectin. Pectins are considered an effective way to treat diabetes. With the technological position, pectins are used as functional and technological ingredients that possess structure-specific properties.

Based on the obtained literature data and a series of earlier experiments, organoleptic evaluation of products such as sour agents of natural origin have been selected, which are soup and puree of black chokeberry and puree of cornel. The choice was due to the chemical composition and properties of plant primary material, content of minerals and vitamins. Fruits of cornel are distinguished with pleasant taste, aroma, presence of nutrients, as well as they are rich in biologically active compounds, due to which cornel has healing properties.

Organoleptic evaluation of various forms of cornel fruits indicates that that they have a sour-sweet taste and pleasant aroma, which depends on the content of essential oils.

Fruits of some varieties (Eugene, Elegant, Yantarnyi, Yelena) have a sweet-sour taste, content of glucose and fructose in them reaches 85- 95% of the total content of sugar.

A large amount of sugar and high acidity from 1.0% to 2.5% makes fruits of cornel a promising material for use in diets. Content of fibers in an average amounts to 1.5 -1.9%, and pectin substances up to 0.8 -1.18%. Fruits of cornel are a source of biologically active substances - bioflavonoids, presented in groups: anthocyanins, catechins, flavonols, and leucoanthocyanins.

We presented a goal of studying the content of polyphenols in varieties of cornel common in Donetsk region. Polyphenolic compounds of cornel are presented with catechins, leucanthocyanins, anthocyanins, and flavonols. A number of catechins in fruits of different forms varies slightly and it amounts to 282,0-370,0 mg%, except for the variety of Syretsky that contains 188.0 mg%. A number of anthocyanins in fresh cornel fruit amounts to 674% ... 850mg, flavonols 62,5-87,8 mg%. The data is presented in Table 1.

Table 1. Polyphenols of fruits of different varieties of cornel (in-equivalent to primary material, mg%)

Form, variety	Catechins	Flavonols	Leucoanthocyanins	Anthocyanins	
				pulp	skin
Lukyanovskiy	252,0	112	212,0	102,0	707,0
Elegant	310,0	72,0	110,0	102,0	773,0
Syretsky	188,0	87,0	175,0	36,0	802,0
Vydubetsky	82,0	72,5	180,0	110,0	766,0
Eugenia	317,0	75,0	187,0	70,0	766,0

Analyzing the data it is possible to say that the content of leucoanthocyanin in fruits is less than catechins, that is from 112.0 to 212.0 mg%. The greatest number of leucoanthocyanins is contained in the varieties of Lukyanovskaya and Eugene. Flavonols in different varieties of cornel vary from 72.5 mg - 87.0% to 112 mg% in the variety of Lukyanovskaya.

Taste features of cornel are defined by content of carbohydrates, organic acids, tannins, which increase acid. At the same time, other substances and their correlation significantly influence formation of flavor characteristics of cornel fruits.

As a source of non-traditional plant materials, a special attention is drawn to black chokeberry, which is widespread in Ukraine. Black chokeberry is superior to other berries by its economic efficiency. It is resistant to diseases; its fruits are not showered and characterized by high and stable yields.

Fruits of different varieties of black chokeberry have sweet and sour taste due to concentration of organic acids and tannins. Chemical composition of black chokeberry has been studied in detail, with particular attention to vitamin composition of primary materials: vitamin C is 50 - 170mg/100g, vitamin B is 0.02 mg/100 g, vitamin E is 0.5 mg/100 g, carotenoids are 7.5 - 9 ,0 mg/100g. Phenolic compounds with P-vitamin activity is from 2000 to 150 000 mg/100g, tanning substances is 0.3- 0.6%, sugar is 6.2 -10.8%, and organic acids are 0.7 - 1 3%, pectin substances are 0.5 -0.8%. It was established that black chokeberry; due to biologically active substances in its composition have antioxidant and

immunomodulatory effects. Catechins of Black chokeberry bind and excrete radioactive substances from a body, which are metal ions (such as cesium, strontium).

Such a rich composition of bioactive substances makes black chokeberry a promising material for use in the technology of many dishes. Black chokeberry is used in the processing industry; it is used for manufacturing conserve, jam, comfiture, syrup, extract, juice, compote, non-alcoholic drinks, wine, brandy, liqueur, balsam and jelly for the confectionery industry. High medicinal properties and nutritional value explain its use in the manufacture of canned food diet.

In order to study parameters of acid hydrolysis of quince poly-fructans and establish regularities of the process of accumulating fructose, possibility of using natural plant materials has been studied.

As a control, conditions of acid hydrolysis of the quince model system in a 1% solution of citric acid were selected. The process of hydrolysis was controlled by the content of fructose in the mixture. The experimental data are shown in Figure 1.

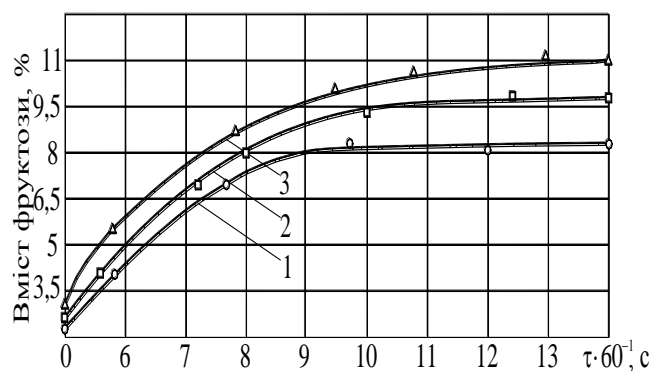


Fig. 1. Effect of herbal additives on the kinetics of hydrolysis of quince poly-fructans: 1 – black chokeberry puree; 2 - cornel puree; 3 - control.

The experimental data evidences that the process of poly-fructans hydrolysis in an medium which is created by acidic agents is different from the process that takes place in aqueous solution with a fixed acidity. It was established that with introduction of black chokeberry puree in the model system it was formed 7.0-8.0% of fructose, and with introduction cornel puree it was formed 9.5-11.0% of fructose.

Thus, it can be stated that presence of organic acids of cornel fruit contributes to intense accumulation of poly-fructans at the quince hydrolysis.

In order to determine optimal conditions for hydrolysis of poly-fructans model system "quince-cornel," we have carried out studies of their acid hydrolysis. The content of cornel puree was 10, 20, 30% from the amount of quince puree. The hydrolysis process was controlled by the content of fructose in the mixture. The experimental data are shown in Figure 2.

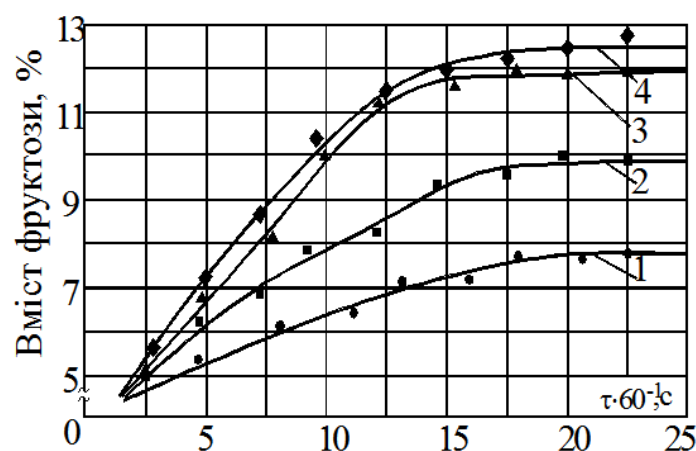


Fig. 2. Effect of cornel puree on the kinetics of hydrolysis of quinces poly-fructans: 1,2,3 - content of cornel puree 10,20,30% respectively, 4 - control.

Thus, from the experimental data we can conclude that with increase of cornel puree amount up to 28.0 - 30%, fructose is the most actively formed in the system.

Optimal parameters of the process of acid hydrolysis of quince poly-fructans are the following: time of hydrolysis is 17 -20 minutes, the acidity of the medium is 3.3-5, temperature is 87 ... 90 C°.

A particular attention should be paid to the fact that when adding cornel puree to the semi-finished product on the base of topinambour in the amount of 28 ... 30%, the product is enriched with food dyes of anthocyanin origin. Presence of anthocyanin pigments in the amount of 220 - 240mg/100g gives a possibility to obtain natural dyes, which have high biological value and nonhazardous for health. Considering the high cost of natural dyes, we believe that we must continue to seek stable and cheap natural dyes of natural origin.

The obtained data allow predicting creation of culinary products with functional load, especially sweet dishes and confectionery, without the use of artificial acidic substances.

Analysis of the existing technological schemes of manufacturing fruit and vegetable purees indicates multi- staging and complexity of the technological process. Taking into consideration that the highly specialized food companies, such as bars, restaurants, cafes, pastry shops, processing of fruit and vegetable primary materials does not conform the technological process, thus creation of semi-finished products based on quince and cornel is expedient.

Semi-finished products based on vegetable primary materials, especially multi-component ones, are complex systems, which are depleted in a variety of chemicals. Under the influence of technological factors, such as blending, heating, grinding, and whipping a variety of chemical processes occur: hydrolysis, fermentation, condensation, oxidation, and reconditioning. Taking into consideration this fact, we deemed it appropriate to learn how to change the organoleptic quality of a semi-finished product based on the quince and cornel, after heat treatment and during storage. The main organoleptic indicators of semi-finished products are shown in Table 1.

Table 1. Organoleptic semi-finished product based on quince and cornel.

Indicators	Characterisric
Appearance, consistency	A homogeneous mass of soft puree, no fibers
Aroma	Pleasant, typical to the used primary materials, free of foreign smell. Aroma of natural cornel fruit.
Taste	Pleasant, sweet and sour
Color	Natural, bright crimson throughout the mass

The derived semi-finished product based on quince and cornel is a creamy, finely divided mass, homogeneous throughout the volume with the pleasant aroma of cornel. The color is bright purple, uniform throughout the mass, the taste is sweet and sour, with the taste of cornel, without foreign flavor, the texture is pasty, and can be easily spread and formed when putting on the surface, and does not flow outward.

Qualimetry methods are widely used for assessing the quality of fruit and vegetable semi-products. We have designed a comprehensive indicator of quality, which reflects the nutritional and biological value, organoleptical, structural - mechanical, and physical - mechanical properties of a semi-finished product based on the quince and cornel. A comprehensive assessment of quality of semi-finished products have shown that the developed semi-finished product has a better quality than its analog in all groups of properties. The overall integrated assessment of quality of the semi-finished product based on the quince and cornel is 7% higher, mainly due to the improved organoleptic and structural and mechanical properties.

Thus, analyzing the obtained data the following conclusions can be drawn:

1. Optimal parameters of acid hydrolysis of quince poly-fructans have been obtained: time of hydrolysis is 17-20 minutes, acidity of the medium is 3.3-5, and temperature is 87 -90C °.
2. Expediency of introducing cornel puree in the amount of 25 - 30%, as an acid agent under acidic hydrolysis have been proved.
3. Biological value and functional properties of cornel makes it use in the technology of hydrolysis expedient.
4. Semi-finished products based on quince and cornel for sweet dishes and confectionery products have high quality indexes.

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ANTIOXIDANT ACTIVITY OF SWEET PRODUCTS WITH ANTHOCYANINS EXTRACTS USE AS A NATURAL FOOD COLORANT

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Abstract. Consumers are increasingly avoiding foods containing synthetic colourants, which lead food industries to replace them by natural pigments, such as carotenoids, betalains, anthocyanins and carminic acid. The objective of this research was to elucidate the influence of the substitution of synthetic colorants (carmoisine) with extracts of grape anthocyanes on the organoleptic, physico-chemical and antioxidant properties of some confectionery products (marmalade, jellies).

Keywords: Antioxidants, natural colourants, grape seeds anthocyanins, confectionery products.

One of the major hazards of this beginning of the millennium is the larger use of various synthetic food additives. Firstly, it includes synthetic colorants, which are present practically in most types of processed food. The danger is even greater when it comes to children who consume, having a small body weight, a mass of sweets, brightly colored, so the contribution of additives per kg of body weight is often dangerous to health, causing various allergic reactions.

Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticancers [1]. Plant extracts obtained from some fruits and vegetables have been reported to be effective antioxidants. In most cases, phenols mediate their anticancer effects by inhibiting all stages of chemical carcinogenesis, initiation, promotion and progression as well as formation of carcinogens from dietary precursors.

Grape (*Vitis vinifera*) is one of the world's largest fruit crops, while grape seed is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins, and 7% complex phenols including tannins, in addition to sugars, mineral salts, etc. Proanthocyanidins of grape seed are a group of polyphenolic bioflavonoids, which are known to possess large pharmacological activities and therapeutic potentials [2,3]. Proanthocyanidins, the major polyphenols found in red wine and grape seeds, have been reported to show cardio protective effects against ischemic reperfusion injury. In addition, grape seeds are rich sources of monomeric phenolic compounds, such as (+)-catechins, (+)-epicatechin, (+)-epicatechin-3-o-gallate, and, dimeric, trimeric and tetrameric procyanidins, which have antimutagenic and antiviral effects. Recognition of such health benefits of catechins and procyanidins has facilitated the use of grape seed extract as a dietary supplement [4].

The objective of this research was to elucidate the influence of the substitution of synthetic colorants (cramoisie) with extracts of grape anthocyanes on the organoleptic, physical-chemical and antioxidant properties of some confectionery products (marmalade, jellies).

Materials and methods

Spectrophotometer measurements were performed by UV-Vis spectrophotometer **Pye Unicam UV4-100 UV-Visible**.

The study group was made a jelly quartz cell size of 10 x 10 mm (working volume of 4 ml), which was placed in the sink-holder device. The measuring process starts automatically after closing the sample compartment (= 546 nm).

The method of determining the ability of inhibiting oxidation activity of hydrogen peroxide (HPSA - hydrogen peroxide scavenging activity).

The ability to inhibit the activity of hydrogen peroxide oxidation (HPSA) is determined according to the method published in NAGULENDRAN et al., 2007 [5].

The principle of the method. The ability to recover hydrogen peroxide is determined by titration method of substitution (the test solution does not come into direct reaction so transformed into a chemical compound, which is then titrated with a solution of known concentration).

Reagents: hydrogen peroxide H_2O_2 (0,1 mM), a solution of ammonium molybdate $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (3%), sulfuric acid, H_2SO_4 (2M), sodium thiosulfate $\text{Na}_2\text{S}_2\text{O}_3$ (5,09 mM), potassium iodide KI (1,8 M), concentrated nitric acid, HNO_3 , distilled water [5].

3. Experimental results

Production of jelly products can not only be considered as a combination of the mechanical action of raw materials. During cooking there is a series of very important physical and chemical changes, which to some extent affect the bioavailability of the raw materials used and the finished product. When cooking, always follow the technology and production conditions in order to get a finished product that meets the quality requirements specified in the regulations, and exhibits antioxidant properties.

Preservation of color - an important indicator of the quality of the product manufactured from natural dye. Change the color speaks of the destruction of natural dyes, which reduces the nutritional value of the product.

Given these conditions it has been determined the optimum pH at which the maximum intensity of the color is preserved and the optimum temperature at which the anthocyanins may be introduced into the product, avoiding their destruction. For marmalade optimum value is the range of pH 2.5-2.7, and the optimum temperature + 60 °C.

The measurements were performed using a spectrophotometer UV/vis "UNICAM", length 1 cm cuvette at wavelength = 546 nm. The results are presented in figure.1.

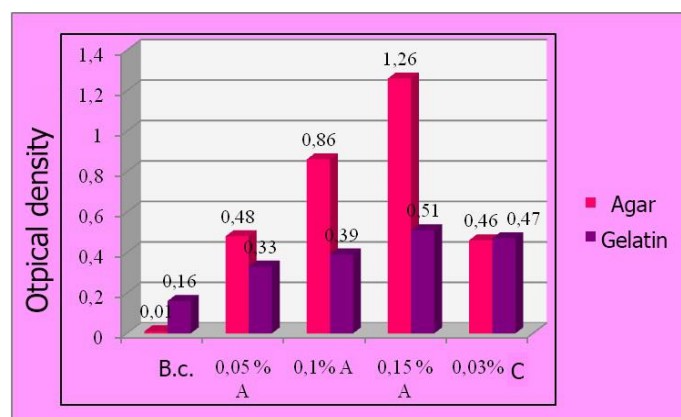


Fig. 1. The study of the intensity of staining jelly gelatin-based and agar, depending on the nature of the dye

The study of intensity jelly products, depending on the nature of paint shows that gelatin products with natural paint color intensity is less than the intensity karmuazinom jam on synthetic gelatin. The intensity color by adding 0.05% anthocyanins equals 0.33 units and adding synthetic karmuazina intensity equal to 0.47 units. For the agar jelly also the color intensity of the product by adding 0.05% solution is almost the natural dye intensity karmuazine sample (0.48 and 0.47, respectively). Thus, marmalade, made on agar with natural dye has a higher color intensity than jam on gelatin. When adding the synthetic dye to the color intensity of the two samples is almost the same marmalade 0.46 and 0.47 units. For the agar jelly, based on natural dye intensity range is between 0.48 1.26 units and for marmalade on gelatin - between 0.33 0.51 units depending on the number of added anthocyanin.

The concentration of anthocyanin affect color intensity can be considered on figure 2.

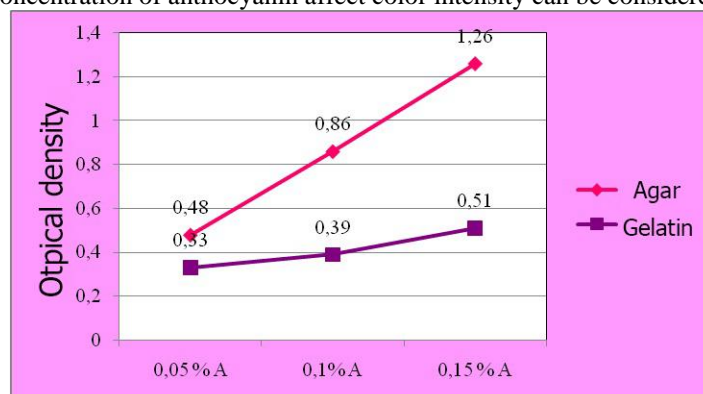


Fig. 2. The study of the intensity of the colors of jelly products depending on the concentration of anthocyanin that were added to the product.

The intensity of the color of jelly products on gelatin and agar grows with increasing concentration of added anthocyanin. Maximum color intensity is observed when adding 0.15% anthocyanin - 0.51 units of samples on gelatin and 1.26 units of samples on agar. The intensity of the color of the samples on agar are 1.5-2.5 times higher than that of samples in gelatin. This can be explained by the fact that initially (even without dye) the

marmalade on agar jelly is a colorless product but marmalade on gelatin has a yellowish color. Therefore, the addition of the dye to the samples on the basis of gelatin, dims out the color of marmalade, and the color intensity of the product falls.

The color intensity is a composite index, which depends on a combination of factors: the nature of the dye, natural ingredients and the concentration of the dye. In order to more accurately describe the intensity of the color of the product is necessary to consider the impact of each factor separately.

Study of changes in the color stability during storage

Anthocyanins - are plant pigments whose color depends on the acidity of the medium. At pH <6 anthocyanins are red variable intensity, more vivid and more dense at pH 1-2, at a pH of 6 - violet at a pH of 8 - blue at pH = 10 - green. Color intensity increases with decreasing pH, thus adding citric acid items increases color stability.

It was found that the acidity of the investigational product is pH = 2.5. In order to determine the color stability over time it has been defined the intensity of the colors of jelly products on the day of manufacture and after 15 days of storage. The measurements were performed in the laboratory on photocolormeter at wavelength = 540nm. The results are presented in figure 3.

The intensity of the color jelly product within 15 days varies slightly. The color of jelly products based on synthetic dyes does not change.

More clearly it can be represented the change of the intensity of the color of jelly products in the diagrams. It can observe changing the the color stability over time, depending on the nature of the dye, the nature of ingredients and the quali of natural dyes.

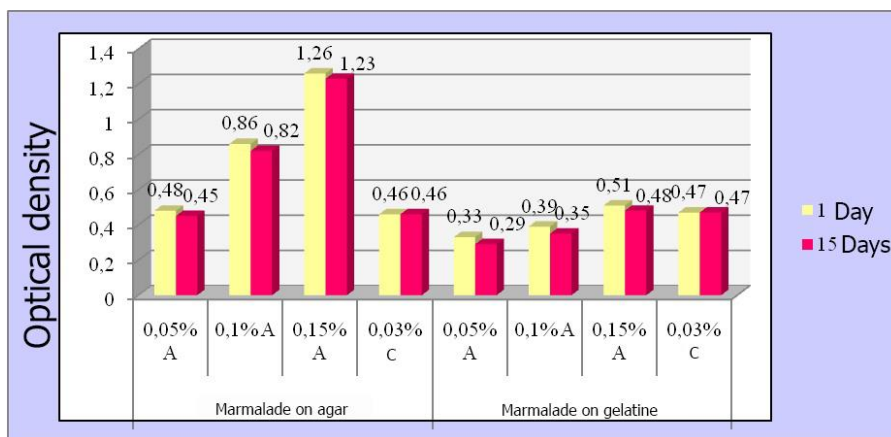


Fig. 3. Changing the color stability of jelly products in time depending on the nature of the dye and natural ingredients.

Undesirable property of natural dyes is to reduce the color of the product during storage. Regardless of the nature of the ingredients in both cases, the products with natural dyes decrease the color intensity of the product for 15 days. But this reduction is not as significant. In the study jelly products reducing the intensity does not exceed 0.04-0.06 units.

If in samples with natural dyes color intensity decreases, then in the specimen with a synthetic dye color intensity of the product over time will not change at all. In jelly products

prepared on the basis of gelatin it is observed the greatest decrease of the intensity by 0.05 units (at 0.05% content of anthocyanin). The highest intensity of staining characteristic of agar jelly, where the color intensity equals 1.26 and in storage decreases by only 0.03 units.

Thus, the products of synthetic dyes can be considered more stable. But the use of natural dyes is very important due to their many favorable properties. In addition, the charts data suggest that if a product is made of natural dyes concentration of 0.15% or higher, the intensity will decrease slightly.

Determination of the inhibition of oxidative activity of hydrogen peroxide.

Many fans of sweet like jelly products (lemon drops, chewing marmalade). Jelly products (lemon drops, chewing marmalade) like many fans of sweet. Their unusual taste pleases kids and adults. Jam and jelly decorate cakes and desserts, ice cream and biscuits. It is very important that of all the sweets the marmalade is the most useful. The fact is that it includes nutrients such as agar-agar, pectin, gelatin and applesauce.

Agar-agar is a product of plant origin which improves liver. Pectin is a soluble dietary fiber, helps normalize the digestive tract. Marmalade is a low-fat confectionery product; it contains no fat, and therefore is considered a dietary product. It is also widely known that the marmalade is an excellent anti-depressant, as it helps to relieve stress and improve mood.

But most importantly, marmalade can be also an excellent antioxidant, for example, if it is composed of natural dye anthocyanin.

The antioxidant properties of marmalade from natural dye can be determined by the ability to inhibit the action of anthocyanin hydrogen peroxide.

The obtained data are recorded in Table 1.

Table 1. The results of the study of antioxidant activity of jelly products

Nr P.p.	Ingredients	The concentration of the dye, %	\bar{V}_0	\bar{V}_1	$HPSA(\%H_2O_{2inhibit}) = \frac{V_0 - V_1}{V_0} \cdot 100, \%$
1	2	3	4	5	6
1.	Marmalade on agar	Without dye	4,65	4,40	5,38
2.		0,03% crimson	6,15	6,05	1,63
3.		0,05 % anthocyanin	5,20	4,80	7,69
4.		0,1% anthocyanin	5,65	4,90	13,27
5.		0,15 % anthocyanin	6,30	5,15	18, 25
6.	Marmalade on gelatin	Without dye	3,90	3,85	1,28
7.		0,03% crimson	5,10	5,00	1,96
8.		0,05 % anthocyanin	4,10	3,90	4,88
9.		0,1% anthocyanin	3,85	3,50	9,09
10.		0,15 % anthocyanin	4,80	4,25	11,46

The nature of the dye has a large effect on the antioxidant properties of the product. In both types of marmalade natural dye products, the antioxidant activity (AOA) of the

finished product increases. The higher the concentration of the dye in the product, the higher the antioxidant activity is. Contrary, the synthetic dye may reduce or increase slightly the antioxidant activity depending on the nature of the ingredients.

In the marmalade on agar, not yet painted, initially there is a rather high content of antioxidants, so the addition of anthocyanins, the AOA of products increases significantly with 5.38% in the unpainted product and to 18.25% in the product, which contains 0.15% anthocyanins.

In the marmalade on gelatin the initial antioxidant content is very low only 1.28%, therefore the highest AOA rises to 11.46% in the richest anthocyanins sample.

Synthetic dyes have an undesirable effect on AOA of the product. The sample on agar synthetic dye reduced AOA by almost 3.5 times from 5.38% to 1.63%.

In this paper, it is important to understand and analyze how and how much natural dye - anthocyanin affected significantly on AOA of the product. To do this, consider the following diagram.

The AOA of the value marmalade products is directly proportional to the amount added to the product of anthocyanin. With increasing concentration of anthocyanin rises AOA of the product. In both types of jelly products AOA increases by 2.5 times from 7.69% to 18.25% in the products on the agar and 4.88% to 11.46% in products in the gelatin. But regardless of that, marmalade on agar with anthocyanin concentration of 0.15% contains 1.5 times more antioxidants than marmalade on gelatin with the same concentration of anthocyanin. Because in the marmalade on agar initially there is a high level of antioxidants, it can be considered as its antioxidant properties lushimi and jellies more useful.

4. Conclusion

The addition of extracts of grape anthocyanes gave an excellent antioxidant effect on the marmalade compared with the effect of synthetic colorant. In addition, natural antioxidants are safe and impart health benefits to the consumer. Increased knowledge of their bioavailability and therapeutic effects will result in better adoption of anthocyanin-based products as functional foods.

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FOOD INDUSTRY WASTE AS RESOURCE OF BIO-ACTIVE COMPOUNDS

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Abstract: Up-cycling of agro-alimentary industry waste is getting more attention from the scientists due to the fact that most of vegetable residues may be considered as a resource of high added value products, such as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants. This paper focuses on the opportunities opened by grapes (*Vitis vinifera*), one of the largest and most common fruit crop in Europe. Identification and/or isolation of bioactive natural products from this waste, as well as their chemical and/or enzymatic modification, are the main directions to obtain valuable natural compounds from up-cycled food industry waste.

Keywords: up-cycling, agro-alimentary waste, bio-active compounds, *Vitis vinifera*.

1. Introduction

Organic chemicals play an important role in our everyday lives. Since the middle of the 20th century, fossil oil and natural gas have been serving as the main raw material resources for chemicals production. Generally, almost all organic compounds can be derived from several basic building blocks, including syngas from methane, ethylene, propylene, butane, butylenes, butadiene and BTX (benzene, toluene, xylene). These building blocks are obtained from natural gas, petroleum and coal [1].

There is a growing interest in the replacement of fossil-based chemicals with biochemicals or “green” chemicals. Several factors, including awareness of finite petroleum resources, availability of renewable resources, environmental imperatives and recent advances in processing technologies, are driving chemical industry to shift the feedstocks from classic to renewable counterparts.

The advantages of using biomass include opportunities for less pollution, more biodegradable and sustainable products and, in some cases, lower costs. It has been found that many biomass derived chemicals have economical advantages, particularly for some functionalized chemicals. In addition, recent advances in processing technologies, especially in fermentation (such as: enzymatic engineering, metabolic engineering and genetic manipulation), may provide new opportunities for producing a wide variety of industrial products from renewable plant resources. A key to the chemical industry gradual shift toward the use of renewable resources is the implementation of the biorefinery concept. Similar to a petroleum refinery, a biorefinery integrates a variety of processing technologies to produce multiple bioproducts from various biomasses. Such an approach will help maximize the value of the biomass and minimize low or no value by-products [2].

The constant search for cheap and renewable bioresources led researchers to investigate the opportunities offered by the annual vegetable waste which accumulates worldwide: millions of metric tons of various pomace waste originate from viticulture, vegetal oil production, tomato processing, fruits processing, as well as from sugar or cassava production.

Up-cycling of agro-alimentary industry waste is getting more attention from the scientists due to the fact that most of vegetable residues may be considered as a resource of

high added value products, such as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants.

Two different strategies are, basically, used to capitalize vegetable by-products: a) identification and isolation of bioactive natural products in vegetable waste as a possible resource of important compounds or enriched fractions and b) chemical and/or enzymatic modification of main compounds to obtain optimized analogues, food additives, drugs or cosmetics. Thus, this is an example of integrated approach between the query for waste up-cycling of agro-food industry and the search for bioactive products from renewable resources.

This paper focuses on the opportunities opened by grapes (*Vitis vinifera*), one of the largest and most common fruit crop in Europe, used mainly for wine production. After harvesting, destemming, crushing and pressing, mainly two different by-products accumulate: grape stems and grape pomace (skins, seeds and lees) in a global amount of aprox. 20% of the harvested grape.

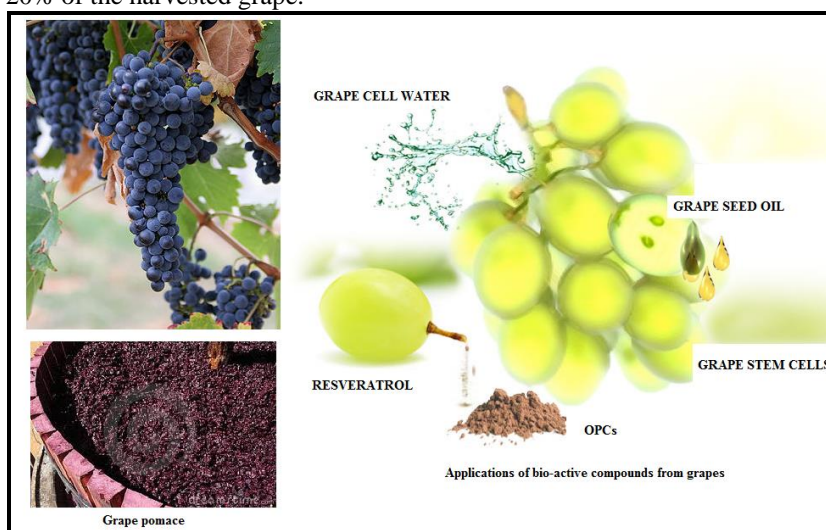


Fig. 1. Grapes, pomace and some applications of the bio-active compounds

Identification and isolation of bioactive natural products from this waste, as well as their chemical and/or enzymatic modification are the main directions to obtain valuable natural compounds from up-cycled food industry waste.

2. Bioactive natural compounds in grape by-products

2.1. Bioactive compounds from grape stems

As by-product from winemaking, grape pomace is frequently used for distillation and production of wine-derivatives alcoholic distillates, with an alcoholic concentration depending on the type of final product. In example, *tescovina* (Romania) has an alcohol content up to 45%, while italian *grappa* contains 30-37% alcohol. Unlike pomace, it seems there is no real use for grape stems other than animal food and composting.

Literature data [3] indicate that it is possible to isolate and identify antiproliferative compounds from an EtOAc (ethyl acetate) crude extract of grape stems, using

chromatographic and spectroscopic analyses, including NMR spectra. Considering the amount in the mixture as criterion, the identified products were divided into two groups. Major compounds and their structures are listed in Fig. 2.

Oleanolic and betulinic acids are among them, as well as the stilbenoid *trans*-resveratrol and *trans*- ϵ -viniferin, a resveratrol dimer. A more polar antiproliferative constituent was also found, it was shown to be a mixture of sitosterol 6'-*O*-acylglucosides, as proved by GC-MS analysis of the methanolysis products. As a matter of fact, the glucose moiety was esterified by fatty acids (linoleic, linolenic, palmitic and stearic, in decreasing percentages). One of the main products was a mixture of inseparable analogues, namely 2,3-di-*O*-acyl-glycerol galactosides. Methanolysis followed by GC-MS analysis of the methyl esters established the acyl residues. The optical rotation measurements on the galactoglycerol obtained from methanolysis allowed establishing the configuration of the stereogenic centre C-2 as *R* in this product and, consequently, as *S* in the natural substrate.

Further minor constituents with antiproliferative properties were isolated and identified from grape stems, their structures being shown in Fig. 3. They are daucosterol, gallic acid, catechin and gallocatechin.

All these compounds were found as pure constituents or inseparable mixtures. Submitted to specific tests [4, 5], they showed different growth inhibitory properties for tumoral cells. In example, one of the most effective compounds was betulinic acid, but *trans*-resveratrol showed interesting results also. Some other compounds showed weak antiproliferative activity, such as: ϵ -viniferin, gallic acid, oleanolic acid and gallocatechin.

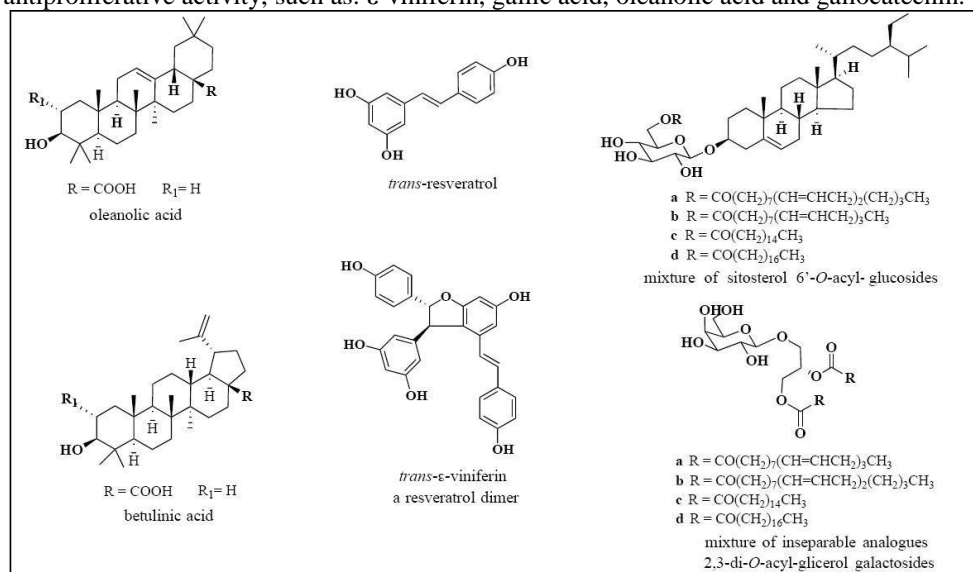


Fig. 2. Organic compounds with biologic activity obtained from grape stems

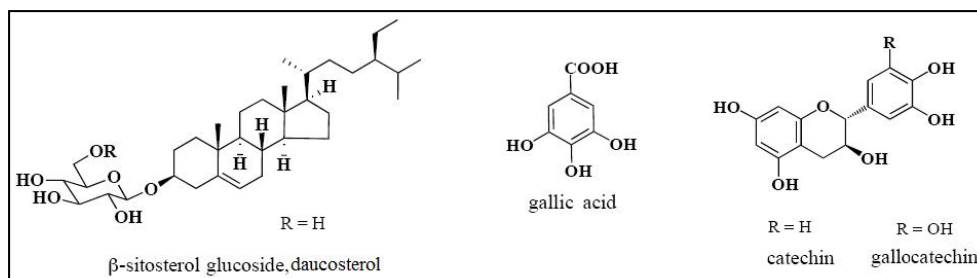


Fig. 3. Minor organic compounds with biologic activity obtained from grape stems

Almost all constituents isolated from grape stems are already known in literature for their benefic properties.

Resveratrol is also known as a grape phytoalexin and is considered one of the most important phenolic compounds in red wines [6], having the capability to prevent coronary syndrome, as well as tumors. It can be extracted from grape stems in an amount of 130 mg/kg dried stems (approx.).

Betulinic and oleanolic acids, as well as sitosterol 6'-*O*-acyl-glucosides and 2,3-di-*O*-acyl-glycerol galactosides have been proved to be effective antiproliferative principles towards tumors [7] or potent antitumor-promoting [8] and anti-inflammatory agents [9].

Gallic acid, catechin and gallocatechin are well-known as constituents of black or green tea and proved antioxidant [10] and anticarcinogenic properties [11].

2.2. Bioactive compounds from grape pomace

The aim of studying grape pomace was to isolate and identify compounds with antioxidant properties, known as "free-radical scavengers". These organic compounds have the ability to trap free radicals, action which would immediately inhibit the auto-oxidation cycle. Under oxygen deficient conditions, alkyl radical scavengers contribute to the stabilization of polymer chains, preventing their decay. Lactones and some phenols are known to be very effective as "free-radical scavengers" in low oxygen media.

A methanolic extract obtained from the destemmed grape pomace was analysed and main flavonols, flavonols glucosides, flavanols and their gallate esters, anthocyanins and low molecular weight pro-anthocyanins were identified [3]. Five pyrano-anthocyanins (less common compounds in pomace, normally found in aged wines) were also identified. These compounds and their structures are presented in Fig. 4.

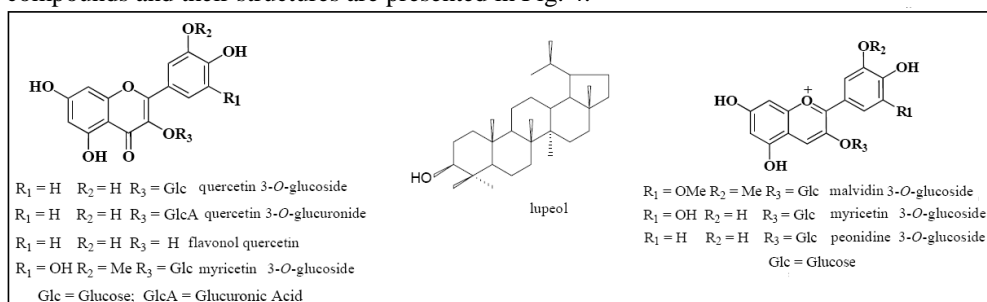


Fig. 4. Most important components with antioxidant properties from grape pomace

Quercetin 3-*O*-glucoside and quercetin 3-*O*-glucuronide resulted to be the major flavonol glycosides (in terms of amount in mixture), while malvidin 3-*O*-glucoside was found to be the main anthocyanin. By specific methods (flash-chromatography), members of the triterpenes family were identified, lupeol and oleanoic acid (Fig. 2), as well as the flavonol quercetin and daucosterol (Fig. 2).

Most of polyphenols identified in grape pomace are well known in the literature [12-14] due to their interesting biological properties, mainly for their antioxidant, free radical scavenging activity. It has been reported that the anthocyanin fraction from red wines was the most effective phenolic fraction in scavenging reactive oxygen species and in lipoprotein oxidation inhibition and platelet aggregation [15]. There are recent studies indicating lupeol as effective anti-inflammatory agent [16], apoptosis inducer [17] and inhibitor of tumoral cells proliferation [18].

The quantitative analysis of the main anthocyanins and flavonols shows that delphinidin 3-*O*-glucoside, myricetin 3-*O*-glucoside, quercetin 3-*O*-glucoside and quercetin, all bearing a catechol moiety, have the highest percentage in the corresponding mixture [3]. With respect to the crude alcoholic extract, these flavonoids are also consistently enriched, whereas a significant reduction in peonidine 3-*O*-glucoside and malvidin 3-*O*-glucoside, without *ortho*-dihydroxy moiety, is recorded.

2.3. Bioactive compounds from grape seeds

Oligomeric proanthocyanidins (OPCs) are naturally occurring substances present in a variety of food and vegetal sources, one of them being the red grape seeds. OPCs are unique members of the flavonols family (i. e., catechin, epicatechin and gallocatechin, Fig. 3) and have powerful antioxidant characteristics and excellent bioavailability. Clinical tests suggested that OPCs may be as much as fifty times more potent than vitamin C, in terms of antioxidative activity. In addition to this, they have the capability to strengthen and repair connective tissues, including at the cardiovascular system, to moderate allergic and inflammatory responses by reducing the histamine production and have antidepressant-like effect [19, 20].

OPCs are present in grape seeds in various amounts (in mg/100 g edible portion), as monomers (171), dimers (170) and trimers (33), depending on the type of grapes. Although they are not consumed as such, seeds are of great importance for wine producers (5 ounces of red wine contain 91 mg OPCs) [21, 22] and as bio-resource of flavonols for the pharmaceutical industry.

3. Study on the chemical and enzymatic modification of resveratrol

Most data on bioactive compounds isolated and identified in grape by-products concern *trans*-resveratrol. *Trans*-resveratrol (3,5,4'-trihydroxy-*trans*-stilbene, Fig. 2) is a stilbenoid found mainly in the skin of red grapes (resveratrol content in grapes skins and [pomace](#) was found to be stable, as shown by samples taken after fermentation and stored for a long period [23]) and in other fruits. Red wine contains very little of it, on the order of 0.1-14.3 mg/l [24]. Resveratrol has been also produced by chemical synthesis [25] and by biotechnological synthesis (metabolic engineered microorganisms) [26, 27].

Recent studies indicated resveratrol as an active principle in biochemical reactions. Thus, it was reported that it significantly extends the lifespan of some yeasts, worms and fruit fly [28, 29]. Many studies have been conducted to investigate its anti-tumoral activity in animals [24]. It was also shown that it can induce apoptosis in platelets and smooth

muscles [30]. Resveratrol seems to act more effective on tumors it can contact directly, as esophageal tumors or melanoma [31]. Furthermore, it seems to have cardioprotective effects, moderate drinking of red wine being known to reduce the risk of heart disease ("the French paradox"). This effect is achieved by the following actions: inhibition of vascular cell adhesion molecule expression; inhibition of vascular smooth muscle cell proliferation; stimulation of endothelial nitric oxide synthase (eNOS) activity; inhibition of platelet aggregation; inhibition of LDL peroxidation [32]. As for resveratrol antidiabetic activity, it was proved in human clinical trials [33] that it determines low blood sugar levels. Other beneficial effects include neuroprotective, antiviral and anti-inflammatory activity. Still, resveratrol has been shown to be ineffective in inhibiting microbial and fungi proliferation [34].

Resveratrol, in common with other polyphenols, was found to be a strong [topoisomerase inhibitor](#), sharing similarities to chemotherapeutic anticancer drugs, such as [etoposide](#) and [doxorubicin](#) [35]. Resveratrol has recently been evaluated as an antiproliferative agent towards prostate tumor cells [36, 37]. Compounds with antiproliferative properties against prostate carcinoma are intensively studied in order to obtain new anticancer drugs or adjuvants of currently used drugs. Various lipophilic analogues of resveratrol were obtained and some of them are presented in Fig. 5.

The most important analogue, 4'-*O*-acetylresveratrol (yield 40%), was synthesized through an enzymatic regioselective direct acylation in the presence of the enzyme *Candida antarctica* lipase (CAL) and vinyl acetate in *tert*-amyl alcohol [3].

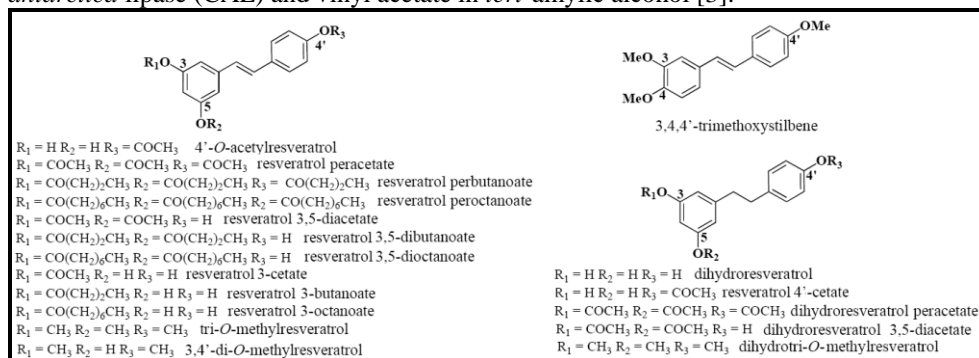


Fig. 5. Lipophilic analogues of resveratrol [3]

The observed regioselectivity of the enzyme towards position 4' was used in order to obtain other resveratrol derivatives. One approach was based on a preliminary peracylation by conventional chemical reagents, such as anhydrides or acyl chlorides, followed by regioselective deprotection of the peracylated resveratrol. Resveratrol esters (peracetate, perbutanoate and peroctanoate) were subjected to lipase-catalysed alcoholysis, employing *n*-butanol as nucleophilic reagent. Butanolysis was more rapid, but less selective than direct acylation. In the presence of CAL, both 4'-deprotected and 5,4'-deprotected derivatives (and acylated butanol as by-product) were obtained.

The enzyme preferentially attacks functional groups bonded to C in position 4' and this enabled synthesis of resveratrol 3,5-diacetate, 3,5-dibutanoate and 3,5-diocanoate. Furthermore, these compounds may become a substrate for the CAL, thus affording the corresponding resveratrol 3-cetate, 3-butanoate and 3-octanoate.

Further chemical and enzymatic modifications on resveratrol aimed to use the central C=C double bond [38] and yielded in dihydroresveratrol, which was then directly acylated in the presence of CAL to afford resveratrol 4'-cetate. The dihydroresveratrol peracetate was submitted to the enzymatic butanolysis and afforded the derivative dihydroresveratrol 3,5-diacetate.

By simple chemical conversion, tri-*O*-methylresveratrol, 3,4'-di-*O*-methylresveratrol and dihydrotri-*O*-methylresveratrol were obtained.

The influence of the methoxy groups position was evaluated through the synthesis of a resveratrol analogue, namely the 3,4,4'-trimethoxystilbene, having a modified substitution pattern of the stilbene skeleton. It was proven that the activity of the trimethoxystilbene analogue was comparable to that of resveratrol, thus suggesting that the substitution pattern is important for the biologic activity.

Other analogues showed potent inhibitory activity against porcine aortic endothelial cells [39], very important feature since the inhibition of angiogenesis in tumor cells is a new frontier in the fight against cancer [40]. Methylated analogues of transveratrol are also showing promising anti-cancer properties [41].

4. Conclusion

Taking into consideration data presented in this paper, it is obvious that food industry waste is a promising resource of bio-active compounds. Its availability and renewable character is giving research a new impulse to elaborate protocols for faster and/or easier isolation and identification of these valuable compounds, as well as for their chemo-enzymatic conversion. One key factor is the preservation, if not the enhancement, of the biologic activity of natural compounds throughout all processes during their functionalization. Using these bio-active compounds as flavours, antioxidants, cosmetic excipients or active principles, drugs or drug adjuvants is adding value and makes all efforts worthwhile. Despite these data, *Vitis vinifera* is not, regrettably, on the *ePlantLIBRA* (EC project number 245199) list of prioritised plants selected for the European Community's Seventh Framework Programme (FP7/2007-2013), although this database will provide a unique comprehensive information resource for expert users: scientists, epidemiologists, health professionals, health educators, food industry professionals, food regulatory authorities and policy makers.

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Section III

Chemistry and Microbiology of Food



STUDIES ON THE COMPOSITION OF ORGANIC AND CONVENTIONAL MILK PRODUCTS

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Abstract: The changes in the basic physicochemical characteristics and the lipid composition of Bulgarian organic and conventional dairy products (cow's yoghurt and cow's cheese) during the winter and summer period have been investigated. There were no significant changes in the basic physicochemical characteristics for organic and conventional milk products (fat content in dry matter, content of milk protein, dry matter, acidity, salt content) in the studied periods. Organic yoghurt has lower acidity in comparison with conventional yoghurt. Organic cheese is the only one with a degree of maturity (21.7% and 20.5%), corresponding to the regulated value in the Bulgarian state standards for cheese (not less than 14%). The lipids have identical fatty acid composition, dominated by saturated fatty acids (68.7% -74.5%) and that their content is higher in winter. During the summer period quantities of trans fatty acids in mono- (C_{18:1}) and polyunsaturated fatty acids (C_{18:2} and C_{18:3}) was higher than in winter. The content of tocopherols in the yoghurt fats is minimal, as their values in summer period is higher, such in the various trademarks cheese tocopherols not detected.

Keywords: organic and conventional dairy products, cow's yoghurt, cow's cheese, physicochemical characteristics, fatty acid composition, trans fatty acids

Introduction

Milk and milk products are highly evaluated for their nutrient properties. Certain organic milk products have been produced lately to feature lower contaminants percent. Comparative studies on the differences between the organic and conventional foods are rare to find in the scientific literature while no data on the composition of the organic and conventional type milk products being distributed through the retail trade system in Bulgaria is available at all. The current study aims to track the alterations across the general and lipid compositions of various organic and conventional milk products (yoghurt and cheese) brands during the winter and summer seasons.

Materials and methods

Milk sample collection

Cow's yoghurt – Organic cow's yoghurt "Harmonica" and conventional cow's yoghurt "Elena"; Cow's cheese – Organic cow's cheese "Kostovi - Saedinenie" and conventional white cow's cheese "Kostovi - Saedinenie" and "Elena". Dairy products were examined during winter and summer.

Methods

Fat content. The Gerber method was used to determine the fat content of the milk [8]. The fat content of the cheeses was determined according to a Shmid-Bondzynski-Ratzlaff method [12].

Moisture content and total solid. The determination of moisture was done by oven drying at 100°C until a constant weight was obtained [1]. The dry matter is calculated by 100 – moisture content.

Titrateable acidity. The titrateable acidity was determined by titrating of the milk and the solution of cheese with 0.1 M sodium hydroxide solution in presence of phenolphthalein indicator [1].

Protein content. The protein content of the milk was determined by the Kjeldahl method and was calculated from the nitrogen content using factor 6.38 [6]. **Soluble protein.** Soluble proteins were extracted with hot water and subjected to digestion, distillation and titration in similar manner to total protein in cheese.

Salt. Salt (sodium chloride) was extracted with hot water, and then titrated with standard solution of 0.1N AgNO₃ according to BS 8274:1982 [7].

Analysis of fatty acids. The total fatty acid composition as well as the fatty acid composition of oils was determined by gas chromatography (GC) after transmethylation with 2 N NaOH in absolute CH₃OH at 50°C [3]. GC was performed on a HP 5890 gas chromatograph equipped with a 30 m x 0.25 mm capillary EC-Wax column and a flame ionization detector [4]. **Total content of Trans isomeric fatty acids** was determined in the oils by IR- spectrophotometry [2], which takes into account quantitatively the presence of trans double bonds as elaidic acid.

Analysis of tocopherols. Tocopherols were determined directly in the oil by high performance liquid chromatography on a "Merck-Hitachi" instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column and fluorescent detector "Merck-Hitachi" F 1000 [5].

Results and discussion

The basic physicochemical characteristics of the yoghurt and Bulgarian white brine cheese brands under study during both seasons are given in a Table 1 below.

The organic and conventional yoghurts under study have failed to comply with certain required Bulgarian standard measures such as fat contents, moisture contents and dry matter respectively. No significant differences in the basic measures during both seasons (summer and winter) have been detected while changes within protein contents and titrateable acidity of the yoghurt depending on the season have been recorded only.

The organic cheese has shown higher fat contents (23.4 and 27.7% respectively) during either season compared to the conventional cow cheese (21.5 - 23.4% respectively). Higher fat contents during the summer season has been found with the organic cheese only while both conventional cheeses „Elena” and „Saedinenie” have demonstrated higher fat contents during the winter season. The organic and conventional cheese brands under study have shown greater fat contents (16.0% - 18.4%) than that specified for the milk products (14.0 - 16.0%). The total amount of protein involves the most important cheese production measure - namely the degree of maturity. Based on the degree of maturity (21.7%) and moisture content (54.0%) only the organic cheese meets the Bulgarian state standard requirements in either seasons. The results obtained on the salt contents have shown measures close to the required standard one (3.5±0.5%). All milk products brands under study have shown titrateable acidity to comply with the required one.

Table 1 Physicochemical characteristics of studies cow's yoghurt and white cow's cheese

Physicochemical characteristics		Cow's yoghurt					
		Winter time		Summer time		Requirements for Bulgarian cow's yoghurt [9]	
		Organic	Elena	Organic	Elena		
Fat content, %		3.4±0.1	3.5±0.1	3.5±0.1	3.2±0.1	not less than 3.6	
Moisture content, %		88.7±0.1	87.8±0.1	88.4±0.1	87.7±0.1	no more than 88.2	
Dry matter, %		11.3±0.1	12.2±0.1	11.6±0.1	12.3±0.1	not less than 11.8	
Protein content, %		3.3±0.01	3.8±0.02	3.2±0.01	3.5±0.01	not less than 3.2	
Titratable acidity, °T		80.0±2.0	102±1.0	89.0±2.0	124.0±1.0	from 90 to 150	
Physico-chemical characteristics	Cow's cheese						
	Winter time			Summer time			Requirements for cow's cheese [10]
	Organic	Saedinenie	Elena	Organic	Saedinenie	Elena	
Fat in product,%	23.4±0.1	23.4±0.2	21.6±0.4	27.7±0.1	21.5±0.1	21.7±0.2	-
Fat in dry matter, %	50.9±0.1	52.9±0.2	55.4±0.4	51.8±0.1	49.9±0.1	48.1±0.2	not less than 44.0
Moisture content,%	54.0±0.1	55.8±0.4	61.0±0.1	54.3±0.3	54.8±0.2	59.5±0.2	54.0
Dry matter, %	46.0±0.1	44.2±0.4	39.0±0.1	45.7±0.3	45.2±0.2	40.6±0.2	not less than 46.0
Protein content,%	16.5±0.1	17.2±0.1	16.0±0.1	18.4±0.1	16.3±0.1	17.5±0.1	14.0-16.0
Degree of maturity, %	21.7±0.1	13.5±0.1	9.5±0.1	21.7±0.2	11.5±0.1	5.3±0.1	not less than 14.0
Titratable acidity, °T	240±5.0	243±3.0	210±5.0	293±8.0	253±3.0	213±5.0	200-270
Salt content,%	3.5±0.1	3.5±0.1	5.3±0.2	2.9±0.1	4.1±0.2	3.6±0.1	3.5±0.5

The fatty acid composition of the milk fats from the tested organic and conventional milk product brands during both seasons is given in the Table 2 below.

The caprylic acid quantity varies from 0.4% to 0.7% during the winter only at that while its quantity has been found to be lower than that recorded by other authors [13]. The capric acid varies from 0.7% to 3.7% while its quantity during the winter is found to be 2 to 5 times higher than in the summer. The quantity of the palmitic and oleic acids in lipides being isolated out from organic and conventional milk products brands dominates. The palmitic acid contents within the fat phase ("Saedinenie" cheese in the summer season) varies from 32.9% to 37.3% ("Elena" yoghurt in summer season) while the oleic acid content (organic yoghurt in the winter season) varies from 20.8% to 28.9% (organic yoghurt in the summer season). The linoleic acid quantity varies from 0.7% to 1.5% while in the summer is twice as high compared to that in the winter season and the essential linoleic acid is scarcely present varying from 0.1% to 0.3%.

Table 2 Fatty acid composition of the fat from cow's yoghurt and cow's cheese

Fatty acids %		Cow's yoghurt				Published data [13]
		Winter time		Summer time		
		Organic	Elena	Organic	Elena	
C _{4:0}	Butanoic	-	-	-	-	3.6
C _{6:0}	Caproic	-	-	-	-	2.2
C _{8:0}	Caprylic	0.4±0.05	0.7±0.1	-	-	1.2
C _{10:0}	Capric	3.6±0.1	3.7±0.3	0.7±0.1	1.8±0.1	2.8
C _{12:0}	Lauric	5.7±0.1	5.3±0.1	2.4±0.1	4.4±0.1	2.8
C _{12:1}	Lauricoleic	0.2±0.01	0.2±0.01	0.1±0.01	0.1±0.01	-
C _{14:0}	Myristic	17.8±0.03	15.2±0.3	12.5±0.3	14.2±0.2	10.1
C _{14:1}	Myristoleic	1.3±0.1	1.1±0.1	0.5±0.01	0.9±0.05	-
C _{15:0}	Pentadecanoic	1.6±0.1	1.6±0.01	1.5±0.1	1.5±0.1	-
C _{16:0}	Palmitic	33.9±1.0	36.2±0.5	33.3±0.5	37.3±0.5	25.0
C _{16:1}	Palmitoleic	1.6±0.1	1.7±0.05	1.5±0.1	1.8±0.1	2.6
C _{17:0}	Margaric	0.6±0.1	0.7±0.01	0.9±0.1	0.7±0.1	-
C _{18:0}	Stearic	10.8±0.5	10.7±0.4	15.9±0.2	11.9±0.2	12.1
C _{18:1}	Oleic	20.8±1.0	21.2±0.4	28.9±0.3	23.7±0.4	27.1
C _{18:2}	Linoleic	0.7±0.1	0.7±0.01	1.5±0.1	1.5±0.1	2.4
C _{18:3}	Linolenic	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	2.1
C _{20:0}	Arachidic	0.1±0.1	0.1±0.05	0.2±0.01	0.1±0.01	-
C _{20:1}	Gadoleic	0.3±0.05	0.2±0.1	-	-	-
C _{20:2}	Eicosadienoic	0.5±0.05	0.6±0.05	-	-	-
Fatty acids %	Cow's cheese					
	Winter time			Summer time		
	Organic	Saedinenie	Elena	Organic	Saedinenie	Elena
C _{8:0}	0.5±0.05	0.7±0.05	0.5±0.01	-	-	-
C _{10:0}	1.4±0.2	3.1±0.1	2.2±0.1	0.7±0.05	1.9±0.1	1.3±0.05
C _{12:0}	2.4±0.2	4.1±0.1	3.5±0.1	2.6±0.1	4.3±0.2	3.4±0.1
C _{12:1}	0.1±0.01	0.2±0.01	0.2±0.01	0.1±0.01	0.2±0.01	0.2±0.01
C _{14:0}	10.6±0.2	14.1±0.3	14.2±0.01	13.0±0.2	14.6±0.2	13.6±0.3
C _{14:1}	0.5±0.01	1.0±0.01	0.7±0.01	0.6±0.05	0.8±0.05	0.8±0.1
C _{15:0}	1.4±0.01	1.5±0.01	1.9±0.1	1.6±0.1	1.8±0.1	1.8±0.1
C _{16:0}	33.6±0.5	36.1±0.5	35.2±0.2	36.5±0.2	32.9±0.4	34.1±0.3
C _{16:1}	1.4±0.01	1.5±0.01	1.5±0.01	1.4±0.1	1.6±0.1	1.7±0.2
C _{17:0}	0.9±0.1	0.8±0.01	1.0±0.01	0.9±0.1	0.8±0.05	0.9±0.1
C _{18:0}	17.4±0.1	12.5±0.1	14.1±0.1	15.6±0.2	14.5±0.1	14.7±0.1
C _{18:1}	26.7±0.2	21.7±0.4	21.4±0.2	25.5±0.3	25.0±0.3	25.4±0.2
C _{18:2}	0.7±0.1	0.7±0.1	0.9±0.1	1.1±0.1	1.2±0.1	1.4±0.1
C _{18:3}	0.3±0.01	0.2±0.01	0.2±0.01	0.1±0.01	0.1±0.01	0.3±0.05
C _{20:0}	0.3±0.01	0.2±0.01	0.3±0.01	0.3±0.05	0.3±0.01	0.4±0.05
C _{20:1}	0.2±0.1	0.3±0.1	0.2±0.1	-	-	-
C _{20:2}	1.4±0.1	1.3±0.1	1.8±0.1	-	-	-
C _{22:0}	0.2±0.01	-	0.2±0.01	-	-	-

The ratios between the saturated and unsaturated acids as well as the trans isomeric fatty acids contents are illustrated in the Fig. 1.

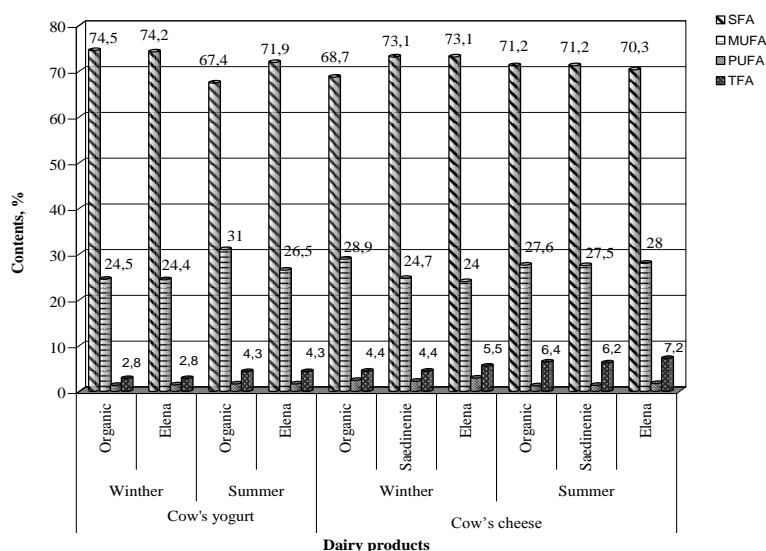


Fig. 1. Content of saturated (SFA) and unsaturated (MUFA - monounsaturated, PUFA - polyunsaturated) and trans fatty acids (TFA) in fat from cow's yoghurt and cow's cheese

High content of saturated fatty acids (over 67.4%) has been observed with all lipides being isolated out of the milk products brands under study. During the winter season both organic and "Elena" yoghurts have demonstrated the highest percent of saturated fatty acids (74.5% and 74.2% respectively) while both organic and "Saedinenie" cow's cheese have demonstrated higher content of saturated acids during the summer season (71.2%). The organic yoghurt has demonstrated the lowest percent of saturated fatty acids during the summer season – 67.4% and the organic and "Elena" cheeses – 68.7% during the winter season respectively while showing higher percent of monounsaturated acids (31.0% and 31.3% respectively). The quantity of the monounsaturated acids with the milk products under study is higher in the summer season which can most probably be accounted for by the grass grazing as the grass contains certain quantity of diene and triene fatty acids.

In the winter season lower percent of trans fatty acids has been found with the organic and conventional milk products (2.8% with the milk and 4.4 - 4.5% with the cheese respectively) in comparison with that in the summer: 4.3% with the yoghurt and 6.2 - 7.2% with the cheese respectively. The milk lipids under study have not demonstrated higher percent of trans isomeric fatty acids in comparison with the cited data (1.0 - 7.0%) [11]. The percent of the trans fatty acids content in yoghurt varies from 0.1% to 0.15% while in cheese its within 1.0 to 1.8%.

The organic and conventional yoghurts are found to contain tocopherols in minimal percent at that (Organic – 14.6 mg/kg winter time and summer time – 23.6mg/kg and for "Elena" 5.9 and 25.5 mg/kg respectively). The α - tocopherol (100%) is the basic representative in the tocopherols list. Cheese is not found to contain tocopherols. Most probably this is due to the low percent of tocopherols in the milk itself and their probable decomposition while the milk is being processed into cheese.

Conclusions

Most of the Bulgarian milk products do not comply with the Bulgarian state standard requirements based on their physicochemical measures. The moisture contents, fat contents, titratable acidity, degree of maturity and salt content are found to deviate from their allowable measures. The organic and conventional milk products have not been found to show differences between their composition as well as between the different milk products under study during both seasons. The lipides being isolated out of the organic and conventional milk products type brands under study are found to show identical fatty acid composition dominated by the saturated fatty acids (67.4 - 74.5%). Mono-, polyunsaturated and tran sisomeric fatty acids are found to show higher percent in the summer season.

Acknowledgements

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STUDY ON THE DEVELOPMENT OF THE MATERIAL BALANCE FOCUSED ON THE METAL TRANSFER BETWEEN THE SYSTEM CAN-LACQUERING AND CANNED VEGETABLES

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Abstract: The paper presents an assessment of the transferable substances amount that is leaking from the packing materials at the food-metallic packing interaction interface by analysing the material balance of the components. The starting point is the theoretical balances of materials. We investigated the transferable materials for rehydrated peas and tomato paste cans. In both cases it was found that an interaction occurs between the organic acids that are found in vegetables as salts and their derivatives, and the metallic structure of the packing material - packaging varnishing system of the can. The real balance of migrated materials gives us an indication concerning the total content of heavy metals accumulated in the cans during the validity period, and allows us to establish precisely a storage period, therefore assuring maximum safety for consumers.

Key words: food safety, material balance, packaging food, canned vegetables

Introduction

In the past years there has been an increasing concern for the food packagings regarding the foods safety due to the possibility of chemical substances migration from the package material in the food stuff. It is known the fact that along with the food stuff the consumer ingests the chemical substances from the packagings and other food contact materials that can migrate into the protected food stuff. The term “migration” usually describes a diffusion process that takes place at the interaction surface between the food and the package, which may be influenced by the compatibility between the food stuff and the packagings and the hygienic quality of the package (Arvanitoyannis & Bosnea, 2004). To insure the food safety by the monitoring of the migration process at the interaction surface between the food stuff and the package has become of a great interest for the entire scientific world. The substances that can affect the human health by migrating from the package into the food undoubtedly depend on the nature of the packaging material (Sendón García & al., 2006).

The substances that leave the package and are transferred into the foodstuff at the interaction surface between the metallic package/ can and the food are of great concern. A correlation between the substances that transfer from the can into the food stuff at the interaction surface between the food stuff and the can be realized by creating a balance of the package material on their components. To create this balance we started with the stoichiometric equation of a chemical process written under the following form:

$$\nu_{A_1} \cdot A_1 + \nu_{A_2} \cdot A_2 + \dots + \nu_{A_n} \cdot A_n = \nu_{A'_1} \cdot A'_1 + \nu_{A'_2} \cdot A'_2 + \dots + \nu_{A'_m} \cdot A'_m \quad (1)$$

In which: ν_{A_i} - the stoichiometric coefficient of the reactants; $\nu_{A'_i}$ - the stoichiometric coefficient of the reaction products; A_i , A'_i - reactants, and reaction products.

At the interaction surface between the food stuff and the package several main and secondary chemical as well as electrochemical reactions take place. To express the quantitative state of the interaction between the food stuff and the metallic can we appeal at a

certain moment at the transformation degree η_{A_k} of a valuable or invaluable component η_{A_i} . This scale is defined as transformed fraction of the valuable component and is expressed by the relation:

$$\eta_{A_i} = \frac{n_{A_i}^0 - n_{A_i}}{n_{A_i}^0} = \frac{n_{A_i}^0 - n_{A_i}}{\frac{V_{A_i}}{V_{A_k}} \cdot n_{A_k}^0} = \frac{n_{A_i}^0 - n_{A_i}}{\frac{V_{A_i}}{V_{A_k}} \cdot n_{A_k}^0} \quad (2)$$

Where: $n_{A_i}^0$, $n_{A_k}^0$, $n_{A_i'}^0$ - is the initial reactional mass composition of the component (A_i), of the valuable component (A_k), and of the reaction products (A_i'); n_{A_i} , $n_{A_i'}$ - the reaction mass composition at a given moment for the reactants (A_i), and for the reaction products (A_i').

The using of the variable transformation degree for the quantitative characterization of the process allows the development of the stoichiometric calculation in a simple form and the creation of a system of balance equations in a proper form for their using for the quantitative description of the process (Gutt & Gutt, 1993).

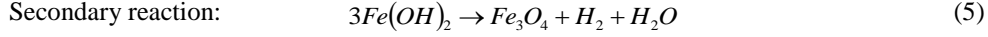
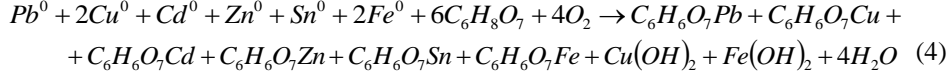
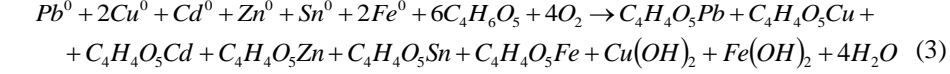
The aim of this study was to correlate the substances (metals) quantities that migrate from the package and are transferred into the food stuff at the interaction surface between the food and the metallic can by realizing the materials balance on their metallic components.

Experimental

Materials – The experimental studies and researches regarding the interaction between the food stuff and the metallic can were led on the food products Rehydrated pea and Tomato paste both canned in specific cans made from varnished metallic materials. It was tested a number of approximately 131 cans of each canned product with the dimensions of $\varnothing 99 \times 118$ mm and varnished with the following types of lacquers: on the inner can surface - white lacquer PL 1333-16, on the outer surface - lacquer PL 1014-59 grace. **Digestion procedures** - Samples in rehydrated peas and tomato paste cans were prepared by dried mineralization according to the Committee Regulation no.333/2007, using a Berghof equipped with temperature and pressure sensors. **Instrumentation** - A Shimadzu Atomic Absorption Spectroscopy (AAS) - AA- 6300, equipped with a flame atomization system, electrochemical atomization, hydride generator, autosampler, data acquisition and processing soft was used for the analysis of metals in samples of rehydrated peas and tomato paste cans. Hollow cathode lamps of Cd, Pb, Cu, Fe, Zn and Sn emitting at characteristic wavelengths of the elements ($\lambda_{Cu} = 228.8$ nm, $\lambda_{Pb} = 283.3$ nm, $\lambda_{Cd} = 324.8$ nm, $\lambda_{Fe} = 248.3$ nm, $\lambda_{Zn} = 213.9$ nm, $\lambda_{Sn} = 286.3$ nm) were used as the radiation source.

Results and discussion

In the case of the cans with vegetable products - *Rehydrated peas can* and *Tomato paste* can the initial reaction mass is considered to be formed by the organic acids that are present in the vegetables. The organic acids are found in the vegetables as salts and their derivatives and influence the taste and preservation of vegetables. The initial reaction mass in the case of Rehydrated peas cans and Tomato paste cans is considered to be formed by metals (lead, cadmium, copper, iron, zinc and tin) that are part of the structure formed by the metallic material - the lacquering system of the cans and the malic acid from the food product in the case of the peas and the citric acid in the case of the tomatoes. The stoichiometric equations of the chemical processes that take place at the interaction surface between the food stuff and the metallic can are represented by equation (4) in the case of the Rehydrated peas cans and by equation (3) in the case of the Tomato paste cans.



To express the quantitative state of the interaction reaction between the food stuff and the metallic can at one moment we appealed to the degree of transformation η_{pb} of the valuable component or of an invaluable component $\eta_{C_4H_6O_5}$. The mass of each component at a certain moment is expressed by the equations of the materials balance in table 1:

Table 1. The relations of the materials balance for the components that migrate from the structure metallic material- cans lacquering system into the canned food stuff

Rehydrated peas canned	Tomato paste
Entered materials	
$m_{Pb^0} = m_{Pb^0}^0 - m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Pb^0} = m_{Pb^0}^0 - m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Cu^0} = m_{Cu^0}^0 - \frac{M_{Cu^0}}{M_{Pb^0}} \cdot \frac{V_{Cu^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Cu^0}^0 - 2 \cdot \frac{M_{Cu^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Cu^0} = m_{Cu^0}^0 - \frac{M_{Cu^0}}{M_{Pb^0}} \cdot \frac{V_{Cu^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Cu^0}^0 - 2 \cdot \frac{M_{Cu^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Cd^0} = m_{Cd^0}^0 - \frac{M_{Cd^0}}{M_{Pb^0}} \cdot \frac{V_{Cd^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Cd^0}^0 - \frac{M_{Cd^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Cd^0} = m_{Cd^0}^0 - \frac{M_{Cd^0}}{M_{Pb^0}} \cdot \frac{V_{Cd^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Cd^0}^0 - \frac{M_{Cd^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Zn^0} = m_{Zn^0}^0 - \frac{M_{Zn^0}}{M_{Pb^0}} \cdot \frac{V_{Zn^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Zn^0}^0 - \frac{M_{Zn^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Zn^0} = m_{Zn^0}^0 - \frac{M_{Zn^0}}{M_{Pb^0}} \cdot \frac{V_{Zn^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Zn^0}^0 - \frac{M_{Zn^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Sn^0} = m_{Sn^0}^0 - \frac{M_{Sn^0}}{M_{Pb^0}} \cdot \frac{V_{Sn^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Sn^0}^0 - \frac{M_{Sn^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Sn^0} = m_{Sn^0}^0 - \frac{M_{Sn^0}}{M_{Pb^0}} \cdot \frac{V_{Sn^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Sn^0}^0 - \frac{M_{Sn^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Fe^0} = m_{Fe^0}^0 - \frac{M_{Fe^0}}{M_{Pb^0}} \cdot \frac{V_{Fe^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Fe^0}^0 - 2 \cdot \frac{M_{Fe^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Fe^0} = m_{Fe^0}^0 - \frac{M_{Fe^0}}{M_{Pb^0}} \cdot \frac{V_{Fe^0}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{Fe^0}^0 - 2 \cdot \frac{M_{Fe^0}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_6O_5} = m_{C_4H_6O_5}^0 - \frac{M_{C_4H_6O_5}}{M_{Pb^0}} \cdot \frac{V_{C_4H_6O_5}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{C_4H_6O_5}^0 - 6 \cdot \frac{M_{C_4H_6O_5}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_6O_5} = m_{C_4H_6O_5}^0 - \frac{M_{C_4H_6O_5}}{M_{Pb^0}} \cdot \frac{V_{C_4H_6O_5}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{C_4H_6O_5}^0 - 6 \cdot \frac{M_{C_4H_6O_5}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{O_2} = m_{O_2}^0 - \frac{M_{O_2}}{M_{Pb^0}} \cdot \frac{V_{O_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{O_2}^0 - 4 \cdot \frac{M_{O_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{O_2} = m_{O_2}^0 - \frac{M_{O_2}}{M_{Pb^0}} \cdot \frac{V_{O_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = m_{O_2}^0 - 4 \cdot \frac{M_{O_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
Out materials	
$m_{C_4H_4O_5Pb} = \frac{M_{C_4H_4O_5Pb}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Pb}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Pb}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Pb} = \frac{M_{C_4H_4O_5Pb}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Pb}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Pb}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_4O_5Cu} = \frac{M_{C_4H_4O_5Cu}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Cu}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Cu}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Cu} = \frac{M_{C_4H_4O_5Cu}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Cu}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Cu}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_4O_5Cd} = \frac{M_{C_4H_4O_5Cd}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Cd}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Cd}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Cd} = \frac{M_{C_4H_4O_5Cd}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Cd}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Cd}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_4O_5Zn} = \frac{M_{C_4H_4O_5Zn}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Zn}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Zn}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Zn} = \frac{M_{C_4H_4O_5Zn}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Zn}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Zn}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_4O_5Sn} = \frac{M_{C_4H_4O_5Sn}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Sn}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Sn}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Sn} = \frac{M_{C_4H_4O_5Sn}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Sn}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Sn}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{C_4H_4O_5Fe} = \frac{M_{C_4H_4O_5Fe}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Fe}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Fe}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{C_4H_4O_5Fe} = \frac{M_{C_4H_4O_5Fe}}{M_{Pb^0}} \cdot \frac{V_{C_4H_4O_5Fe}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{C_4H_4O_5Fe}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Cu(OH)_2} = \frac{M_{Cu(OH)_2}}{M_{Pb^0}} \cdot \frac{V_{Cu(OH)_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{Cu(OH)_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Cu(OH)_2} = \frac{M_{Cu(OH)_2}}{M_{Pb^0}} \cdot \frac{V_{Cu(OH)_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{Cu(OH)_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{Fe(OH)_2} = \frac{M_{Fe(OH)_2}}{M_{Pb^0}} \cdot \frac{V_{Fe(OH)_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{Fe(OH)_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{Fe(OH)_2} = \frac{M_{Fe(OH)_2}}{M_{Pb^0}} \cdot \frac{V_{Fe(OH)_2}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = \frac{M_{Fe(OH)_2}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$
$m_{H_2O} = \frac{M_{H_2O}}{M_{Pb^0}} \cdot \frac{V_{H_2O}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = 4 \cdot \frac{M_{H_2O}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$	$m_{H_2O} = \frac{M_{H_2O}}{M_{Pb^0}} \cdot \frac{V_{H_2O}}{V_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0} = 4 \cdot \frac{M_{H_2O}}{M_{Pb^0}} \cdot m_{Pb^0}^0 \cdot \eta_{Pb^0}$

On the basis of the chemical equations and the relations in table 1 we obtained the numerical values for the theoretical materials balance and for the real one for the processes at the interaction surface between the food stuff - Rehydrated peas and Tomato paste - and the metallic package/ the metallic cans during the shelf life. The graphic representations are given in the figures 1, 2, 3 and 4.

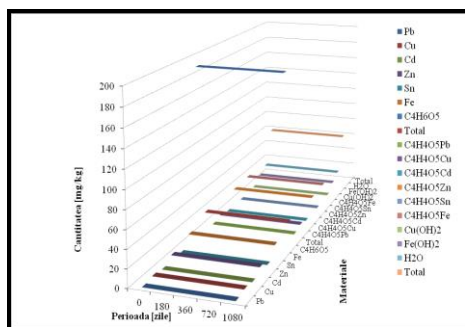


Fig. 1. The theoretical materials balance for the components that migrate from the structure metallic material- cans lacquering system into the Rehydrated peas canned food stuff

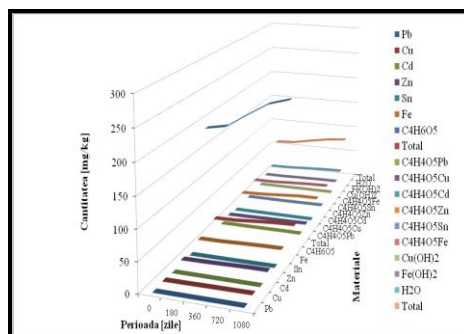


Fig. 2. The real materials balance for the components that migrate from the structure metallic material- cans lacquering system into the Rehydrated peas canned food stuff

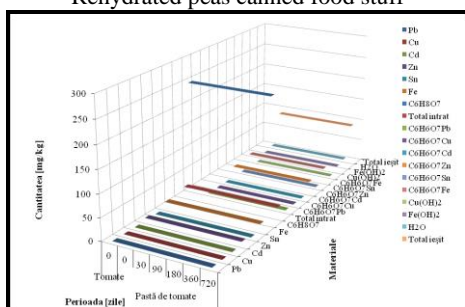


Fig. 3. The theoretical materials balance for the components that migrate from the structure metallic material- cans lacquering system into the Tomato paste canned food stuff

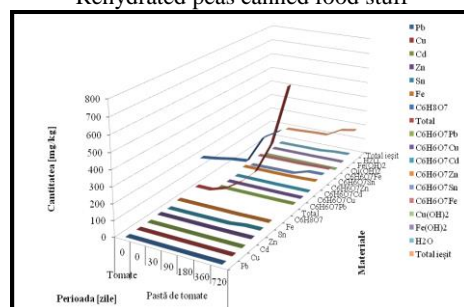


Fig. 4. The real materials balance for the components that migrate from the structure metallic material- cans lacquering system into the Tomato paste canned food stuff

The lead and cadmium are toxic metals that can be very dangerous ingested for a long period of time even at low concentrations. The lead can have negative effects on the peripheric nervous system and can induce severe encephalopathies, and cadmium is accumulated in the liver and kidneys determining kidney dysfunction, skeletal damage and reproductive deficiencies. According to the CF/5 INF/1/2011 Document of Codex Alimentarius Commission, there are traced certain limits for the metallic contaminants in the food stuff: 0.05 – 0.1 mg Cd/kg vegetables and 0.2 – 0.3 mg Pb/kg vegetables; 1.0 mg Pb/kg fruits and canned vegetables, 1.5 mg/kg processed tomato concentrates. Copper is known to both vital and toxic for many biological systems and may enter the food materials from soil through mineralization by crops, food processing or environmental contamination, as in the application of agricultural inputs, such as copper-based pesticides which are in common use in farms in some countries (Onianwa et al., 2001). Zinc is one of the

most important trace metals for normal growth and development of humans. The average daily intake has been estimated to be maximally 20 mg Zn/day for adults. It is known that adequate iron in a diet is very important for decreasing the incidence of anemia. Food is the main source of tin for man. Larger amounts of tin may be found in foods stored in plain cans and, occasionally, in foods stored in lacquered cans. The maximum tin level permitted for canned vegetables samples is 250 mg/kg according to the CF/5 INF/1/2011 Document of Codex Alimentarius Commission.

According to the data from the real materials balance, in the case of the two canned products from figure 2 and figure 4 as a consequence of the chemical processes that took place during the shelf life at the interaction surface between the food stuff and the metallic package the levels of their own metals and salts increase in time. An intense increment of the level of the migration metals from the structure metallic material- cans lacquering system into the food stuff can be observed in a storage period between 180 - 720 days. In this period their can be overpassed the maximum limits admitted for the metals and so the safety of the food stuff can be altered.

Conclusion

In general, the levels of trace metals in canned foods were higher than vegetable samples and it increases during the cans shelf life. The real balance of the migration materials offers us indications regarding the total metals and salts content accumulated in the cans during the products shelf life giving the possibility of action for to obtain and establish a storage period in total safety conditions for the consumers. The work allows concluding that, in order to keep contamination to minimum, canned foods must be consumed in the firsts two months after packaging. The levels may be reduced by more careful handling practices, processing of raw vegetable and using the good quality of packing.

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IMPROVEMENT OF TEXTURAL PROPERTIES OF FERMENTED MILK BY USING STREPTOCOCCUS THERMOPHILUS STRAINS

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Abstract: Monitoring of pure cultures of *Streptococcus thermophilus* strains from Branch Collection of industrial lactic acid bacteria (LAB) was made in the Laboratory of Food Biotechnology. LAB are useful in the food industry for their contribution to the organoleptic properties of the final fermented food. Extracellularly secreted sugar polymers, or EPS, are partly responsible for this. In general, the food industry is particularly interested in natural viscosifiers and texture enhancers, so-called biothickeners. EPS production is an important feature of *Streptococcus thermophilus* characterization in forming starter cultures for fermented milk products with suitable texture and specific rheology.

Key words: *Streptococcus thermophilus*, exopolysaccharide, texture, starter cultures, dairy products

Introduction

Lactic acid bacteria (LAB) are useful in the food industry, not only because of their ability to acidify and hence preserve food products from spoilage, but also for their contribution to the organoleptic properties of the final fermented food. LAB play an important role in the texture development of yogurts and other fermented milks, low-fat cheeses, and dairy desserts [1]. Extracellularly secreted sugar polymers, or exopolysaccharides (EPS), are partly responsible for this. In general, the food industry is particularly interested in natural viscosifiers and texture enhancers, so-called biothickeners. These are mostly plant carbohydrates (e.g., starch, pectin, and guar gum), animal hydrocolloids (e.g., gelatin and casein), or bacterial biopolymers (e.g., xanthan and gellan). Also, the EPS produced by LAB may have technological and health benefits in food products.

Low-fat fermented milk are increasingly popular due to their nutritional and potentially therapeutic characteristics. Fat reduction can cause some defects in yogurt such as lack of flavour, weak body and poor texture. Several studies have discussed the improvement of physical, textural, flavor and rheological properties of low-fat yogurts by incorporating the stabilizers into the milk. This increases the viscosity and reduces syneresis.

The EPS produced by low-fat fermented milk starter cultures affect the textural and physical properties of final product and improve the sensory characteristics such as mouthfeel, shininess, clean cut, ropiness and creaminess [2]. Ropy EPS possess high water binding ability, resulting in increased water retention in yogurt. Using EPS producing cultures when developing a low-fat yogurt with physiological functions is necessary.

Streptococcus thermophilus is considered one of the most commercially important of the lactic acid bacteria. *Streptococcus thermophilus* also produces exopolysaccharides. These are essential to the texture of fermented milk products and also to the production of reduced-fat dairy products that maintain similar characteristics to their full-fat counterparts [3].

Dairy strains of *Streptococcus thermophilus* that produce exopolysaccharides (EPSs) have attracted interest recently, since the EPSs act as in situ-produced natural biothickeners that improve the texture of fermented foods. Thus, a high EPS production in situ during the fermentation of milk to yogurt could be an advantage for the food industry. However, EPSs from *S. thermophilus* strains are produced at relatively low levels, i.e., 50 to 400 mg per liter [4].

The objectives of this study was to monitor of pure cultures of *Streptococcus thermophilus* strains from Branch Collection of industrial (LAB) isolated from dairy products of spontaneous fermentation and to investigate the effect of EPS-producing culture on the textural and physical characteristic of fermented low-fat milk.

Materials and methods

Cultures

Nine strains of *S. thermophilus* were studied on ropy characteristic. They were isolated from different dairy products of spontaneous fermentation from various regions of Moldova and are stored in The Branch Collection of industrial lactic acid bacteria from the Laboratory of Food Biotechnology and The National Collection of Non-Pathogenic Microorganisms ASM.

Manufacture of dairy product of type “Reazhenka”

Ryazhenka is produced through fermentation of bacteria in the milk to “roast”. Roasting process involves heating milk, standardized milk until it reaches a minimum temperature of 95 ° C for about three hours. This roasting process produces a brownish color and flavor of milk, like cream caramel.

The media used for fermentations were low fat milk medium. Was prepared starter culture combination with add of EPS-producing strain of *S. thermophilus*. The transfer inoculum was 5.0% (vol/vol) in milk medium. A Biostat A Sartorius fermenter with a working volume of 1,5 l was used to study the kinetics of exopolysaccharide production. The fermenter was operated at 40 °C. Slow agitation was maintained to keep the fermentation broth homogenous.

Isolation and quantification of EPS

EPS was isolated from the fermented milk sample using a next procedure. Trichloroacetic acid was added to the sample culture to a final concentration of 4% (w/v) and allowed to rest for 2 h at room temperature. After centrifugation (10000×g for 30 min at 4°C) to remove the precipitated proteins and bacterial cells, the supernatant was mixed with a double volume of cold ethanol and then stored at 4°C for 24 h. The precipitated EPS was collected by centrifugation (10000×g for 30 min at 4°C), dissolved in deionized water and mixed with a double volume of cold ethanol and stored at 4°C for 24 h. EPS was precipitated with ethanol. It was recovered by centrifugation at 4°C 10000×g for 30 min. Total EPS (expressed as mg/L) was estimated in each sample by phenol–sulphuric method.

Spontaneous whey separation analysis

A cup of set fermented skim milk was removed from refrigerator at 4°C, weighed and kept at approximately 45°C to allow the whey on the surface to be collected on the side of the cup. A needle connected to a syringe was used to withdraw the liquid whey from the surface of the sample, and the cup of fermented skim milk was weighed again. The process lasted for less than 10 s to avoid further leakage of whey from the curd. The syneresis was expressed as the percentage weight of the whey over the initial weight of the fermented skim milk sample.

Results and discussion

Nine strains of *S. thermophilus* were studied on ropy characteristic. Was selected one strain of *S. thermophilus* which demonstrated viscosity of gel. Characteristics of the ropy strain are listed in Table 1.

Table 1. Characteristics of the ropy strain of *S. thermophilus*

Characteristics	EPS-producing strain of <i>S. thermophilus</i>
Appearance of gel	Ropy, homogenous, dense, creaminess
Taste and smell of gel	Are clean, sour-milk, moderately sweet, without any foreign taste and smell
Fermentation duration, h	4,5

In the present study we observe that ropy strain of *S. thermophilus* (Figure 1) showed faster growth capacity compared with other studied strains.

The EPS produced by low-fat fermented milk starter cultures affect the textural and physical properties of final product and improve the sensory characteristics such as mouthfeel, shininess, clean cut, ropiness and creaminess. Figure 2 shows the changes in spontaneous whey separation of fermented skim milk. The use of ropy EPS-producing strain reduced the level of syneresis in fermented skim milk. Non-EPS-producing strain of *Streptococcus thermophilus* from Branch Collection of industrial lactic acid bacteria has demonstrated this capacity on the low level. This may be due to high water binding capacity of EPS and reduce permeability of serum through skim milk gel.

In this study we are selected one EPS-producing strain of *S. thermophilus* and was made quantification of EPS. The amount of EPS produced by ropy strain reached 29.1 mg/L. According to some authors, the production of exopolysaccharides by the culture is very low, maximum of ~31,4 mg/L, but it plays a major role in the development of texture of the final product [5].

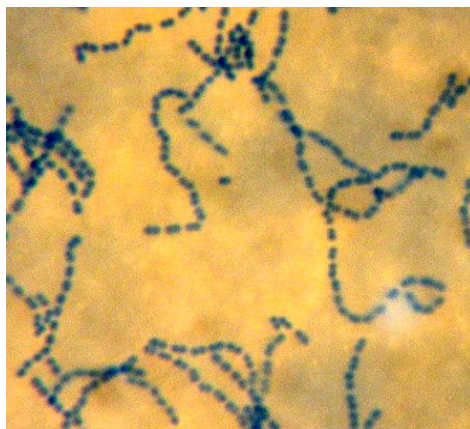


Fig. 1 *Streptococcus thermophilus* EPS-producing strain

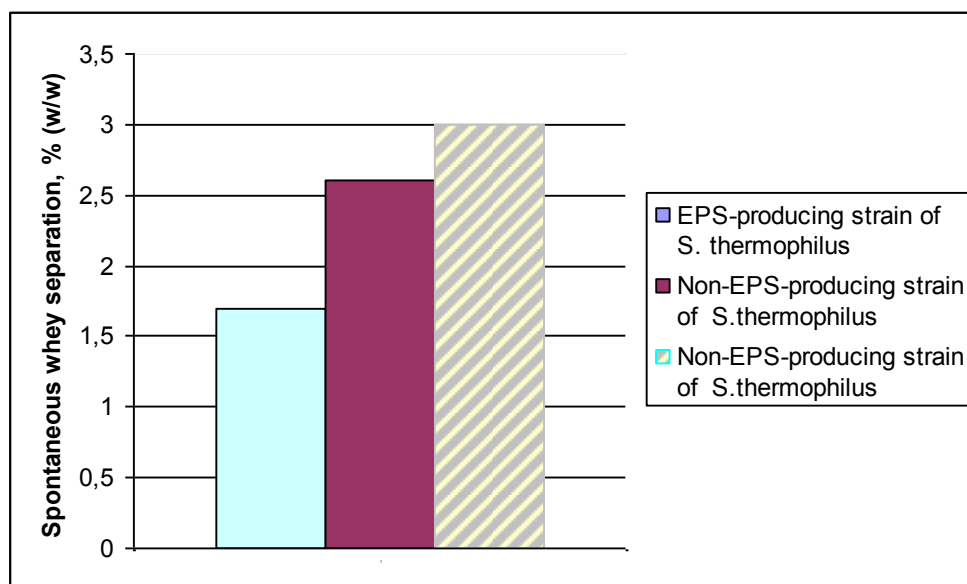


Fig. 2. Effects of ropy EPS-producing strain of *S. thermophilus* and non-EPS-producing strain of *S. thermophilus* on spontaneous whey separation of the fermented skim milk.

Was prepared samples of fermented skim-milk using added ropy EPS-producing strain of *S. thermophilus* in combination of culture starter and non-EPS-producing culture of *S. thermophilus* + commercial stabilizer.

Table 2. Characteristics of the fermented milk tipe “Ryazhenka” obtained with ropy EPS-producing culture and with non-EPS-producing culture+ commercial stabilizer

Characteristics	Fermented milk tipe “Ryazhenka” with ropy EPS-producing culture	Fermented milk tipe “Ryazhenka” with non-EPS-producing culture+ commercial stabilizer
Appearance	Ropy, homogenenious, dense, creaminess	Homogenenious, dense, creaminess
Taste and smell	Are clean, sour-milk, without any foreign taste and smell	Are clean, sour-milk, , without any foreign taste and smell
Fermentation duration, h	4,5	5,0
Acidity, °T	76	78

From table 2 we observe that “Ryazhenka” obtained with culture starter with added EPS-producing strain of *S. thermophilus* demonstrate characteristics identical “Ryazhenka” produced with commercial stabilizer. However, fermentation duration passed quicker.

Hence, the using of ropy EPS-producing starter culture reduced the level of syneresis in fermented skim milk significantly. This may be due to high water-binding capacity of EPS and reduce permeability of serum through skim milk gel. [6]

Conclusions

Results indicate that exopolysaccharides produced by *S. thermophilus* have a considerable effect on the physiochemical properties and the textural characteristics of fermented skim milk by EPS-producing culture compared with the samples using with non-EPS-producing culture+commercial stabilizer. EPS-producing culture may be effective in improving the viscosity and the water holding capacity as well as reducing spontaneous whey separation. Described selected strain recommended and may be used like added strain in composition of culture starter for manufacturing dairy products.

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SOME 3D-METALS DIOXIMATES– BIOSTIMULATORS FOR ENZYMES PRODUCTION OF INDUSTRIAL IMPORTANCE

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Abstract: A range of cobalt(III), copper(II) and zinc coordination compounds with oxime ligands were synthesized. For obtained complexes the composition and structure were determined by modern physicochemical methods: elemental analysis, IR and UV-Vis spectroscopies, ^1H , ^{12}C , ^{19}F NMR, as well using the single crystal x-ray diffraction. After a conducted screening some complexes were selected which were used as stimulators of biosynthetic processes in some strains of fungi of *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium* genus, for intensification of enzymogenesis (amylases, pectinases, lipases, proteases, cellulase, xylanases, etc..), biomass accumulation, shortening of time of technological process, as well as stabilizers of fungi activity in adverse conditions.

Key words: coordination compounds, stimulators, biosynthetic processes, fungi

Coordinative compounds of transition metals with chelating ligands occupy an important place in contemporary chemistry. This class also includes the dioximates of transition metals. The complexation capacity of α -dioximes with metals of d -type draws the researchers' attention not only from the perspective of the hemoglobin or vitamin B₁₂ pattern synthesis [1], but also from one of a broad spectrum of synthetic, analytical and structural possibilities. Dioximates can be used as catalysts for industrial processes [2], stimulators of erythropoietin functions [3], anti-hypoxic preparations [4], preparations with antidote properties [5], basis to obtain new semiconducting materials [6], for the separation and purification of the metals that are complex generators etc.

The interest towards the complexes of $3d$ elements with α -dioximes is subject to their stereo-chemical and electronic structural diversity, presenting perspective objects to elucidate the general principles between composition, structure and properties. The varied structure of dioximates is due to different synthesis conditions (the pH of the solution, etc.), to the nature of axial ligands, to anions in the external sphere and other factors.

From the point of view of practical applicability of the dioximates of transition metals, there are now clearly outlined several directions in which the representatives of this class can successfully be used: elaboration of some artificial models of biological molecules of vital importance; elaboration of efficient bio-technologies to obtain enzyme preparations used in various branches of food and pharmaceutical industries, obtaining some compounds with useful medical properties, synthesizing materials with valuable physical properties (semiconductors, photo-luminescent materials); obtaining some systems to use in hydrogen production, etc.

Modern bio-technology pays a special attention to directed synthesis of bio-active substances by micro-organisms based on valuable fundamental studies with implications for medicine, micro-biological, chemical, pharmaceutical, cosmetic industries etc. Micro-organisms, due to specific features, as enhanced response to ambient changes, adaptive metabolism, short development cycle, are convenient objects for various researches. From

another point of view, organisms are recognized as advantageous economical sources for obtaining a wide range of important bio-active substances.

The ligands also play an important role in the manifestation of metal complexes properties as a part of their composition and, due to the wide set of donor atoms, create stable complexes with transition metal ions, different in composition, structure and properties. The biologically active substances, included as ligands in the composition of the metal complexes, essentially increase their efficiency [7, 8].

Previously, Co(III) dioximate with nicotinamide was tested in the treatment of hemolytic anemia and other diseases [3]. Co(III) dioximates can also be used as an antidote to extract various toxins (cyanide, phosphamide etc.) from the body [9].

There was performed the oriented synthesis of a series of dioximates of Co(III) with the general formula $[\text{Co}(\text{DioxH})_2\text{L}_2]_n\text{X}\cdot n\text{H}_2\text{O}$, where DioxH is the monoanion of the dioxime: dimethylglyoxime (DH_2), methylglyoxime (MH_2), 1,2-cyclo-hexandiondioxime (NioxH_2), α -benzildioxime (DfH_2); L - thiocarbamide (Thio), pyridine (Py), aniline (An), nicotinamide (PP), sulphanilamide (Sam); X - $[\text{BF}_4]^-$, $[\text{PF}_6]^-$, $[\text{SiF}_6]^{2-}$, $[\text{ZrF}_6]^{2-}$, $[\text{TiF}_6]^{2-}$ etc., crystal structure and spectral characteristics of which have proven *trans*-octahedral configuration of the complexes. There have been studied the chemical and physical properties. It was stated that the anions in the external sphere and crystallization water molecules have an important role in the field of in the formation of the crystalline structure. The analyzed complexes were used as additives in the nutrition environment for some strains of fungi. The testing of the dioximates of cobalt(III) in the Laboratory of Enzymology of the Institute of Microbiology and Biotechnology of the ASM on the gender strains of *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium* showed that these complexes beneficially influence on the development of some biological processes of the respective fungi [10-14].

Structural and compositional complexity, the metal presence as central atom shows the perspective of using the coordinative compounds as stimulators and regulators of biological processes in the cell of microorganisms. The inclusion of several micro-elements (eg: Co, Si, F; Co, P, F; Co, B, F and Co, Ti, F) in the composition of the same complex contributes to the manifestation of the bio-stimulating properties of the respective dioximates. Cobalt is a necessary element for the biological systems. Other elements are equally necessary for organisms. Boron, for example, participates in regulating membrane functions, biosynthesis of nucleic acids. Titanium is an indispensable element for many organisms, with vital functions: increases erythropoiesis, and catalyzes the synthesis of hemoglobin, immunogenesis [15]. Complexes of titanium are not only stimulating agents of phagocytosis, but also substances that activate cellular and humoral immune reactions. Titanium chelates, as we know, affect the reproduction function of some animal species. Experimentally, it has been proved that titanium is an element that is easily removed from the body and does not present danger to get accumulated in large quantities. Moreover, being considered a bio-compatible element it is used in medicine to join fractured bone tissue.

The synthesis of tested complexes was conducted in an aqua-methanol medium. As a result of the analysis of the IR spectra for thiocarbamide there were observed some changes in the values of the absorption bands of valence and deformation vibrations related to the respective oscillation values in the spectrum of free thiocarbamide. The frequencies $\nu_{\text{as}}(\text{NH})=3310\text{-}3370$, $\nu_{\text{s}}(\text{NH})=3210\text{-}3240$, $\delta(\text{NH}_2)=1615\text{-}1625$, $\delta(\text{NCS})=412\text{-}415\text{ cm}^{-1}$ indicate the presence of thiocarbamide coordinated to the metal.

In the IR spectra of the complexes that contain pyridine there are manifested the characteristic bands to the *trans*-dioximates Co (III): $\nu(\text{C}=\text{N})=1556\text{--}1588$ and $\sim 1455\text{ cm}^{-1}$, the bands of deformation vibrations $\delta(\text{C}-\text{H})=676\text{--}765\text{ cm}^{-1}$ of the mono-substituted aromatic ring, $\nu(\text{Co}-\text{N})=510\text{--}520$ and $460\text{--}465\text{ cm}^{-1}$. The valence frequencies changes $\nu(\text{C}-\text{H})=3120\text{--}3230\text{ cm}^{-1}$ and $\nu(\text{C}=\text{C})=1610\text{--}1615\text{ cm}^{-1}$ confirm the coordination of pyridine molecules to the metal. These two bands are shifted to higher values compared to their position in the spectrum of uncoordinated pyridine.

The dimethylglyoxime monoanion in complexes is confirmed by the bands $\nu(\text{CN})=1560\text{--}1580$, $\nu_{\text{as}}(\text{NO})=1230\text{--}1245$, $\nu_{\text{s}}(\text{NO})=1080\text{--}1095$, $\delta(\text{OHO})=1825\text{--}1750$, $\gamma(\text{OH})=970\text{--}985$, $\gamma(\text{CNO})=730\text{--}745$, $\nu_{\text{as}}(\text{Co}-\text{N})=505\text{--}525$ and $\nu_{\text{s}}(\text{Co}-\text{N})=425\text{--}440\text{ cm}^{-1}$. The coordination of the dioximes to the central atom is proved by the appearance of new bands or by intensity changing of the existing bands in the spectrum. For example, the band that appears in the region $1220\text{--}1240\text{ cm}^{-1}$ refers to $\nu(\text{NO})$ of ionized N-OH group of the dioxime. This band does not appear in the spectrum of the free dioxime. The intensity decrease of the band $\gamma(\text{OH})=970\text{--}985\text{ cm}^{-1}$ and the appearance of a weak band $\delta(\text{OHO})=1825\text{--}1750\text{ cm}^{-1}$ proves the deprotoning of dioximes during coordination and the formation of intra-molecular hydrogen bonds of O-H...O type in the equatorial plane.

It was studied the influence of some dioximates of cobalt(III) on the strains of fungi of the genera *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium*. In parallel it was tasted the influence of the complexes and their parts (original salt, dioxime, neutral ligands) on the bio-synthetic processes. It was found that the influence of the complexes is higher, a fact which points to the possibility of using them as bio-stimulators of the enzymogenetic processes and bio-mass accumulation.

The results obtained on the basis of the influence testing of the dioximates Co(III) on physiological processes of some strains of micromycete allow concluding that in the series of the analyzed complexes it can be selected:

- bio-stimulators of enzymogenetic processes of fungi (eg: the complex $[\text{Co}(\text{DH})_2(\text{Thio})_2]_2[\text{SiF}_6]\cdot 3\text{H}_2\text{O}$ increases pectolytic activity of the fungi *Rhizopus Arrhizus* with 184.08% if compared to the control sample);
- catalysts for biomass accumulation processes (for example: at the introduction of the compound $[\text{Co}(\text{DH})_2(\text{Thio})_2][\text{BF}_4]\cdot 3\text{H}_2\text{O}$ with a concentration of 5-10 mg/l in the cultivation medium of the fungi strain of *Aspergillus niger 412* it was recorded a double increase of the biomass productivity - with 188.3-197.07% if compared to the control sample);
- stabilizers of the vital bio-chemical processes in unfavorable conditions (for example, in unfavorable thermal conditions, introducing complex $[\text{Co}(\text{DH})_2(\text{Py})_2]\text{BF}_4$ in the nutritional medium of fungi *Aspergillus niger 412* increased bio-mass accumulation process with 132.93% if compared to the control sample);
- accelerators of the biological development of micro-organisms, reducing the technological cycle with 24-48 hours, that is, to $\sim 30\%$ of the entire cycle length (when introducing the complex $[\text{Co}(\text{DH})_2(\text{An})_2]_2[\text{TiF}_6]$ in the nourishing medium of the micromycete *Aspergillus niger 33-19 CNMN FD 02A*), a fact that presents economic interest.

When using copper and zinc dioximic compounds as additives in the nourishing media of some strains of fungi it has also been established an intensification of the bio-synthetic processes.

The effect of metal complexes varies according to the origin of micro-organism, type of the synthesized enzymatic system, the composition and concentration of the compound [10-14]. Summarizing the obtained results, we can conclude that the tested dioximates show properties of stimuli (bio-mass accumulation, increased enzymatic activity), stabilize the bio-synthetic processes in unfavorable conditions of activity, reduce the technological cycle. All these properties are influenced by the presence of the ion Co^{3+} as a generating complex, as well as by the nature of the ligands in the internal sphere and that of the anion in the external one. These experimental results create favorable conditions for the development of economically advantageous technologies of cultivation some types of strains of fungi of industrial importance.

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THE STUDY OF THE PHTHALATES REMOVING PROCESS FROM ALCOHOLIC PRODUCTS

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Abstract: In the laboratory of National Center for Quality Testing of the Alcoholic Beverages there were investigated phthalates sorption processes in the most widely applicable sorbents in alcoholic products. Among them – activated carbon, bentonite, kieselguhr. Also the sorption capacity and a number of other synthetic drugs were investigated. In addition to determining the level of phthalates sorption, the impact on other components of a complex matrix of alcoholic products was defined. The conclusions on the potential use of adsorbents for removal of phthalates range of products were done.

Keywords: Phthalates, alcoholic beverages, sorbents, gas-chromatography mass-spectrometry.

Introduction

With the appearance of problems of phthalates in alcoholic products, it was necessary to remove these contaminants. Studies of sorption processes - degradation of phthalic acid esters are not new, and most of them study the impact of natural microorganisms in the soil [1,2], water (sewage sludge) [3,4], also some studies of removal from the aqueous phase onto activated carbon [5], in addition photocatalytic degradation of phthalate in aqueous TiO₂ suspension under UV illumination has been investigated by Baoling Yuan *et al* [6]. Food stuff and alcoholic beverages in particular in this sense, were ignored. In the laboratory of National Center for Quality Testing of the Alcoholic Beverages some investigations in the field of processes of natural removing phthalates from alcoholic products in different stages of productions were done. Several sorbents, which commonly used in wineries and distillery, were studied. Among them: activated carbon, bentonite and kieselguhr. Additionally, few specimens were examined as sorbents, such as silicagel, synthetic adsorbent, ion exchange resin, also yeasts influence on phthalates content in fermenting mash were determined.

Experimental

Material and methods

Investigations were done on the base of wines: white - dry& sweet, red - dry& sweet (4 types), hydro alcoholic solutions (40% v/v) and eau de vie aged.

For samples contamination were used solvents of phthalates prepared from individual substances – DMP (dimethylphthalate 99.6%), DEP (diethylphthalate 99.6%), DBP (dibutylphthalate 99.8%), DEHP (Bis(2-ethylhexyl)phthalate 99.7%), DOP (dioctylphthalate 99.7%), DDP (didecylphthalate 99.8%) PESTANAL from SIGMA-ALDRICH.

As a sorbents used Bentonite (CLARIT PLV-45), activated carbon (FA SIHA), silicagel L 40/100µ (for column chromatography), kizelgur (BECOGUR-3500), furthermore three types of synthetic polymers were tested, - anion exchange resin RELITE RAM1,

synthetic adsorbent RELITE SP 411 (adsorbent based on styrene and DVB copolymer) and cation exchange resin FIBAN K-1.

Instruments

GCMS-QP-2010S (IS) (SHIMADZU) with a COMBI PAL autosampler (CTC ANALYTICS) with fused silica column RESTEK - Rtx-5MS (30m/0.25mm/0.25 μ m 5% diphenyl / 95% dimethylpolisiloxane phase).

Capillary Electrophoresis System – CAPEL – 105M (LUMEX).

Alcolyzer Wine (ANTON PAAR) plus density meter DMA-4500.

Gas chromatography–mass spectrometry

Measuring the concentration of phthalates in wine and base-wine was based on its elimination by chloroform extraction, chromatographic separation on a capillary column, identify by retention time and mass spectrum, and quantify with the characteristic ions m/z. Measuring the concentration of phthalates in alcoholic beverages such as vodka, brandy, cognac alcohol, rectified ethyl alcohol was based on chromatographic separation of the sample on a capillary column using aldrin with a purity above 99.3% and supplied by SUPELCO as an internal standard, the identification was made by retention times and mass spectrum, quantification – of characteristic ions m/z for phthalates and for aldrin respectively.

Results and Discussion

Activity of sorbents described below in relation to phthalates was studied on the different matrices. Firstly, sorption capacity was investigated for six phthalates in the base of hydro-alcoholic solution (40% v/v). With this purpose all types of sorbents were prepared in accordance with relevant procedures – INTERNATIONAL OENOLOGICAL CODEX – Bentonites COEI-1-BENTON: 2003, diatomite (kieselguhr) COEI-1-DIATOM: 2002, Oenological Carbon COEI-1-CHARBO: 2007. Three synthetic specimens were prepared conform manufacturer's recommendations. Silicagel has been washed with alcohol (not contain phthalates). Batch of phthalates solutions with sorbent was obtain. In this series ratio of phthalate to sorbent (by weight) were approx. 1:5000, 1:10000, 1:50000, 1:100000, 1:500000. Concentrations of phthalates in the solutions were equimolar to each other, and ranged from $0.05 \cdot 10^{-6}$ to $5 \cdot 10^{-6}$ molL⁻¹. Sorption mixture was agitated for a pre-determined time period (150min) using orbital shaker (BIOSAN PSU-20i), agitation - 120rpm, temperature - 25°C. The residual phthalate concentration in the hydro-alcoholic phase was analyzed by gas chromatograph with mass spectrometer. Sorption capacity of different natural and synthetic sorbents is shown in tab.1. Capacities are given in ppm (mg of phthalates in kg of sorbent).

Tab.1. Sorption capacity of different natural and synthetic sorbents

Sorbent	DMP, ppm	DEP, ppm	DBP, ppm	DEHP, ppm	DOP, ppm	DDP, ppm
Carbon	0	0-8,5	4,85-180,0	16,0-675,0	31,5-2060,0	50,7-3670,0
Bentonite	0,2-3,0	0,2 - 4,0	0,25 - 4,7	2,25-12,5	4,9 - 24,4	6,6 - 41,2
Kieselgur	0 - 0,1	0 - 0,3	1,55 - 35,0	2,2 - 41,5	3,6 - 44,0	10,0 - 62,5
Silicagel	0 - 0,8	0 - 1,1	0 - 3,4	0 - 11,8	0,5 - 31,0	1,1 - 32,1
Fiban K-1	0	0	0,5 - 10,0	2,0 - 70,0	17,2 - 290,0	30,9 - 2910,0
RAM 1	0,9 - 11,0	0,95 - 45,0	1,6 - 85,0	5,7 - 495,0	16,1 - 810,0	37,8 - 9500,0
SP 411	2,4 - 70,0	3,0 - 13,0	3,2 - 55,0	14,7 - 975,0	30,0 - 2460	59,0 - 10100

The most of the capacities values are given in the form of intervals, because sorption depends on the concentrations of phthalates and phthalates content in the solutions – for one or all six together in the same solution. As it can be seen from the table, the most natural specimens do not have a high sorption activity with the exception of carbon. The highest activity was showed by synthetic materials. All examined sorbents showed a common tendency. Sorption capacity of each sorbent is proportional to molecular weight of phthalate isomers. Perhaps this is due to the labiality of the phthalate's molecules. This picture takes place in the case of hydro-alcoholic base only. With complication of this alcoholic matrix – to make it more closer to eau-de-vie or brendy by reproduction the mineral composition (basic cations) according to the Skourikhin I. M. [7], the picture changes significantly. For this purpose hydro-alcoholic solutions with phthalates (concentrations above) were prepared with addition of salts. Concentrations of basic cations (Na, K, Mg, Cu, Fe, Pb) were created by corresponding salts ($\text{NaKC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$). As a result, for a twenty-four hours in static conditions, $T=25^\circ\text{C}$, the sorption of DBP and DEHP decreased on 100% with carbon, kiselgur and bentonite. The following proportions sorbent / wine distillate were used – 0.25g/25ml, 0.50g/25ml.

The action of sorbents was subjected to real aged wines distillates in the similar conditions and with the same proportions. The degree of carbon's and bentonite's exposure on the aged wines distillates were established by capillary electrophoresis. The results of studies of sinapaldehyde, coniferaldehyde, syringaldehyde and vanillin are given in the tab.2.

Tab.2. Effect of sorbents on the content of aromatic aldehydes, mg L⁻¹.

		Sinapaldehyde	Coniferaldehyde	Syringaldehyde	Vanillin
Carbon	control	1,76	1,60	3,20	1,48
	0,25g/25ml	1,40	1,14	2,80	1,26
	0,50g/25ml	0,84	0,70	2,60	1,14
Bentonite	control	1,66	1,54	2,80	1,34
	0,25g/25ml	1,72	1,54	2,80	1,38
	0,50g/25ml	1,68	1,58	2,80	1,44

As a result, concentrations of DBP and DEHP were down on 3-5%, the most significant impact had a carbon to "heavy" phthalates – DOP and DDP.

By GC and GCMS methods were established that carbon, kiselgur, silicagel and bentonite did not affect the flavor of wine and eau-de-vie, due to the bad sorption of light ethers (esters) and aldehydes as well as the fusel alcohols.

Furthermore, the influence of some sorbents on phthalates in wine also was investigated. With this purpose samples of wines with six phthalates were created. Four types of wine were given for the experiment – white dry, white sweet (sugar - 80g L⁻¹), red dry and red sweet (sugar - 160g L⁻¹). Concentrations of phthalates were in the range of 0.01-0.03mg L⁻¹. On the basis of previous studies, the following proportions of the sorbent-wine were taken: bentonite, kiselgur, silicagel – 1/100, carbon – 1/1000. Process of sorption was carried out within 5 days in static conditions, $T=25^\circ\text{C}$. As a result the sorption by bentonite, kiselgur, silicagel and carbon of DMP and DEP were also close to zero. Sorption ability of bentonite, kiselgur and silicagel for DBP was approximately equal to 5-10%. In the case of carbon, despite the fact that carbon is 10 times less, sorption of DBP was 20-

25%. DEHP was sorbed on the bentonite, kizelgur and silicagel by 10-15%, on the carbon (1/1000) – 40-50%. DOP and DDP were sorbed on the carbon by 40-50% too. Bentonite and silicagel showed high activity toward DOP and DDP – 70-80%, kizelgur – 40-60%. In general, we can conclude, that difference in the sorption of different types (four) of wines is not significant.

Research on the instrument by ANTON PAAR shows changes of density, total extract and alcohol content of wines in tab.3.

Tab.3. Effect of sorbents on the density, total extract and alcohol content of wines.

Bentonite 2g/200ml (1:100)	parameter before treatment/after treatment		
	alc % v/v	density g/l	total extract g/l
white/dry	11,88/11,09	0,9898/0,9909	18,90/18,88
red/dry	11,16/11,01	0,9927/0,9929	24,25/24,22
white/sweet	13,60/13,09	1,0178/1,0182	96,87/96,41
red/sweet	16,44/15,30	1,0448/1,0455	175,72/174,28
Carbon 0,2g/200ml (1:1000)	parameter before treatment/after treatment		
	alc % v/v	density g/l	total extract g/l
white/dry	11,88/11,81	0,9898/0,9897	18,90/18,73
red/dry	11,16/11,14	0,9927/0,9927	24,25/24,06
white/sweet	13,60/13,57	1,0178/1,0175	96,87/96,00
red/sweet	16,44/16,39	1,0448/1,0448	175,72/175,57
Silicagel 2g/200ml (1:100)	parameter before treatment/after treatment		
	alc % v/v	density g/l	total extract g/l
white/dry	11,88/11,50	0,9898/0,9904	18,90/18,81
red/dry	11,16/11,16	0,9927/0,9929	24,25/24,13
white/sweet	13,60/13,32	1,0178/1,0180	96,87/96,57
red/sweet	16,44/15,84	1,0448/1,0459	175,72/175,69
Kizelgur 2g/200ml (1:100)	parameter before treatment/after treatment		
	alc % v/v	density g/l	total extract g/l
white/dry	11,88/11,46	0,9898/0,9904	18,90/18,89
red/dry	11,16/11,16	0,9927/0,9929	24,25/24,17
white/sweet	13,60/13,40	1,0178/1,0178	96,87/96,28
red/sweet	16,44/16,09	1,0448/1,0456	175,72/175,62

Also, in the future, we plan to estimate the change of mineral composition of the wines and aged wines distillates after sorbents treatment.

Conclusions

As a result, it can be concluded that sorbents relatively effective show themselves on a simple matrix. Study of complex organic and inorganic bases, such as wines and real aged wines distillates, shows that besides target reactions with phthalates, there are many competing processes.

Also, it should be noted that in all cases sorption capacity of each sorbent is proportional to molecular weight of phthalate isomers. In addition, at this point, we can conclude, that the most effective way to get the pure product is to prevent contamination.

Acknowledgments

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ACTION UV RADIATION ON MICROBIOTA

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Abstract: Vegetables and fruits with their high nutritional value have a favorable effect on the human body. The term conservation of vegetables and fruit fresh is very short. In this work we studied the influence of the most effective conservation methods: UV radiation treatment with conservation in modified atmosphere for mushrooms and cabbage, the carrots, conopides, latues. For the assessment of treatment efficacy was studied index microbiological, chemical and sensorial phisco vegetables.

Keywords: Mushrooms, cauliflower, salad, conservation, microbiota, irradiance.

Introduction

Vegetables belonging to different groups are rich in carbohydrates, minerals (Fe, K, P, Ca, Mg, Na), fat-soluble vitamins (A, E, K).

Nutritional value of vegetables and fruits has a positive effect on body metabolism, the immune, etc.

Shelf-life of vegetables (mushrooms, cauliflower, lettuce), fresh is quite short, so time work aims to extend research on retention (preserving fresh) vegetables of these methods:

1. Treatment with UV;
2. Refrigeration and chilled storage;
3. Modified atmosphere storage.

Aim

Research and optimization of conservation of mushrooms and cauliflowers. In the experiments were taken 3 methods:

- Modified atmosphere;
- Cold treatment;
- Ultraviolet irradiation.

Accumulation of CO₂ in packaging quantities, avoid the use of chemical preservatives so little now want to preserve food.

Ratio of O₂ and CO₂ in the atmosphere to keep breathing slows reducing the concentration of O₂ needed to slow respiration varies with temperature.

Materials and methods

For placing the product in a modified atmosphere bags must be used with the following features:

- Good impermeability to gases;
- Physical and mechanical strength;
- Compatibility throughout the product shelf life packaging.

In the experiments performed were used bags of a size appropriate volume of product of complex material polyamide polyethylene (PE / PA).

La etapa I a experiențelor au fost efectuate determinări, pentru a cunoaște încărcarea microbiană a ciupercilor proaspete și conopidelor înainte de ambalare în AM și iradiere.

Table 1 Microbiota dynamics mushrooms and cauliflower before and after irradiation

Product tested	Microbiota before irradiation		Irradiation conditions fungi	Microbiota after irradiation		%, microorganisms inactive or destroyed
	Mesophilic bacteria	Yeast and mold		Mesophilic bacteria	Yeast and mold	
Fresh Champignons Mushrooms	$3,2 \cdot 10^4$	$3,6 \cdot 10^4$	Lamp 12 W power for 10 min at 40 cm from the source of radiation.	1200	900	69
			Lamp power 12 W for 15 minutes with the radiation source.	900	700	76,5
			Lamp 12 W power for 20 minutes	700	600	81,5
Cauliflower	$3,4 \cdot 10^4$	$6,5 \cdot 10^3$	Lamp 12 W power for 10 minutes at 40 cm from the source of radiation.	$8,1 \cdot 10^3$	$1,3 \cdot 10^3$	23,8
			Lamp power 12 W for 15 minutes with the radiation source.	$4,6 \cdot 10^3$	$1,06 \cdot 10^3$	13,5
			Lamp 12 W power for 20 minutes	$1,9 \cdot 10^3$	$2,9 \cdot 10^3$	4,6

At stage II samples irradiated identically packaged and were examined for different storage parameters.

Table 2 Champignons respiration intensity and cauliflower

Product analyzed	Weight, kg	Storage temperature, °C	Retention time, h	Intensity of breath, mg CO ₂	V CO ₂ eliminated mg CO ₂
Mushrooms Champignons	0,200	2-8 ambient	121	59,2	1438
	0,200		121	90,1	2367
Cauliflower	0,200	2-8 ambient	11-14 h	34	145
	0,200		11-14 h	95	89

Research has shown that the process of breathing CO₂ removal by mushrooms and cauliflower depends on storage temperature. The temperature is higher, the breath grows louder.

Activity of the enzyme, polyphenol oxidase, which produces a yellow product was determined by standard method. Research has shown that polifenoloxidazei activity remains almost unchanged after treatment for several days.

Results and discussion

Follow mushrooms stored at room temperature (25-27 ° C) and refrigerated (2-8 ° C) was performed by analysis organoleptic, microbiological and physico-chemical properties.

Table 3 Research activity Champignons mushrooms polifenoloxidazei

Product Name examined	Storage condition	Poliphenol oxidase activity, enzymatic units
Mushrooms Champignons	CO ₂ control, 2-8 ° C, ambient	17
	CO ₂ UV, τ = 20 min. ambient	16
		16
		17

In table 1 are listed the results obtained on microbial load of fresh mushrooms and cauliflower and after treatment.

Table 2 includes the results of the analysis volume and intensity of breath CO₂ removed during storage at ambient temperature and mushrooms in the refrigerator.

Table 3 includes the results of determination polifenoloxidazei activity in different storage conditions.

Conclusions:

All these results allowed us to make the following conclusions on keeping fresh mushrooms and cauliflowers:

- Storing mushrooms and cauliflowers in containers of 2 kg bags packed in PA / PE.
- Irradiation of packaged mushrooms and cauliflowers.
- Storage temperature between 2-8 ° C.

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SYNTHESIS AND STUDY OF SOME DIOXOVANADIUM(V) COORDINATION COMPOUNDS BASED ON ISONICOTINOYLHYDRAZONE OF SALICYLALDEHYDE AND ITS DERIVATIVES

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Abstract: Three mononuclear dioxovanadium(V) complexes, $[\text{VO}_2(\text{HL}^1)]$ (**1**), $[\text{VO}_2(\text{HL}^2)] \cdot 1.2\text{H}_2\text{O}$ (**2**) and $[\text{VO}_2(\text{HL}^3)] \cdot \text{DMF} \cdot \text{H}_2\text{O}$ (**3**) with tridentate ONO ligands derived from isonicotinoylhydrazide and salicylaldehyde (H_2L^1), *o*-vanillin (H_2L^2) and 3-carboxy-salicylaldehyde (H_2L^3), respectively, have been synthesized and characterized by elemental analysis, IR-spectroscopy and single crystal X-Ray diffraction studies. The V(V) in all complexes has a distorted square pyramidal geometry with ONOO2 donor atoms set.

Keywords: oxovanadium(V) complexes, ONO donors, Schiff base, X-Ray crystal structure

1. Introduction

The catalytic properties of vanadium compounds and their effects in biological systems or as therapeutic agents have led to an increasing interest in the coordination chemistry of this element. Among the vanadium complexes that have been synthesized as models for biologically active compounds or as therapeutic agents with insulin-mimetic properties the oxovanadium (V) chelates demonstrate an essential activity [1-3]

In most cases oxygen–nitrogen donor ligands, which have a tendency to stabilize the vanadium in its highest oxidation state, are used[4,5]. Hydrazone ligands derived from the condensation of an acid hydrazide with aromatic carbonyl compounds are important tridentate ONO-functional ligands containing a combination of donor sites such as protonated/deprotonated amide oxygen, an imine nitrogen of hydrazone moiety and additional donor site (usually N or O) provided from the aldehyde or ketone forming the Schiff base. The number of coordination compounds with isonicotinohydrazone of salicylaldehyde and its derivatives is comparatively limited. [4, 6-8] These ligands possess a fourth nitrogen donor atom in the isonicotinoyl part that could link metal moieties into higher dimensional structures[7].

In this paper we report the preparation and characterization of three new dioxovanadium (V) complexes $[\text{VO}_2(\text{HL}^1)]$ (**1**), $[\text{VO}_2(\text{HL}^2)] \cdot 1.2\text{H}_2\text{O}$ (**2**) and $[\text{VO}_2(\text{HL}^3)] \cdot \text{DMF} \cdot \text{H}_2\text{O}$ (**3**) of salicylaldehyde isonicotinoylhydrazone (H_2L^1), *o*-vanillin isonicotinoylhydrazone (H_2L^2) and 3-carboxy-salicylaldehyde isonicotinoylhydrazone (H_2L^3) (Scheme 1).

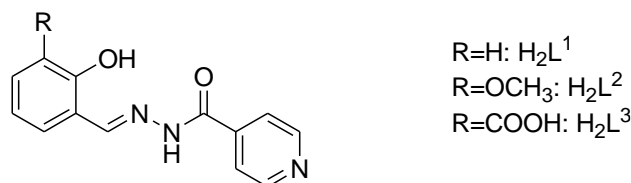


Fig. 1. Salicylaldehyde based isonicotinoylhydrazone Schiff base ligands.

2. Experimental

2.1 Materials

Solvents and reagents (isonicotinic acid hydrazide, salicylaldehyde, *o*-vanillin) were obtained from commercial sources and used without further purification. $[VO(acac)_2]$ and was prepared as described in the literature [9, 10].

2.2. Physical measurements

Elemental analyses of carbon, nitrogen and hydrogen were carried out on a Vario EL III CHNOS Elemental Analyzer. IR spectra were recorded with a Perkin-Elmer FTIR spectrometer. NMR spectra for ligands were performed on a Bruker 400 spectrometer in DMSO-*d*₆ solution using TMS as internal reference.

2.3. Synthesis of the ligands

The tridentate Schiff base ligands, salicylaldehyde isonicotinoylhydrazone (H_2L^1), *o*-vanillin isonicotinoylhydrazone (H_2L^2) and 3-carboxy-salicylaldehyde isonicotinoylhydrazone (H_2L^3) were prepared by condensing isonicotinic acid hydrazide with salicylaldehyde, *o*-vanillin or 3-carboxy-salicylaldehyde as previously reported procedure [11, 12] and were characterized by IR and NMR spectroscopy.

2.4. Synthesis of dioxovanadium complexes 1-3

Synthesis of $VO_2(HL^1)$ (**1**)

A mixture of 0.22g (1 mmol) of $VOSO_4 \cdot 3H_2O$ and 0.21g (1 mmol) of 2-hydroxybenzaldehyde isonicotinoylhydrazone (H_2L^1) in 20 mL of a MeOH/DMSO mixture (1:1) was stirred at room temperature to give a clear brown solution, which was filtered and allowed to stand 2-3 days. The brown prisms crystals which appeared were filtered off and air-dried.

Yield: 67%. *Anal.* Calcd. for $C_{13}H_{10}N_3O_4V$: C, 48.31; H, 3.12; N, 13.0. Found: C, 48.17; H 3.24; N 12.43.

The complexes **2** and **3** were prepared in the same way as for **1**, except using $VO(acac)_2$, *o*-vanillin isonicotinoylhydrazone (H_2L^2) and 3-carboxy-salicylaldehyde isonicotinoylhydrazone (H_2L^3) ligands, and MeOH and MeOH/DMF (1:1) mixture for **2** and **3**, respectively.

The complexes are soluble in polar and/or coordinating solvents such as pyridine, DMF and DMSO, and sparingly soluble in MeOH.

For **2**: Yield: 62%. *Anal.* Calcd. for $C_{14}H_{14.4}N_3O_{6.2}V$: C, 44.86; H 3.87; N 11.21. Found: C, 45.13; H 4.21; N 11.22.

For **3**: Yield: 58%. *Anal.* Calcd. for $C_{17}H_{19}N_4O_8V$: C, 50.12; H 4.70; N 13.75. Found: C, 49.65; H 4.21; N 13.22.

3. Results and discussion

Three novel V^V isonicotinohydrazone complexes with the formula $[VO_2(HL^1)]$ (**1**), $[VO_2(H_2L^2)] \cdot 1.2H_2O$ (**2**) and $VO_2(HL^3) \cdot DMF \cdot H_2O$ (**3**), where L = salicylaldehyde isonicotinohydrazide (H_2L^1), *o*-vanilin isonicotinohydrazide (H_2L^2) and 3-carboxy-salicylaldehyde isonicotinohydrazide (H_2L^3), have been synthesized in good yields and high purity. The products are characterized by spectroscopic data, elemental analyses and single crystal X-Ray diffraction studies.

3.2 Spectral characteristics

In the IR spectra of complexes **1-3**, a strong band at about $1630\text{--}1650\text{ cm}^{-1}$ may be attributable to the carbonyl resonance and it is red-shifted from $1680\text{--}1700\text{ cm}^{-1}$ in the free ligands. Deprotonation of the hydrazone and salicylaldehyde OH groups enhances the electron delocalization across the ligand system, reduces the double bond character and increases the donor strength of the carbonyl group as a consequence[13]. For the oxovanadium(V) complexes, two new bands appear at about $900\text{--}930$ and $950\text{--}970\text{ cm}^{-1}$. These two bands for the previously reported VO^{2+} complexes are assigned to the ν_{asim} and ν_{sim} stretching of the *cis*-dioxovanadium(V) moiety, respectively[14].

3.3 Crystallographic study

X-ray crystallography revealed that compound **1-3** have a molecular structure built from mononuclear $[VO_2(HL^{1-3})]$ entities (Figure 1) [15]. and solvate water[16] and DMF/water molecules for compound **2** and **3**, respectively. In each complex vanadium(V) atom adopts a square-pyramidal geometry coordinated by the tridentate Schiff base ligand stabilized in E form and two oxygen atoms in *cis* positions.

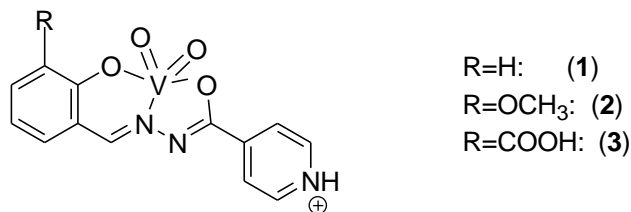


Fig. 2. The structure of **1-3**, solvent molecules are omitted

The N–N, N–C and C–O bond distances observed in the $=N-N=C(O-)$ -fragment of the ligands put in evidence the enolate form of the amide functionality. The average $V=O$ and $V-O$ bond lengths are comparable to those observed in other vanadium(V) Schiff base complexes [17, 18]. X-ray diffraction study has demonstrated the protonation of the pyridine ring, which was also observed earlier in V^V complexes with a related ligand [13,18]. The crystal structure of **1** is stabilized by a strong intermolecular $N-H \cdots O$ H-bonds, involving the protonated pyridinic nitrogen and the equatorial oxo ligand. The crystal structure of **2** represents a supramolecular 3D network, sustained by the $N-H \cdots O$, $O-H \cdots O$ hydrogen bonds and $\pi \cdots \pi$ stacking interactions.

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RESEARCH ON THE IODIZED OIL

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Abstract: the objectives of this work are researches for obtaining iodized oil that would satisfy the requirements of body in iodine. The sunflower seed oil is a product with the most important value, thus the production of oil fortified with iodine would be a cheap and accessible variant. Production of oil fortified with iodine constitutes a considerable iodine supplement, which, associated to iodine contribution from kitchen salt, would contribute to the eradication of deficiency in iodine.

Key words: iodine, sunflower oil, π type compounds, triglycerides

1. Introduction

At present iodine deficiency is one of main problems that have to be solved. The iodine is an essential oligo-element, whose contents in human body varies from 15 to 23mg, being indispensable for the synthesis of thyroid hormones, which have a primordial importance in the metabolism of cells, especially of cerebral and bony tissue. The deficit of iodine is established in conditions of diminishment of the contribution of this mineral both with food factor, and with drinking water. The deficit of food contribution in iodine has as consequence the deregulation of thyroid function, restraints in physical and psychical development of individual.

The objectives of this work are researches for obtaining iodized oil that would satisfy the requirements of body in iodine. The sunflower seed oil is a product with the most important value, thus the production of oil fortified with iodine would be a cheap and accessible variant. Production of oil fortified with iodine constitutes a considerable iodine supplement, which, associated to iodine contribution from kitchen salt, would contribute to the eradication of deficiency in iodine.

Insufficiency of iodine represents a nutritional problem that severely affects the health of the entire population. The deficit of iodine is an especially actual problem for Moldova as in fine it negatively affects not only the public health but also the entire social-economic development of the country. It is conditioned by the reduced levels of iodine in the country's natural geographic environment – soil, water, air, plants [1, 2].

Iodine is an essential oligo – element, its content in human organism varies from 15 to 23 mg, it is indispensable for the synthesis of thyroidal hormones (thyroxine – 65%, tri-iodinetrionine – 59%), that are of primordial importance for the cellular metabolism, especially for the cerebral and bone tissues [3, 4].

Sunflower oil is a very common product in Moldova, so the production and consumption of oil enriched with iodine (40 - 50 $\mu\text{g/day}$), equivalent to the quantity of iodine received with the salt, would allow to reduce the iodine deficiency.

The objective of this work consists in the elaboration of sunflower oil iodination and evaluation of its quality indicators (physical-chemical properties).

2. Preparation of iodinated oil

For the purposes of these investigations we used double refined and deodorized sunflower oil of local production. In order to obtain iodinated oil per one liter of product we added 1g of iodine (I_2) in crystals of type „X χ ”. After the establishment of equilibrium the

oil was further investigated. The iodinated oil had an intensive brown color due to the presence of free iodine. The obtained oil (A) with a total content of iodine of 1mg/ml was diluted in the proportion of 1:100 and so was obtained the product (B) with content of iodine 10 μ g /ml. From the product (B) by dilution (1:500) was prepared the sample (C) with content of iodine not exceeding 2 μ g/ml i.e. much lower than recommended daily rate. The iodinated oils with different content of iodine (A,B,C) served as objects of research for this thesis. All measurements have been done in conformity with the norms STAS – 1129 – 93 [5].

3. Results and discussions

The use of iodinated sunflower oil is an accessible and low cost method. The particular advantage of this method consists in the liposolubility of iodine that facilitates its incorporation in oil. However, it is a very complex phenomenon associated with modifications of physical-chemical properties of the finite product as thereafter discussed in the present work.

Addition of iodine and positioning of double bonds takes place according to a mechanism that implies the formation of compounds of type π , without the breakage of the double bond from the unsaturated fatty acids molecules. There was established that the reaction of formation of π triglycerides-iodine compounds is heat-absorbing reaction. The reaction is described by a kinetic reaction of first order.

Experimentally it was determined that the iodine index decreases gradually depending on the quantity of iodine added (fig.1).

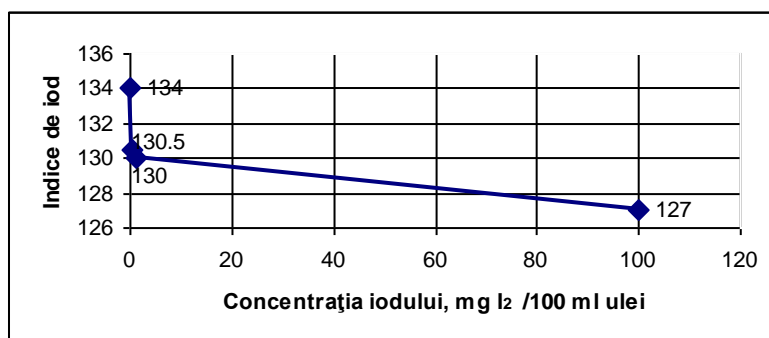


Fig. 1. Evolution of iodine index of oil depending on the quantity of iodine added

The saponification index of iodinated oil doesn't vary considerably compared to the reference sample. This fact demonstrates that the molecular mass of triglyceride doesn't vary during the process of oil iodination. A small increase in the index of saponification for the oil containing 100 mg I₂/100 ml oil (sample A) can be explained by the possible chemical interaction between iodine and hydroxide.

It was determined that the content of free fat acids in the iodinated oil increases for both samples A and B in line with the increase of quantity of iodine added. This fact can be explained by the consumption of certain quantities of KOH for interaction with iodine. The only sample in which the AGL index was exceeded was the sample A that contains 100 mg

I₂/100 ml oil. For iodinated oil containing 0,2 mg I₂/100 ml (sample C) the AGL index doesn't vary compared to the reference sample.

Variation of peroxide index of iodinated oil doesn't vary significantly for oils with small concentration of iodine (samples B and C) compared to the reference sample while for the oil with high content of iodine 100 mg/ 100ml (sample A) it far exceeds the maximum admissible value.

This can be explained by the presence of free iodine in the iodinated oil - sample A.

4. Conclusions

This work demonstrates the possibility of iodination of sunflower oil; iodine doesn't affect the main physical-chemical indices of sunflower oil. Formation of compounds of type π , without the breakage of the double bond from the unsaturated fatty acids molecules assures a high stability of resulting compounds (samples B, C). For this reason the use of iodinated oil for the production of highly accessible foodstuffs (margarine, mayonnaise) represents a particular interest. A small quantity of iodinated oil in foodstuffs doesn't affect the organoleptical and physical-chemical properties of finite products. Use of these products in combination with iodinated salt would allow to prevent the deficit of iodine and the associated incidence of iodine deficiency diseases.

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MODERN METHODS FOR IDENTIFICATION OF COUNTERFEIT FOOD PRODUCTS

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Abstract: There are presented the commonly encountered concepts and forms of food tampering. The study of quality indices for wide range of foods, including apple and grape juices, vegetable and dairy fat, was performed. The expertise methodology was developed to identify the counterfeit products. The investigation results apply to production laboratories and food inspection.

Key words: adulterated products, gas chromatography, HPLC, juices, vegetable oils, butter.

Problem of revealing of adulterated products and their removal from circulation has centuries-old roots. However, this problem got more stringent aspect at the beginning of 1990's when considerable amounts of low quality and adulterated food products both domestic and imported ones have penetrated to food markets of many countries [1].

Adulteration of food products and raw materials as a fraudulent action manifests itself in production and realization of counterfeited foods, which do not correspond with name and marking, while dubious quality and nutritional value of such products can constitute a threat for the consumer health.

Adulteration types that are considered as most spread are: *assortment-related* one, which manifests itself in realization of goods under foreign trademark and with foreign commercial parameters; and *qualimetric* one representing falsification of qualities through resorting, replacement of some expensive raw constituent by another cheaper one, addition of some additives in order to increase the product quantity, reconditioning of degraded products in order to conceal their defects and others.

At the same time, falsification can be accompanied by incorrect and incomplete information qualified as *informational falsification* intended to mislead consumers with regard to essential characteristics of a product.

Falsification by itself presents not only economic threat through illegal commercial economic activities, but also social one that can manifest itself through effect on the health of population within a country (sanitary fraud).

Alcoholic and refreshing drinks, juices, meat and fish preserves, fats and oils, milk and milk products, confectionary and many other products are subjected to counterfeiting.

Thus, every year, counterfeiting of food products is perfected from simple examples (dilution with water, addition of cheap ingredients) to elaboration of complex methods of creation of “chemical cocktails”, which contain necessary amounts of substances ensuring concealment of falsifications.

For this reason, exporters or importers of food products in many countries carry out for many years investigations oriented toward elaboration of reliable falsification revealing methods: selection of optimal controlled indices for identification of product naturalness; accessible experimental-statistical argumentation of controlled indices; studying of

corresponding methods and means of control; elaboration of legal systems for termination of production of adulterated food products and their removal from circulation.

In the Republic of Moldova, investigations are carried out that are oriented toward revealing of adulteration of juices, vegetable oils, milk fats, as well as adulterations in coffee, cacao and products prepared on the basis of these agricultural food raw materials.

Material and methods

Natural apple and grape juices, vegetable oils of soya, sunflower and rape and grape seeds, as well as spreads both imported and produced in the Republic of Moldova have served as a subject of research.

Standardized quality control methods were used:

- juices: pH indices; dry matter mass fraction; absorbancy; titrable acidity; organoleptic indices;
- vegetable oils: relative density at 15°C; coefficient of refraction at 20°C; saponification indices; iodine, acidic and peroxidic indices;
- butter: under GOST 37-91 „Butter. Technical Specifications”.

Also, methods for determination of organic acids, glucides and alcohols in grapes by means of liquid chromatography (HPLC), as well as chromatographic methods for determination of α -tocopherol and higher fatty acids in oils and milk fats in gaseous state were adopted.

Macro- and microelements were determined by means of flame absorption spectroscopy.

Results and discussions

For identification of adulterations of fruit juices, methods for determination of specific indices defining naturalness of juices were selected, approved and partially modified; variation ranges of every index of domestic products were determined; rated content and values of indices of the similar imported products were verified. All controlled indices are in conformity with legislation of the Community states, Codex Alimentarius standards and normative acts of the Association of juice and nectar producers.[2].

Basing on the obtained data, methodological guidelines for determination of authenticity of apple and grape juices were elaborated. At the same time, scheme for identification of products was determined. Under this scheme, examined juice passes several stages of investigation. At first stage, product packing as well as label inscriptions – product name and content, name of manufacturing company – are examined. Initially, factors of inconsistency can be revealed. For example: product is named as “juice”, while it is indicated that the content of fruit base constitutes 25%. It is an apparent informational falsification. Then, organoleptic testing of a product is performed through estimation of its appearance, smell, taste and presence of foreign inclusions and indices are determined by traditional chemical and physical-chemical analytic methods under normative acts.

Second stage provides determination of biochemical indices, including organic acids, glucides, proline and formol index. Said indices are determined under liquid and gas chromatography methods, as well as fermentative methods. Following these analyses are performed, basing on results obtained in respect of composition of organic acids, it is possible in some cases to establish the fact of replacement of one juice by another or addition of another juice (for example, replacement of grape juice by apple one or addition of apple juice into grape one). Results obtained at the second stage of examination are compared and

interpreted in accordance with standards and requirements to the juice quality. Should parameters of the juice sample presented for examination meet corresponding requirements, examination is interrupted.

If obtained result do not allow univocal conclusion on the quality of presented sample to be made, it becomes necessary to pass to the third stage of examination – determination of the content of mineral substances and microelements (ions of potassium, sodium, magnesium, calcium, as well as chlorides, sulfates, phosphates, nitrates). To this end, ratio of calculated values and the determined content of ions (for example, between K and Mg, K and Ca) is used. In order to obtain more ample characteristic of a product, volatile substances that define its aroma can be studied. Such kind of examination makes it possible to reveal adulterated products more reliably. However, practice demonstrates that first two stages of examination are sufficient to provide an answer in respect of the product quality.

General scheme of identification of adulterated products is presented in Fig.1.

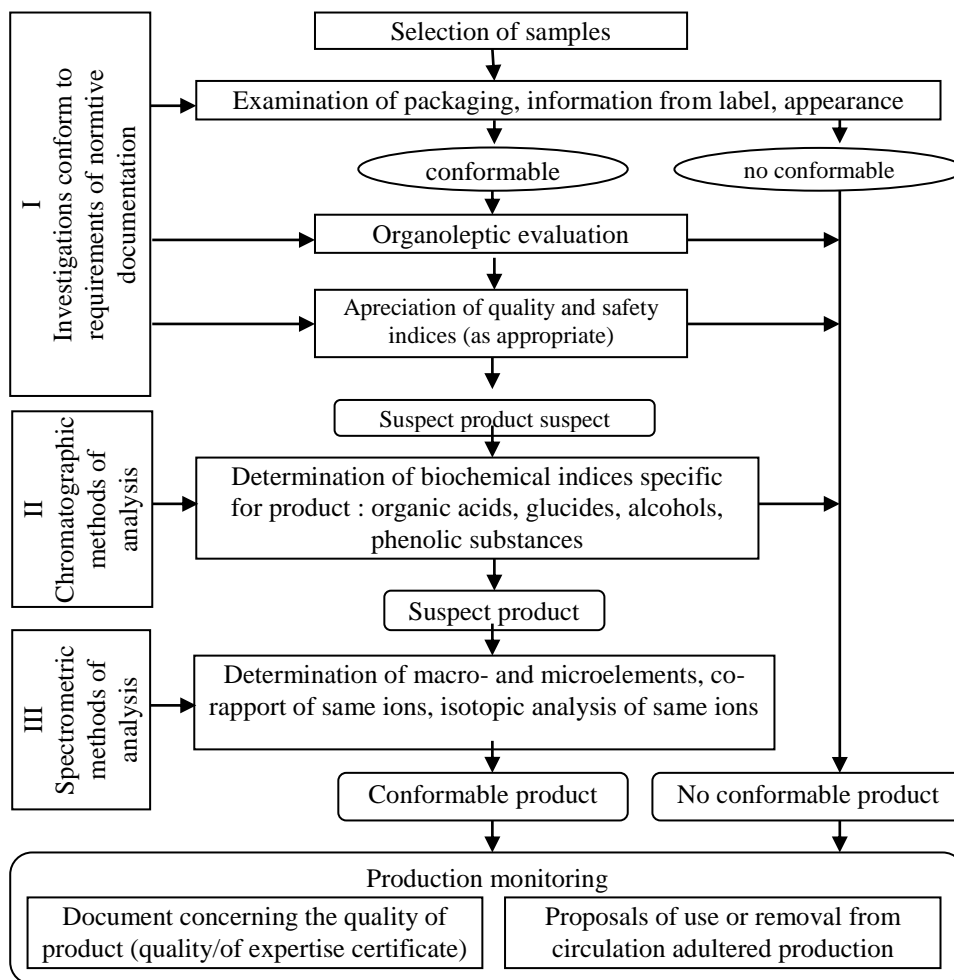


Fig. 1. Identification scheme of no-conformable/adulterated food product

Problems concerning adulteration of vegetable oils were resolved within the Institute basing on examination of composition of fatty acids [3,4]. Oils produced by enterprises of the Republic of Moldova, Ukraine and Romania were studied. For this purpose, stage-by-stage scheme of examination similar with one described for juices was used.

It was established that vegetable oils both produced in the Republic of Moldova (of sunflower, sunflower with increased content of oleinic acid, soya, rape, grapeseeds) and imported ones correspond with quality indices under normative acts and Codex Alimentarius standards for natural products. At the same time, cases of adulteration were revealed especially with samples selected from individual sector. These samples had bitter taste and additions of soya-bean and rape-seed oil to sunflower-seed oil.

These studies were implemented directly in production through scientific-technical support in determination of composition of fatty acids and identification of the product naturalness provided to producers of vegetable oils.

Modern market of food products offers wide range of butter both domestic and imported one packed in different packing of different weight. In the Republic of Moldova, as well as in CIS countries, composition and quality of butter is regulated by the GOST 37-91 „Butter”. Water content can be increased and mass fraction can be decreased, but not more than by 0.2%.

In recent years, in the light of modern concept of healthy alimentation, requirements to composition and nutritive value of food products were transformed essentially. It means revision of requirements to butter quality: reduction of traditional fat/plasma ratio in order to improve biologic values and dietetic properties; admission of adjustments in composition of fatty acids of fatty phase, including by means of partial use of vegetable fats [5].

Possibility of combining composition and properties of these products, as well as their economic expediency has provoked considerable interest in different countries. Such products continue to be named traditionally as sweet butter with improved composition and properties - „lightened” or „light”. However, principles of healthy alimentation remained the predominant economic reasoning. Addition of nondairy fats remained unjustified and fats of not so good quality are used many times. Many products similar with domestic and imported ones have appeared at the market. But, products only where there are no nondairy fats at all can be called as “sweet butter”. As a rule, there is no impartial information on these products, which is qualified correctly as apparent falsification both informational and qualimetric.

For revealing of adulterations of butter, new methods were elaborated or existed ones were modified in order to find out additions of vegetable fats or their substitutes in butter.

To reveal adulterations of butter, methods were selected, tested and modified for analysis of the specific quality indices of these products: the content of water-soluble volatile fatty acids; their sterols; higher fatty acid; and ratio of acids [6].

Convincing results were obtained during application of gas chromatography method for determination of higher fatty acids and Reihert- Meissel chemical method for analysis of water-soluble volatile fatty acids (capronic and butyric).

The last one is known, though it was not used for revealing of butter adulterations due to the absence of standards for these milk products.

These standards were established on the basis of simulation of mixture of milk fats with vegetable ones and substitutes of milk fats. Currently, analyses are performed that are

directed toward revealing of adulterations of butter produced by enterprises of the Republic of Moldova.

It should be noted that products are examined stage by stage similarly with adulterations of juices paying special attention to determination of specific components.

Problem of adulteration of food products became the national one in every country. Presence of the large volume adulterated products leads to economic losses both for the state and fair producers of high quality products [7,8].

This situation appeared in the result of absence of the state system preventing production and circulation of counterfeited goods in the course of merchandise logistics, absence of administrative and legal responsibility for production, introduction in circulation and realization of adulterated products.

Conclusions

By application of standardized methods and original methods elaborated within the frames of study, methods of (liquid and gas) chromatography were modified for determination of organic acids, glucides, proline and formol index in juices, composition of fatty acids in vegetable oils, higher fatty acids $C_{10} - C_{20}$ and water-soluble volatile fatty acids $C_4 - C_8$ in margarine and butter.

Methodology of examination of food products with revealing of adulterated ones (informational, assortment-related or qualimetric falsification) provides several consecutive stages.

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USE OF GRAPE OF VITIS LABRUSCA VARIETY IN THE REPUBLIC OF MOLDOVA

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Abstract: Actual situation with grape of Vitis Labrusca varieties, as well as opportunities of their use are described. Results of investigations of the dynamic process of accumulation of the main nutritive substances in grape of Isabella and Noah varieties are presented. Organoleptical, as well as physical and chemical indices of juice and squash samples obtained from raw material harvested at different phases of ripening are determined. Depending on the ratio of glucides, organic acids and polyphenolic substances in grape, eventual range of non-alcoholic products that can be used widely in food industry is proposed. Principal directions of use of grape of Vitis Labrusca from the pre-ripening phase are identified: production of natural acidulants, “green juices” and derivative products (fruit and vegetable matrices).

Keywords: „Vitis Labrusca”, grape juice, glucides, acids,

In the Republic of Moldova, approximately 9.8 thou. ha of plantations of the grape hybrid varieties, including „Vitis Labrusca” (Isabella, Lidia, Noah), have remained mainly (80%) in the central zone. In 2011, it was planned to produce about 50 thou. tons; 20 thou. tons were directed for processing, while average yield amounted to 7-8 t/ha.

Wine-making of grapes of respective varieties was reduced due to the formation of methanol during alcoholic fermentation in amounts exceeding permissible limits, as well as anthocyanins with diglucosides passing into wine during maceration and presenting risk for consumer health [1-3]. Besides, demand for wines of these varieties at markets of the CIS countries has reduced. As a result, above mentioned wines remained in stocks of wine-makers, while purchase prices for grape have decreased by approximately 1.5 times and, thus, viticulturists bear economic losses.

Hence, it becomes reasonable to produce non-alcoholic products of grape of Vitis Labrusca varieties. Favorable pedologic and climatic conditions of the central zone permits cultivation of these varieties and production of high quality ecologically clean products (in this case, agricultural technology does not provide application of phytosanitary substances in amounts comparable with ones for European varieties). Depending on the content of glucides, organic acids, polyphenolic substances, mineral substances and vitamins that varies for the period of ripening, wide range of products like acidulants, „green juices” and blended food products can be produced.

It is also favorable that enterprises dealing with processing of fruits and vegetables, as well as can factories like „Călăraşi”, „Orhei-Vit” and „Eco-Vit” are located in this zone.

Materials and methods

Grape of Noah and Isabella varieties harvested for the period of 23.08-16.09 was used as a raw material to produce juice samples. Titrable acidity expressed in g/l of tartaric acid was determined by titration with NaOH 0.1 N alkaline solution up to the slight pink

tint. The content of glucose and fructose was determined by high-performance liquid chromatography (HPLC) in accordance with Resolution Oeno 23/2003. 25ml juice samples were filtered through blocks of 0.45-0.8-1.2-5 μm filters + pre-filter and were 5-time diluted by homogenization of 20 ml of juice with 80 ml of distilled water in 100 ml measuring flasks. 9ml of every diluted solution of juice were passed through filtering cartridge with last 6ml transferred into the test glass. Mobile phase - acetonitrile: water isocratic eluent in 85:15 ratio; flow rate - 1 ml/min; temperature in column- $+20^{\circ}\text{C}$. Initially, 10 μl standard solutions of fructose, glucose and saccharose of 5 g/l concentration each were introduced. Then, 10 μl work juice solutions prepared of grape of different phases of ripening were introduced. Solutions were kept in Zorbax-NH₂ 150x4.5 column for some period specific for every substance. Quantitative analysis was carried out using refractometric detector.

The content of malic, tartaric and citric acids was determined by high-performance liquid chromatography (HPLC) in accordance with OIV General Methods. 20 ml juice samples were degassed and then filtered through 0.45 μm cellulose membrane + pre-filter. 8ml of every juice solution were passed through filtering cartridge with last 5ml transferred into the test glass. Mobile phase - eluent solution of 0.0035 M sulfuric acid; flow rate – 0.5 ml/min; temperature in chromatographic column- $+20^{\circ}\text{C}$. Initially, 10 μl standard solutions of malic, tartaric and citric acids were introduced. Standard solutions had following concentrations: malic acid – 0.8 g/l; tartaric acid – 2.5 g/l; citric acid – 0.6 g/l. Then, 10 μl work juice solutions prepared of grape of different phases of ripening were introduced. Acids were kept in Zorbax Sax 250x4.6 mm column for some period specific for every substance. Quantitative analysis was carried out using diode-array detector (DAD) at 192, 208 and 210 nm wavelengths.

Results and discussions

Dynamics of accumulation of acids, glucides, phenolic substances, vitamins and mineral substances in grape was studied.

In season of the year of 2011 (23 August – 16 September), grape of „Vitis Labrusca” varieties - Isabella and Noah – were harvested at different stages of their ripening from trial plots of the National College of Viticulture and Winemaking „Stauceni”.

Samples of juice and squash were obtained in laboratory of every batch of harvested fresh grapes under the following technologic process.

For juice, grapes were washed with potable water, destemmed, rinsed and directed into the mechanical screw press, where juice was separated. Then, juice was transferred into the stainless steel vessels and thermally treated for 5 min at $95-98^{\circ}\text{C}$ temperature. Afterwards, hot juice was poured in 650 ml and 1 l glass jars.

For squash, grapes were washed with potable water, destemmed, rinsed and directed into the pulper of 0.3 mm diameter; then, obtained squash was transferred into the stainless steel vessels and thermally treated for 6 min at $95-98^{\circ}\text{C}$ temperature. Afterwards, hot squash was poured in 650 ml and 1 l glass jars.

From the organoleptic point of view, juices produced for the period of 1-7 September were estimated as best ones by appearance, color, taste and aroma.

Results of sensorial analysis of juices produced of grapes of Isabella and Noah varieties for the period of their ripening are presented in Figs. 1 and 2.

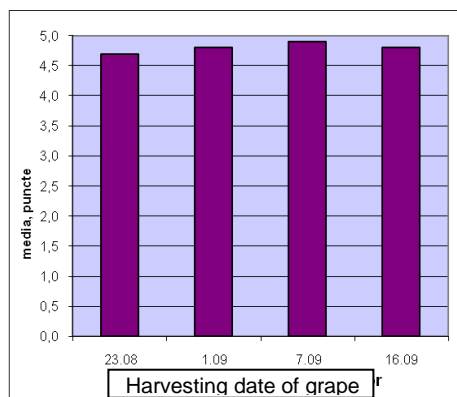


Fig. 1. Organoleptic appreciation of juices variety Isabella

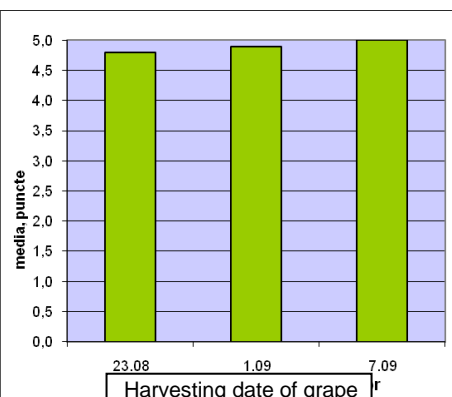


Fig. 2. Organoleptic appreciation of juices variety Noah

Concentration of organic acids and glucides in grape was determined by high-performance liquid chromatography (HPLC) (Figs. 3,4,5 and 6).

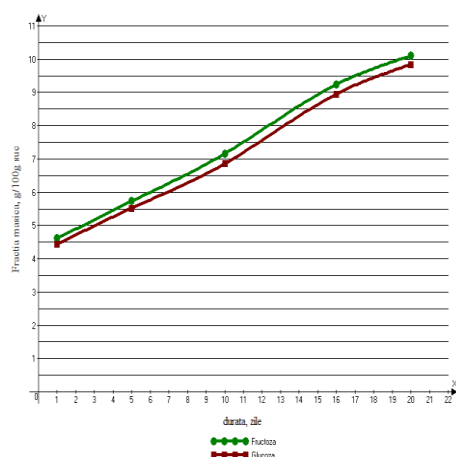


Fig. 3. Accumulation dynamics of glucides to Noah variety

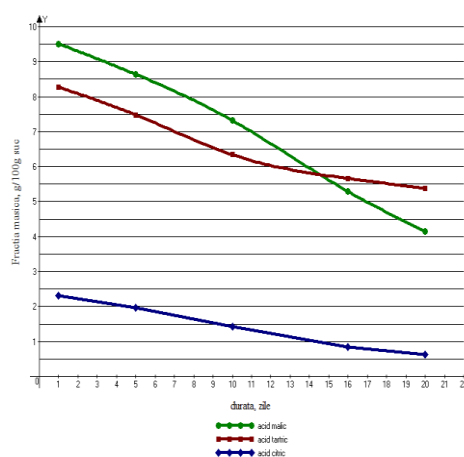


Fig. 4. Content diminution of organic acids to Noah variety

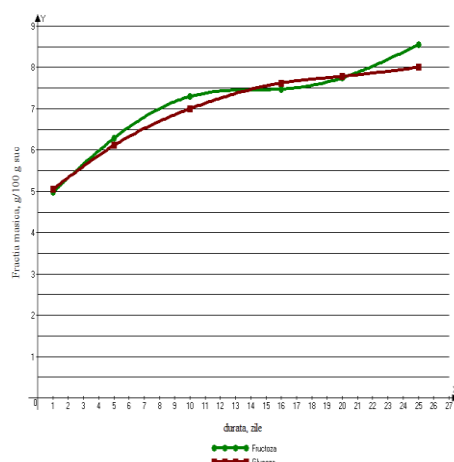


Fig. 5. Accumulation dynamics of glucides to Isabella variety

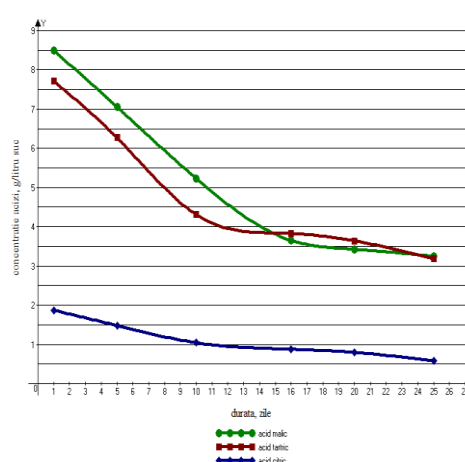


Fig. 6. Content diminution of organic acids to Isabella variety

Basing on the obtained results with regard to the total content of glucides and titrable acidity, sugar/acidity indices in juice samples produced of grape of Noah and Isabella varieties were determined (Table 1)

Table 1. Sugar/acidity indices of grape of Vitis Labrusca varieties

Juice obtained from grapes	Total content of glucides, g/100g	Titrate acidity expr. in tartaric acid, g/100g	Sugar/ acidity indices	Harvesting date
Noah variety	9,05	2,17	4,2	23.08.2011
	14,02	1,70	8,2	01.09.2011
	18,19	1,30	14,0	07.09.2011
Isabella variety	10,03	1,85	5,4	23.08.2011
	14,30	1,10	13,0	01.09.2011
	15,59	0,97	16,1	07.09.2011
	16,55	0,75	22,1	16.09.2011

By ratio between the content of glucides and acidity, sample of Noah variety juice with 14.0 index of 07.09 and samples of Isabella variety juice with 13.0 and 16.1 indices of 01.09 and 07.09 respectively were selected by the taste panel as most optimal ones. They have pleasant sweet-sour taste, characteristic varietal color and well expressed, but not intense aroma. Juices with such properties meet requirements for non-alcoholic products of grape and can be consumed directly.

Grape juices with sugar/acidity indices between 5 and 10 are recommended as acidifiers in preparation of dishes, sauces and marinating of meat products.

Large content of simple glucides like glucose and fructose being presented in almost equal proportions provides an important quantity of energy required for vital activities of an organism.

Organic acids - tartaric, malic and in less degree citric one – determine characteristic taste of grape, create fresh and cool gustative sensation and have a refreshing effect.

The total content of polyphenolic substances (IPT) was determined under spectrophotometric method. For the period of 23.08-07.09, it varied within 350-400 mg/l with Noah variety and within 600-700 mg/l with Isabella variety and has increased up to 1,500 mg/l with Isabella variety for the period of 07-16.09.

Results obtained during studying of samples have contributed conceptual elaboration of the range of new food products of grapes of *Vitis Labrusca* varieties (Fig.7).

Trial lots were obtained in laboratory conditions. Correspondingly, following elaboration and improvement of the technologic scheme of production, some lots will be produced also in industrial conditions.

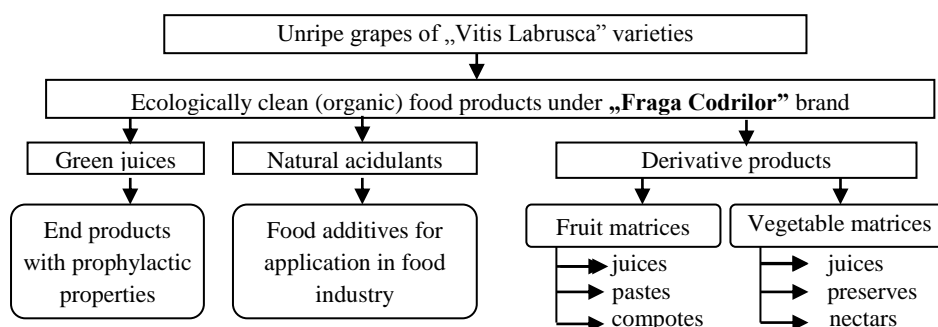


Fig. 7 Range of new food products of grapes of „Vitis Labrusca” varieties

Conclusions

Additional advantages of the use of grapes of *Vitis Labrusca* varieties for production of non-alcoholic products :

- juices and squashes obtained of unripe grapes have larger content of organic acids and, therefore, less strict modes of pasteurization/sterilization can be applied;
- valuable nutritive compositions can be obtained with optimal glucides: organic acids : polyphenolic substances : mineral substances ratios;
- more acid juices can be clarified and concentrated obtaining natural acidulants;
- new range of functional beverages, nectars, pastes, blends with products of fruits/berries with high nutritional properties can be obtained;
- due to optimal content of nutrients, „green juices” possess with biological activity and curative properties.

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AUTOCHTHONOUS SYMBIOTIC CULTURES FOR GOAT'S MILK YOGURT OBTAINING

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Abstract: În ultimii ani în Republica Moldova tot mai mulți fermieri se preocupă cu creșterea caprelor. Avându-se în vedere valoarea biologică și nutritivă înaltă a laptelui de capră, în Laboratorul de Biotehnologii alimentare IȘPHTA se efectuează cercetări privind elaborarea tehnologiilor de utilizare a laptelui de capră la fabricarea iaurtului. În cadrul cercetărilor, pentru obținerea iaurtului din lapte de capră, a fost elaborată o combinație de tulpini autohtone de bacterii lactice termofile *Lactobacillus bulgaricus* și *Streptococcus thermophilus* cu proprietăți biotehnologice valoroase din Colecția Ramurală a IȘPHTA. Combinația prezintă o cultură simbiotică stabilă, cu activitate acidifiantă medie, unite în raport 1:1. Datele obținute ne demonstrează că cultura simbiotică elaborată, compusă din tulpini autohtone de bacterii lactice termofile, poate fi utilizată cu succes la fabricarea iaurtului din lapte de capră.

Key words: simbiotic culture, starter bacteria, yogurt, sheep milk.

In recent years the Republic of Moldova more farmers are concerned with goat rearing. Goat livestock is one of the highest efficiency in milk production [1]. Herd of goats currently lists about 120-130 thousand. Unfortunately, in our country goat milk is used in the food industry and is done in most cases in particular.

In terms of nutritional and dietary quality goat milk is higher than cow's milk quality. Goat milk is more homogeneous deproteinized contains more nitrogen, proteins are of better quality, with a higher content of thiamine than any other food. Thiamine refers to the most important group of vitamins of group "B", without which man can not deprive his lifetime. Goat milk has an important role in traditional medicine, it is a valuable food for children weak and those who suffer from food allergy [2].

Goat milk and dairy products play an important role in the diet of the population in developing countries. In developed countries, where the tendency to eat healthy food there is great interest in goat milk.

Goat milk is used to treat various diseases such as gastrointestinal disease, tuberculosis, poisoning with salts of heavy metals, acids, alkalis, bromine, iodine for cleansing the body from the effects of chemotherapy, as well as baby food. It also should be noted that goat milk has anti-allergic properties. In some cases it is sufficient to relieve allergy symptoms. In addition, goat milk replaces cow's milk, where the latter causes allergy. Helps treat thyroid gland. Raw goat's milk is less dangerous; goats are more resistant to disease than cows.

Goat milk should also be included in the complex therapy of all serious diseases such as gastrointestinal diseases, heart disease, liver after various operations, and atherosclerosis, burns and fractures.

As the research scientist, goat milk compared with cow's milk has a different fractional composition of proteins and virtually no cause allergic reactions and digestive disorders. It is known that α_{s1} -casein, the main protein of cow's milk, is a potent allergen

for humans. Fractional content of this protein in goat milk is 2 times lower than in the cow. Nevertheless, β -casein content is 2-3 times higher, due to which, goat milk clotting softer easily digested in the human stomach. [3]

Worldwide more people appreciate all price quality goat milk as a source of natural health leads not secondary effects.

Goat milk and goat milk products strengthen human immunity. Residents Caucasus and Balkan countries have a long life of years because goat milk included in the daily diet and believes that goat milk prolongs life. Goat milk and products he is himself the strength and health of nature. It is very useful for children and necessary for those who suffer from allergy to cow's milk. [4]

Goat milk fats decompose ferment before it is storing, boost immunity, and helps the body to stop the development of cancer diseases and atherosclerosis.

Taking into account the biological and nutritional value of goat milk high in food ISPHTA Biotechnology Laboratory research is carried out on developing technologies using goat milk in the manufacture of various dairy products.

Yogurt is a dairy product quality acid multiple health benefits known from ancient times. These remove both its nutritional content, complete and balanced, as well as the contribution of active lactic acid bacteria, which improve digestion (including people with lactose deficiency), stimulates immunity. Yogurt has a very important role in maintaining a balanced intestinal flora [5]. In European countries there is a wide range of yoghurts from cow's milk and goat's milk as well. In Moldova occurs only cow's milk yogurt and goat milk yogurt generally no market. Based on the above, the Laboratory of Food Biotechnology research is being done on developing ISPHTA Technology and Document technical standards for goat milk yogurt.

Used in the manufacture of yogurt mixed cultures composed of two species of lactic acid bacteria: *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

In the process of obtaining yogurt fermentation of goat milk were applied indigenous combinations of strains of thermopiles lactic acid bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the collection branch of ISPHTA. Were originally developed and tested three combinations, which was selected biochemical properties optimal combination to achieve a requisite technological yogurt.

The combination presents a stable symbiotic culture, which consists of three strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* two strains of medium acidification activity, united in 1:1 ratio basic. Technological characteristics of selected symbiotic culture are presented in Table 1.

Table 1 Symbiotic Culture features (Growing in sterilized skimmed cow's milk inoculated with 2% culture, cultivated at 42 ° C)

During curd formation	6:00 hours
Titrateable acidity	98°T
Appearance and consistency curd	Smooth, firm, dense, slightly viscous, without removing the whey
Smear microscopic appearance	diplococi separated and long chains (about $\frac{3}{4}$ field), lactobacilli separated and in chains (about $\frac{1}{4}$ field)

The data in Table 1 show that symbiotic culture characteristics developed technology meet the requirements of yogurt starter cultures.

Culture developed under experimental groups were prepared yogurt from pasteurized whole goat milk yogurt and lots witnesses classic cow brought to the same indices and fat mass fraction of dry matter.

Fermentation processes were carried out by means of modern control tank installation program - Sartorius Biostat A plus bioreactor and were monitored by recording continuous-time active acidity.

It is known that the optimum temperature for the development of *Lactobacillus bulgaricus* is comparatively high as the optimum temperature for growth of *Streptococcus thermophilus* (40 °C). *Lactobacillus bulgaricus* is also acidifying more energy than *Streptococcus thermophilus* [6,7]. In this connection, the time required getting a product with moderate acidity and firm but soft consistency, the technological development was important to set some parameters to direct the fermentation process for settling and establishing development *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in repot 1:3-1:4. Directing was done by determining the percentage of inoculation of the mixture of milk (5% culture) and thermostatic temperature (40 °C).

Dynamic processes active during acid is shown in Figure 1.

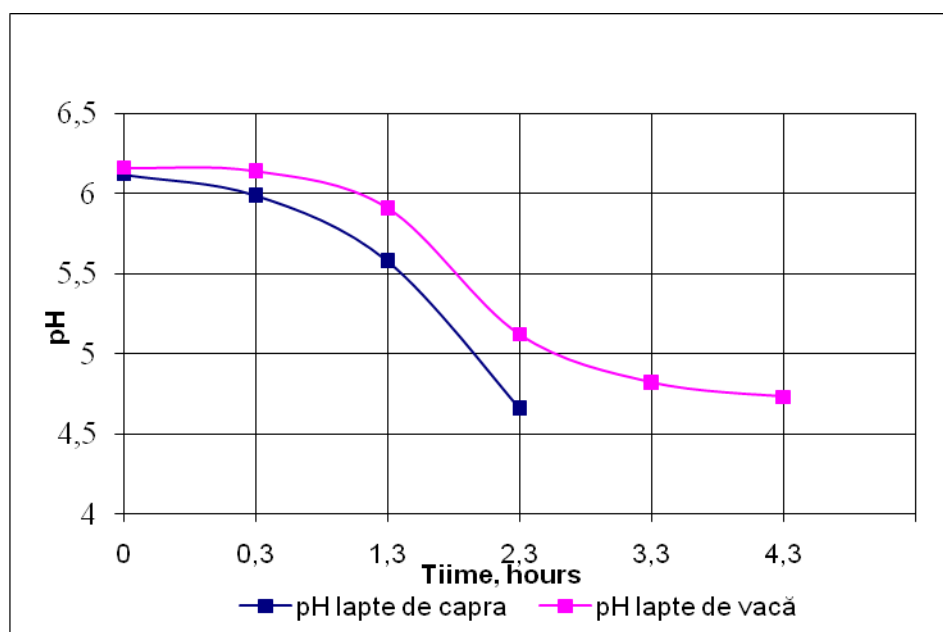


Fig. 1. The dynamics of active acidity during fermentation

Figure 1 shows that the decrease of the pH value up to 4.6 (level at which the coagulation of casein) in goat milk was produced over 2.5 hours, while in cow's milk than 4 hours. So in the process of fermentation of goat milk went faster than in the cow's milk.

Yogurt obtained was poured into containers tank containers like sales (of each 200 ml) and stored for ripening temperature 4 ± 2 °C.

Characteristic of goat milk yogurt and cow's milk are presented in Table 2

Table 2 Characteristics of yogurt from goat milk and cow milk.

Indices	Features		
	Goat milk yogurt	Yogurt from cow's milk	Technical requirements ГОСТ Р 51311-99
Appearance and consistency	Homogeneous, dense, without removing the whey, shiny, viscous, very fine	Homogeneous, dense, without removing the whey, shiny, slightly viscous	Homogeneous, moderately viscous
Taste and smell	Sour milk fermented specifically with pleasant aroma, odor-free goat milk	Sour milk fermented specifically with pleasant aroma	Fermented milk without foreign taste and odor
Titrate acidity, ° T	95	112	75-140
Lactic acid bacteria, UFC/1m	7×10^8	7×10^8	Min 1×10^7

The data of Table 2 it can be seen that after organoleptic and physicochemical made from goat milk yogurt with the technical conditions yogurt product called lactic acid. Unlike cow's milk yogurt, goat milk yogurt has a much finer texture, due to the fact that the fat globules of goat milk are smaller compared to cow's milk.

As a result of research found that symbiotic culture developed actively fermenting goat milk and yogurt quality obtained with the technical conditions given product. Unlike cow's milk yogurt, goat milk yogurt has a much finer texture, due to the fact that the fat globules of goat milk are smaller compared with cow's milk [1].

The data obtained demonstrates that symbiotic culture developed consisting of local strains of thermophilic lactic acid bacteria can be successfully used in the manufacture of goat milk yogurt, which is a very useful body and preferably both children and adults as well.

Goat milk can be successfully used in the manufacture of yogurt, which is a very useful and preferred body both as children and adults.

The results obtained will be developed technical and normative documents: Technology company standards and instructions that will be featured entrepreneurs in the milk processing industry.

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PHYSICO-CHEMICAL CHARACTERISTICS OF SOME BULGARIAN HONEYS

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Abstract: Eight honey samples from Bulgaria with different geographical and botanical origin have been studied in terms of their quality characteristics and element composition. The moisture, determined by a moisture analyzer was found to be $19.0 \text{ g } 100\text{g}^{-1}$ at maximum. The ash content was quantified by incineration in a muffle furnace varied from 0.23 to $0.89 \text{ g } 100\text{g}^{-1}$. The protein, carbon and nitrogen were defined by combustion at 949°C TruSpec CN. The protein in honey was found to be between 0.17 and 0.40 %, where as carbon exceeded 19 %, and nitrogen was not more than 638 mg kg^{-1} . In addition, another four macroelements i.e. potassium, sodium, phosphorus, and magnesium, were quantified. The most abundant appeared to be potassium achieving values of 1044 mg kg^{-1} , followed in almost all samples by sodium, phosphorus and magnesium. In three samples magnesium was not detected. From the microelements, zinc was quantified ($0.6\div 4.9 \text{ ppm}$).

Key words: honey, quality evaluation, minerals, protein

INTRODUCTION

Honey is a traditional product of production and consumption in Bulgaria (Obreshkov et al., 2012b) with an annual production of more than 10 thousand tons. Being a natural product, honey is a product of high interest and its quality characteristics are subject of analyses by many authors (Bobis et al., 2005; Bobis et al., 2006; Bobis et al., 2007; Bobis et al., 2008; Chirife et al., 2006; Pridal and Vorlova, 2002). The pH values influence the texture, stability and shelf life of honey (Terrab et al., 2004). Some minerals in honey like cadmium, lead and zinc serve as environment pollution markers (Przybylowski and Wilczynska, 2001). Recently, the mineral composition of honeys has been determined by Yurukova et al. (2008), Bogdanov et al. (2007), and Lazarova and Yurukova (2007).

The aim of the present study is to determine honey properties related to its mineral composition as well its total protein content.

MATERIAL AND METHODS

Honey samples

Eight honeys – H1 (registration number #60620072), H2 (#81830075), H3 (#41750444), H4 (not available, from the region of Strandzha mountain), H5 (#83190012), H6 (#62270155), H7 (#70820373), and H8 (#86770159) were bought directly from various local beekeepers located in different parts of Bulgaria. The type of honeys was defined by the producers themselves. The honeys were stored at room temperature ($20\pm 2^\circ\text{C}$) in glass jars until analyses.

Determination of pH

The pH of honey was determined according Bogdanov (2009). Briefly, an aliquot of 10 g of honey was diluted in 75 mL double-distilled water ($\text{TOC} = 4 \text{ ppb}$; $18.2 \text{ M}\Omega \cdot 10^{-2} \text{ m}$) and the pH values were read by inoLAB pH720 (WTW, Weilheim, Germany).

Moisture determination

The moisture was determined using Mettler balance & moisture analyser, model LJ16, Type PJ300MB supplied with LC-P45 printer (Mettler-Toledo). The results were expressed in $\text{g } 100\text{g}^{-1}$.

Determination of protein, carbon and nitrogen

The protein, carbon and nitrogen were determined at 949°C , using Helium flow-through carrier gas by TruSpec CN (Leco Corporation, USA). The apparatus was calibrated with EDTA calibration sample (Leco Corporation, USA). The results were expressed as $\text{g } 100\text{g}^{-1}$.

Ash content determination

The ash content of honey was determined after incineration in Muffle furnace Nabertherm (L15/11, Lilienthal, Germany) and was expressed as $\text{g } 100\text{g}^{-1}$ honey (Bogdanov, 2009).

Phosphorus determination

Phosphorus was colorimetrically determined based on the vanadomolybdate procedure by Stufins (1967). The method included incineration at 550°C , solution in 1N nitric acid. Then, the absorbance was read by Specord 200 spectrophotometer (Analytik Jena, Germany). The data was processed by WinASPECT® 1.2 software (Analytik Jena, Germany) and the results were expressed as $\text{g } 100\text{g}^{-1}$.

Determination of K, Na, Mg and Zn

The minerals were determined by flame atomic absorption spectrometer (Model AAS 5-FL, Analytik Jena AG, Germany) equipped with an AS 52 autosampler (Analytik Jena AG, Germany). A calibration standard by Merck (Germany) ICP multi-element standard solution IV (23 elements in diluted nitric acid) was used. The data were processed by WinAAS ver.3.80.

Data processing

The data were processed with the stated software packages. In addition, MS Excel (Microsoft Corporation, USA) was used.

RESULTS AND DISCUSSION

The pH values are presented in Table 1. The pH range is between 3.89 and 4.70. The average value is 4.36 that is in compliance with Yurukova et al. (2008) who reported similar values (4.29 ± 0.20) for Bulgarian honeydew honeys. Vela et al. (2007) reported pH values between 4.00 and 5.08 of Spanish honeys.

Table 1. Characteristics of Bulgarian honeys

	pH	Moisture g 100g ⁻¹	Ash g 100g ⁻¹	Protein g 100g ⁻¹
	-			
H1	4.68 ± 0.02	14.8 ± 0.3	0.89 ± 0.04	0.29 ± 0.00
H2	4.34 ± 0.01	19.0 ± 0.1	0.77 ± 0.04	0.31 ± 0.01
H3	4.70 ± 0.01	13.8 ± 0.2	0.65 ± 0.11	0.22 ± 0.00
H4	4.46 ± 0.01	14.0 ± 0.2	0.82 ± 0.05	0.27 ± 0.00
H5	4.23 ± 0.01	14.1 ± 0.2	0.84 ± 0.03	0.26 ± 0.01
H6	3.89 ± 0.01	18.0 ± 0.1	0.69 ± 0.08	0.40 ± 0.00
H7	4.61 ± 0.00	14.1 ± 0.1	0.79 ± 0.07	0.17 ± 0.01
H8	4.00 ± 0.01	12.6 ± 0.3	0.23 ± 0.01	0.23 ± 0.01

The moisture content in all honey samples is below 20 g 100g⁻¹ (Table 1), thus meeting the national legislation requirements in Bulgaria. The honey from the region of Burgas shows the lowest moisture content (12.6 g 100g⁻¹). Meanwhile, Yurukova et al. (2008), analyzing samples from different parts of the country, found that the honey from the region of Burgas is characterized also with the lowest moisture (14.3 %).

The minimal ash content is 0.23 (H8). Rodriguez (2004) studied the ash in Venezuelan multifloral honeys that ranged within the limit of 0.19 and 0.50. The honey sample H1 from the region of Stara Zagora demonstrates a rather high ash content of 0.89 g 100g⁻¹ (Table 1).

Although, honey is predominated by carbohydrates (Obreshkov et al., 2012a; Obreshkov et al., 2012b), it contains small quantities of protein. Lee et al. (1990) reported 0.19, 0.21 and 0.23 g protein per 100g of honey. The highest protein content is showed by honey is 0.40 g 100g⁻¹ (H6). The most frequent value for the protein is between 0.2 and 0.3 g 100g⁻¹ (Table 1).

Table 2 shows the content of carbon and nitrogen. Carbon varies between 19 and 24 %. These results could be explained with the high amounts of carbohydrates contained in the honey (Obreshkov et al., 2012a; Obreshkov et al., 2012b). The nitrogen in honey is found to be within the limits of 0.0277 and 0.0638 %.

In addition, the macroelements phosphorus, potassium, sodium, and magnesium are also determined (Table 2). According to the Bulgarian national legislation, there are no stated limits for the mineral composition of honey. In all cases, the most abundant is potassium reaching values of 1044 ppm. In almost all cases, the descending order of elements is potassium > sodium > phosphorus > magnesium. The only exception is honey sample H3 where phosphorus (134.4 ppm) exceeds sodium (109 ppm).

Magnesium was not detected in three samples. The only quantified microelement is zinc (Table 2). The range is rather wide varying from 0.6 to 4.4 mg kg⁻¹. Similar to the protein content, the highest zinc content is found in sample H6 (4.4 ppm).

Table 2. Element composition in Bulgarian honeys

	C	N	P	K	Na	Mg	Zn
	g 100g ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
<i>H1</i>	21.13	462	63.6	1044	185	50	2.0
<i>H2</i>	24.35	496	46.2	656	180	29	3.3
<i>H3</i>	20.37	354	134.4	819	109	44	1.7
<i>H4</i>	20.58	436	106.5	852	180	27	2.9
<i>H5</i>	22.00	422	53.9	238	91	nd	0.6
<i>H6</i>	21.77	638	40.8	158	97	nd	4.9
<i>H7</i>	18.81	277	94.7	638	167	41	4.4
<i>H8</i>	19.52	375	34.9	117	81	nd	0.8

nd – not detected

RSD is highest 3.21 %.

CONSLUSION

Honey is an acidic product with pH below 7. Concerning their moisture, all samples meet the requirements of the country (not more than 20 %). Only one sample demonstrated ash content below 0.50 %. In general, the honey is rich in carbohydrates, there are also found small amounts of protein. Data about carbon and nitrogen are also obtained. The descending order of elements is potassium > sodium > phosphorus > magnesium.

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QUALITY STUDY OF WALNUT OIL JUGLANS REGIA L

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Abstract: Insufficient scientific information on the quality of walnuts grown in Moldova and the composition of their oils necessitates carry out a systematic study of the most representative varieties of national production in order to contribute with scientific arguments and establish a major potential for walnut oil production at industrial level. The main objectives of the study were to find out solutions in order to obtain walnut oil relatively stable during storage. This paper presents an argumentation of the factors that determine the quality of walnut oil. It presents the chemical composition of oil from walnuts *Juglans regia* L according to a research conducted in recent years.

Key words: walnut oil, stability, peroxide value, acid value, enzyme activity, Pirson correlation.

1. INTRODUCTION

Walnuts *Juglans regia* L. are known as relevant sources of oil beneficial to health. Although walnut oil is not described by the current Committee on Fats and Oils of the Codex Alimentarius, small amounts are produced and commercialized in countries such as France, Spain, Chile and Argentina. The oil is used directly (without refining) for edible purposes, mainly as a salad dressing. It is also used in the cosmetic industry as a component of dry skin creams and wrinkle and anti-aging products [10]. The oil content in commercial walnut varieties ranges from 620 to 740g /kg kernel [10]. Prasad [14] reported a mean value of 690g/kg in walnuts from different varieties and geographic origins.

Insufficient scientific information on the quality of walnuts grown in Moldova and the composition of their oils necessitates carry out a systematic study of the most representative varieties of national production in order to contribute with scientific arguments and establish a major potential for walnut oil production at industrial level. Being rich in mono and polyunsaturated walnut oil is unstable during storage. It is important to know the factors which determine the quality of walnut oil.

2. MATERIALS AND METHODS

2.1. Samples

Served as a subject:

A - Industrial walnut oil, sold in Moldova; B –Walnut oil, cold pressed (press screw laboratory, Technical University of Moldova, Chisinau); C - Walnut oil, cold pressed (B), without setting.

The presses have used varieties walnuts grown in Moldova. Oil samples were stored at 25-30°C.

2.2. Analytical methods

The regress of lipid oxidation was assessed by measuring Peroxide Value (PV), Acid Value (AV), Activity Polifenoloxidase (A PPO).

The traditional method of determining PV, expressed as mill equivalents of active oxygen per kilogram of oil (meqO₂/kg), involves a titration of the oil contains potassium iodide in a chloroform-acetic acid mixture. The hydro peroxides oxidase the iodide to iodine, which can be determined by titration with sodium thiosulphate (AOAC official method 965.33).

3. RESULTS AND DISCUSSION

Worldwide there are various studies aimed at oil stability during storage. In the reference study conducted found that the stability of walnut *Juglans regia* L. oil it is determined by several factors such as: chemical composition of walnuts (oil), oil extraction method, packaging, storage parameters etc. The oil content in walnut kernel is determined by the genotype but may also be influenced by environmental conditions and irrigation rate [14]. Minor differences were observed in crop year effects [9]. Oil content in different varieties of walnut *Juglans regia* L. reported in various studies it is within 570 ... 710 g/kg [5-10]. The major components of walnut oil are triacylglycerols (TAGs; up to 980 g kg⁻¹ oil), in which monounsaturated Fas (mainly oleic acid) and polyunsaturated FAS (PUFAS; linoleic and α -linolenic acids) are present in high amounts (Table 1), [2, 4, 5, 9, 14]. Nine TAG species have been characterized, of which trilinolein is the most abundant ($\approx 37.7\%$), followed by dilinoleoyl-oleoyl-glycerol (18.5%) and dilinoleoyl-linolenoyl-glycerol (18.4%) [10]. The FA composition of walnut oil has been extensively reported for several cultivars from different geographic origins, including Argentina, [4, 13] the USA, [2] New Zealand, [1] Portugal, [12, 13] Turkey, [9, 11] Greece [18], and Germany [10] and Moldova [3, 15].

Table 1. Oil content and main fatty acids of some commercial walnut varieties from different origins [7-9, 11-13]

Variety	Oil content (g kg ⁻¹)	Fatty acids (mg g ⁻¹)				
		16 : 0	18:0	18:1 Ω 9	18:2 Ω 6	18:3 Ω 3
Chandler	695-725	66-69	15-17	161-178	565-589	165-186
Franquette	623-724	66-75	19-31	170-284	502-592	117-149
Lara	665-712	63-81	16-28	149-197	579-625	122-152
Mayette	665-732	59-70	18-27	161-223	554-575	130-176
Marbot	663-697	63-71	27-28	163-165	589-597	127-143
Mellanaise	630-697	63-70	26-28	145-171	587-616	125-145
Parisienne	631-715	62-63	24-29	174-195	577-624	96-132
Criolla	676-689	77-78	17-19	173-212	573-578	119-156
Hartley	710-714	68-81	9-13	167-179	584-592	146-160
Serr	711-728	66-67	18-19	167-179	167-179	584-592
Sorrento	716-739	72-76	15-17	172-191	589-593	129-150
Tulare	732-736	61-64	20-22	230-241	559-569	114-120
Fatty acid nomenclature: 16 : 0, hexadecanoic acid; 18 : 0, octadecanoic acid; 18 : 1n-9, cis-9-octadecenoic acid; 18 : 2n-6, cis-9,cis-12-octadecadienoic acid; 18 : 3n-3, cis-9,cis-12,cis-15-octadecatrienoic acid.						

The high content of unsaturated fats can lead to oxidative stability and reduced shelf life of walnut oil. Although walnut kernels contain a diverse array of phenolic and polyphenol compounds with strong antioxidant and radical-scavenging properties, only minor amounts could be present in the extracted oils. Protection against oxidative degradation seems to be limited mainly to tocopherol content. Other minor components

characterized in walnut oil include phospholipids, sphingolipids, sterols (principally β -sitosterol), hydrocarbons (particularly C14–C20 n-alkanes) and volatile components [1].

The majority of the volatile components found in walnut oil are produced by oxidative breakdown of linoleic and linolenic acid hydro peroxides. Considering the sensory attributes characterizing such volatiles, they could adversely affect the sensory profile of the raw shelled walnut or its by-products (oil and flour) during handling and storage [10].

The formation of some volatiles (pentanal, 2-heptenal and 2-octenal) can be better explained by an enzymatic pathway, rather than chemical (non-enzymatic) [10]. In walnuts *Juglans regia* L may be present enzyme lipoxygenases (LOXs), which oxidases polyunsaturated fatty acids containing cis, cis-1,4-pentadiene fragments, such as linoleic and linolenic acids [6, 10]. The hydroperoxides produced by LOXs undergo cleavage to give short- and medium-chain hydrocarbons, aldehydes and alcohols (similar to those formed by non-enzymatic autoxidation) that contribute to the headspace volatile flavours [13]. In Figure 1 presents the reactions of autoxidation of linoleic acid and formation of major volatile compounds in walnut oil [10].

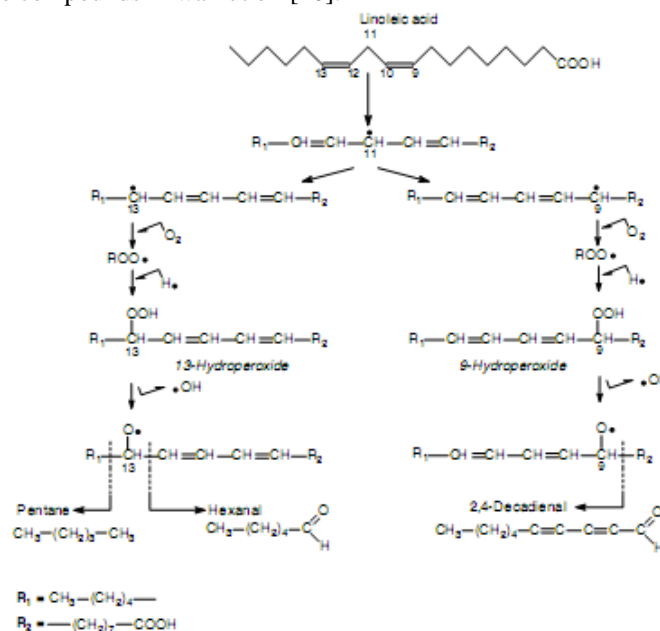


Fig. 1. Reactions of autoxidation of linoleic acid and formation of major volatile compounds in walnut oil [10]

Table 2 includes the experimental data across the physic chemical evolution of different indicators walnuts oil products in Moldova during storage. It was found that peroxide value (VP), acid value (VA) and activity PPO increase during storage. This phenomenon is observed in studies [16-19] attest oxidative and enzymatic instability of walnuts and nut oil.

Table2. Change indicators PV, VA and A PPO in oils produced from different varieties of nuts grown in Moldova during storage

Samples tested	Indicators tested	Term storage (days)					Allowable Values
		0	30	60	90	120	
A	PV	-	7,7	10	10	11	Government decision, Restriction technique
	VA	-	3.30	3.51	3.72	3.90	
	A PPO	-	-	0.6	0.72	0.1	VA - max.4, PV - max 10
B	PV	8.0	9.3	12.6	15.2	28.1	
	VA	3.21	3.36	3.62	3.74	4.03	
	A PPO	0.75	0.92	1.0	1.2	1.5	
C	PV	9.5	18.2	24.5	28.3	35.6	
	VA	3.2	4.7	5.1	5.7	6.0	
	A PPO	1.5	3.0	3.7	4.2	4.3	

PV- peroxide value, meg O₂/kg oil, VA - acid value, mg KOH/1g oil, APPO – activity polifenoloxidase, units activity

In Figures 2, 3 and 4 presents the correlation between the peroxide value (PV), acid value (AV) and activity PPO of walnuts oil. Prisons coefficient was estimated and tested indicators.

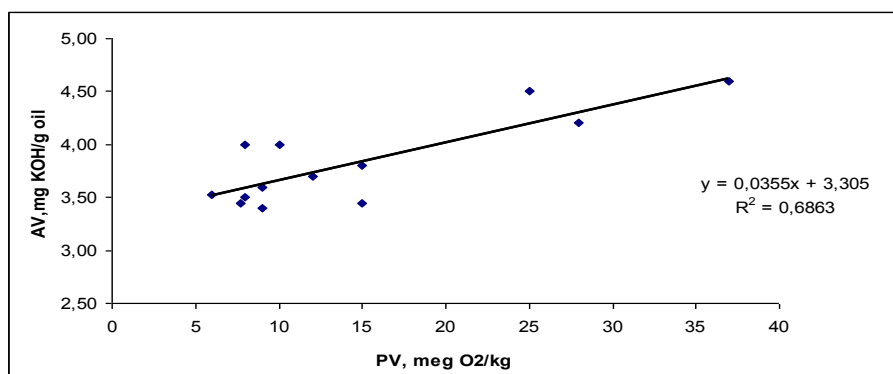


Fig. 2. Correlation between peroxide value and acid value of walnuts oil
Pirson correlation R (f = PV, AV) = 0.8284

High Pirson correlation (R (PV, A PPO) = 0.9622) was found between VA and activities PPO. Perhaps the correlation between VP and the activity of other enzymes, including the lipoxygenases is even more significant.

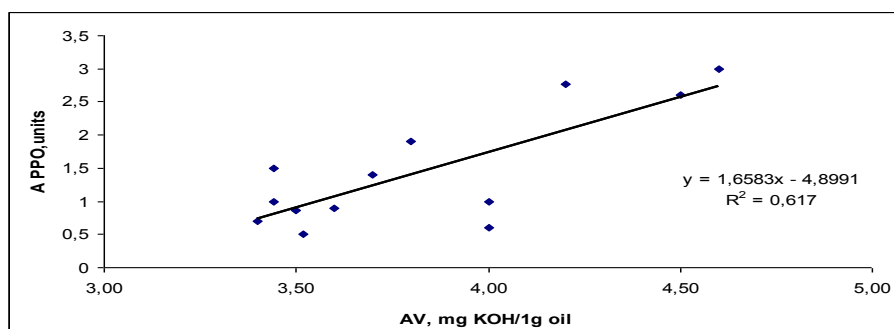


Fig.3. Correlation between activity PPO and acid value of walnuts oil
 Pirson correlation R (f = AV, APPO) = 0.7855

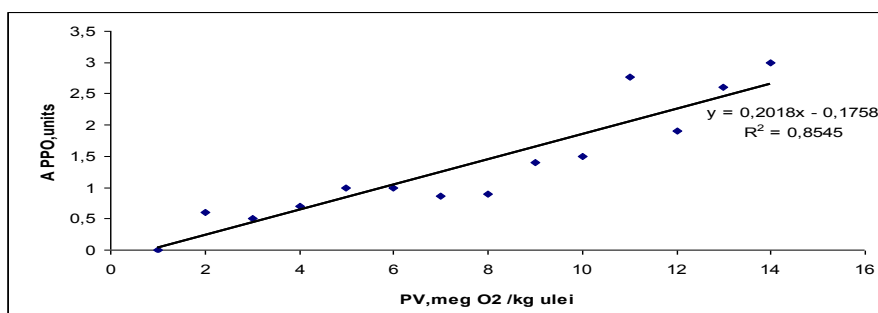


Fig.4. Correlation between activity PPO and peroxide value
 Pirson correlation R(f = PV, A PPO) = 0.9622

It is necessary to study and appreciate the factors that cause oxidative and enzymatic processes in walnuts and walnut oil to extend the oil storage. The oxidative stability is an important parameter in ascertaining the quality of oils, as it gives a good estimation of their susceptibility to oxidative degradation.

CONCLUSION

Walnuts *Juglans regia* L are important sources of vegetable protein and polyunsaturated fatty acids. Nutmeg contains the optimum ratio of $\Omega 6$ and $\Omega 3$. Oxidative stability is an important criterion in ascertaining the quality of oils. The balance of ω -6/ ω -3 ratio (1:1 to 1:4) is also an important determinant in decreasing the risk for coronary. Quality and oil stability depends on its chemical composition, especially the content of unsaturated fatty acids, as well as processing and storage conditions.

During storage in walnut oil held various physicochemical and enzymatic changes that influence the quality.

There was a significant correlation between peroxide value, acid value and enzyme activity. Pirson correlation has the following meanings:

$$R(f = PV, AV) = 0.83; R(f = PV, A PPO) = 0.96; R(f = AV, APPO) = 0.79.$$

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PHYSICO CHEMICAL PARAMETERS OF SOME VARIETIES OF WALNUTS (JUGLANS REGIA L) GROWN IN MOLDOVA

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Currently, in the food industry, it is given attention to processing autochthonous walnuts in order to obtain quality products with high nutritional value. Walnuts present a valuable raw material considering the content of unsaturated fatty acids, saturated acids, vitamins, antioxidants, minerals. During the storage, the quality of walnut kernels decreases and this process alters the organoleptic and physicochemical properties. This paper shows the changes of the chemical composition of walnut (*Juglans regia* L) grown in Moldova during the storage. There were estimated the following indicators: acidity, peroxide index, water activity. The study was conducted on autochthonous nuts which were stored for 1 month, 1 and 2 years.

Key words: walnut, peroxide index, water activity, kernel ratio

INTRODUCTION

Walnuts are of high economic for the food industry and its nuts are highly appreciated for its unique organoleptic characteristics [2], hypocholesterolemic effects [3, 4] and antihypertensive effect [5]. Walnuts *Juglans regia* L. are rich in polyunsaturated fatty acids, especially 18:2 and 18:3 and protein value [6]. Walnuts present a valuable raw material considering the content of unsaturated fatty acids, saturated acids, vitamins, antioxidants, minerals [1, 7, 8].

This paper shows the changes of the chemical properties of walnut (*Juglans regia* L.) grown in Moldova during the storage. There were estimated the following indicators: acidity, peroxide index, water activity. Quality of walnuts is determined by the physical properties such as: nut diameter, nut length, nut thickness, nut shape, nut size, nut weight, shell thickness, shell roughness) and kernel properties (kernel weight and kernel ratio).

1. MATERIAL AND METHODS

Materials

The study was conducted on autochthonous walnuts which were stored for 1 month, 1 and 2 years. Nuts *Juglans regia* L. Were harvested in Telenesti, Moldova, during harvest 2010 and 2011. Storage took place at ambient temperature.

Methods

Physical analysis: Physical analysis includes nut fruit properties (i.e. nut diameter, nut length, nut thickness, nut shape, nut size, nut weight, shell thickness, shell roughness) and kernel properties (kernel weight and kernel ratio) [4].

Shape of the nuts was determined by the following formula:

$$Nut_{index} = \frac{Nut_{length}(mm)}{(Nut_{diam} + nut_{thickness})/2} \quad (1)$$

Nut_{index} < 1.25 were taken as sphere shape, Nut_{index} > 1.25 indicate the oval shape of the nuts

Kernel ratio was determined by the formula:

$$Kernel_{ratio}(\%) = \frac{Kernel_{wt}(g)}{Nut_{wt}(g)} \times 100 \quad (2)$$

Size extra for the nuts if:

Nut diameter > 27mm for sphere; Nut diameter > 26mm for oval

Chemical analysis: For the Chemical analysis AOAC methods were used. Fats contents were determined by using AOAC 22.034.

Free acidity was determined by titration of the dissolved oil in a mixture of alcohol-ether (1:2) with an aqueous solution of sodium or potassium hydroxide (Standard EN ISO 660).

Acidity value represents KOH quantity in mg that is necessary for neutralization of free fat acids in one of fat (oil) [6].

AV was determined by the formula:

$$AV = \frac{28 \times V \times f}{m}; \text{ (mg KOH/g oil)} \quad (3)$$

Where: V= solution volume of KOH used by titration (ml);

f = 0.8416, solution factor of KOH 0.5 N

m = sample quantity, in gram

FA was determined by the formula:

$$FA = 0.5041 \times AV, \quad (\text{g. oleic acid/100g oil}) \quad (4)$$

Peroxide value represents the quantity of peroxide who is found in aliment and who have the capacity to liberate in one oxidative process iodine by potassium iodine [6].

For peroxide value, was used the next formula:

$$PV = \frac{(V_1 - V_2) \times n}{g} \times 100, \quad (\text{meg/Kg oil}) \quad (5)$$

Where: V1 = ml sodium thiosulphate used by sample titration;

V2 = ml sodium thiosulphate used by reference sample titration;

g = quantity of sample, g;

n = solution normality.

Water activity plays an important role in the oxidation of walnuts and walnuts oil in storage. Water activity walnuts and nuts oil was evaluated with the device Novasina LabSwift-aw.

2. RESULTS AND DISCUSSION

Fruit properties of the walnuts: Walnut cultivars in Telenesti, center of Moldova. Table 1 shows the physical characteristic: fruit dimensions and shape properties of walnuts *Juglans regia* L.

Table1. Fruit dimension and shape properties walnuts *Juglans regia* L. grown in Moldova

Physical properties studied	Values	Harvest year		Mean 2010-2011
		2010	2011	
Nut length, mm	Minimum	15.5 ± 0.45	14.5 ± 0.47	15.0 ± 0.55
	Maximum	2.55 ± 0.70	2.70 ± 0.53	26.3 ± 0.60
	Limit values	15.5 – 25.5	14.5 – 27.0	15.0 – 26.3
Nut diameter, mm	Minimum	12.5 ± 0.68	11.5 ± 0.48	12.0 ± 0.62
	Maximum	18.5 ± 0.56	22.0 ± 0.43	20.25 ± 0.72
	Limit values	12.5 – 18.5	11.5 – 22.0	12.0 – 20.25
Shape		11.6 – 17.1	10.4 – 20.1	11.0 – 18.6

The data obtained indicate that the diameter walnuts investigated during the two years, the harvest years 2010 and 2012 was within the limits 11.5 ... 22.0mm; length limits values in 14.5 ... 27.0mm.

Table 2 includes the minimum, maximum and average fruit, nut kernels, and indicators kernel ratio, shell thickness.

It was found that walnuts have investigated various dimensions and physical parameters. So we can assume that the chemical composition of walnuts was diverse.

Table2. The physical parameters of walnut

Harvest year	Values	Fruit weight, (g)	Kernel weight, (g)	Kernel ratio (%)	Shell thickness (mm)
2010	Minimum	11.88	4.78	40.03	0.93
	Maximum	13.68	6.89	50.4	2.0
	Average	12.88	4.82	37.5	1.47
2011	Minimum	13.02	3.85	29.7	1.1
	Maximum	14.01	7.04	50.3	1.9
	Average	13.19	5.02	38.4	1.5
2010 - 2011	Limit values	11.88 - 14.01	4.78 - 7.04	29.7 - 50.4	0.93 - 2.0
	Average	13.01	4.02	30.9	1.5

Quality walnuts *Juglans regia* L. during storage was evaluated following chemical and physical parameters: acidity value (AV), peroxide value (PV) and water activity (Aw) (Table 3). These indicators determine the quality of those walnuts and nut products. It was found that with prolonged storage duration increased values of these indicators.

Table 3. Physic chemical parameters studied nuts, grown in Moldova

Harvest year	During storage, months	AV, mg KOH/g	FA, % acid oleic	VP, meqO ₂ /kg	A _w , %
2010	20	2.403 ± 0,05	1.21 ± 0,05	13.7 ± 0,2	0.500
2011	9	2.299 ± 0,10	1.16 ± 0,10	8.3 ± 0,5	0.491
2010-2011		2.299 - 2.403	1.16 - 1.21	8.3 -13.7	0.401 - 0.500

CONCLUSION

This work is a source of information on physic-chemical walnuts *Juglans regia* L, grown in Moldova, as well as these parameters change during storage. This result can serve as information for the food industry including oil extraction from walnuts.

The data indicate that fruits nuts of these cultivars vary greatly in term of nut weight, kernel weight, kernel ratio, shell thickness, moisture content. The variability observed in these parameters is due to both genetic and environmental parameter describing fruit properties, mineral composition and nutritional parameters including chemical composition and weight distribution of the endosperm.

Experimental data proves that storage affect the physic-chemical parameters of walnuts *Juglans regia* L. Results indicate the values will increase VP, AV, A_w, so there was a decreases quality nuts. Therefore, we recommend for production walnuts oil nuts with 1 year shelf life.

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ENZYMES' IMPACT ON QUALITY OF WALNUTS (*JUGLANS REGIA L.*) AND WALNUT OIL

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Abstract: Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity. Lipoxidases catalyze the oxidation of unsaturated fatty acids such as linoleic, linolenic and arachidonic acids, in this way it form esters and peroxides, this process reduces the product's quality. This article provides a bibliographic study of enzymes and their assessment methods in walnut kernels and walnut oil. There were collected and studied different types of walnuts (*Juglans regia L.*) from Moldova. Nuts were evaluated fresh or after being stored for 1 and 2 years at room temperature. The study presents the changes of enzymes' activity during storage of the walnuts and oil, using titrimetric and spectrophotometric methods.

Key words: enzymes impact, oxidation enzymatic, polyphenol oxidase (PPO), enzymatic degradation

INTRODUCTION

The literature data confirm that walnuts are a rich source of a number of important nutrients that appear to have a very positive effect on human health [9]. Walnuts have a special dietary food value, given their carbohydrate content (11-14%), protein (14 -16%) represented the essential amino acids and lipids (62-65%, of which 44-48% are polyunsaturated fatty acids) [6, 12]. G. Ozkan and M. A. Koyuncu [6] report the chemical composition of 12 genotypes of walnut *Juglans regia L.*: protein (20.92 – 25.95%); ash (1.68 - 2.06%); fat (66.30 - 74.95%), from 62.4 to 68.7%, the oleic acid content of the oils ranged from 14.3 to 26.1% of the total fatty acids, while the linoleic acid content ranged from 49.3 to 62.3% and the linolenic contents from 8.0 to 13.8%.

Walnuts are also a rich source of bioactive compounds: they contain polyphenols, dietary compounds, tocopherols, folic acid, minerals, and manganese and copper [4, 10].

Walnut and walnuts oil quality depends on many factors, including the presence and activity of enzymes in these products [3, 10] his article provides a bibliographic study of enzymes and their assessment methods in walnut kernels and walnut oil.

1. MATERIAL AND METHODS

Material

The study was conducted on autochthonous walnuts *Juglans regia L.*, harvested in Telenesti, Moldova, during harvest 2010 and 2011. Storage took place at ambient temperature. Also served a object study walnuts kernel, stored in light/dark, walnuts oil industrial and obtained laboratory by cold pressing.

Methods

Polyphenol oxidase (PPO) activity in walnuts kernels and oil was performed in accordance with method exposed in [6, 7].

Acidity value represents KOH quantity in mg that is necessary for neutralization of free fat acids in one of fat (oil) [6].

Water activity plays an important role in the oxidation of walnuts and walnuts oil in storage. Water activity walnuts and nuts oil was evaluated with the device Novasina LabSwift-aw.

RESULTS AND DISCUSSION

Walnut kernels contain substantial quantities of triacylglycerol's and polyunsaturated fatty acids, and thus are susceptible to oxidative and hydrolytic rancidity [10].

Enzymatic oxidation of Unsaturated Fatty Acids

Decomposition of unsaturated fatty acids begins with hydrolysis of various glycerides by lipases, lipolytic acyl hydrolases, and phospholipases, during which the polyunsaturated fatty acids are, freed (Fig. 1).

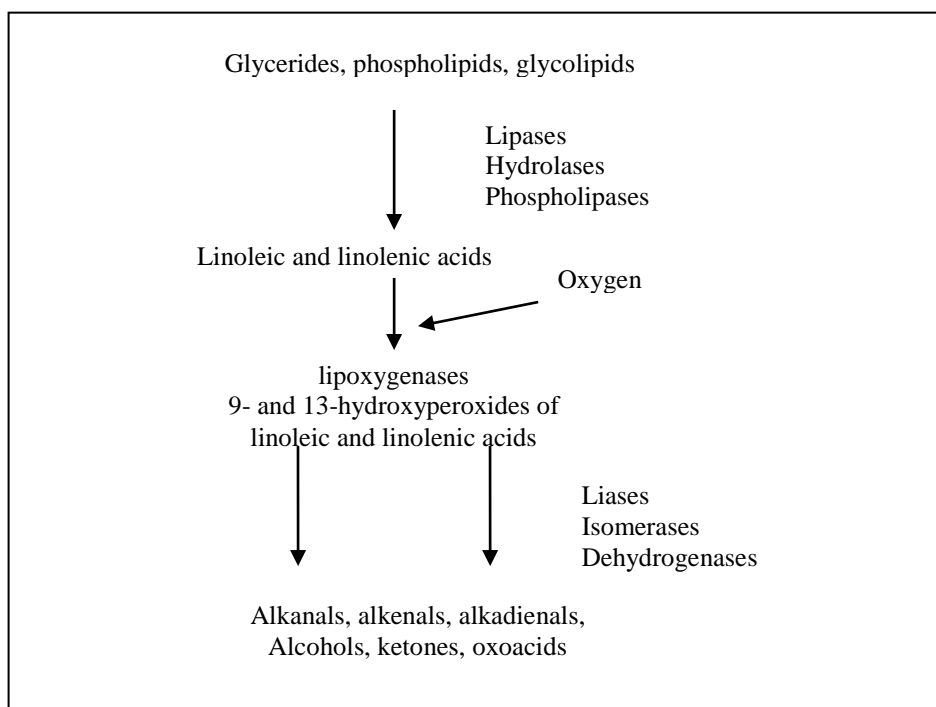


Fig. 1. Enzymatic oxidation of unsaturated fatty acids [1]

Lipoxygenases then convert unsaturated fatty acids into hydroperoxides, mainly 9 and 13 isomers, which are unstable. In last step, lyases, isomerases and dehydrogenases transfer hydroperoxides into a variety of volatile and nonvolatile products. The flavor components formed, such as aldehydes and alcohols, can be directly responsible for off-flavor [3].

Free unsaturated fatty acids, particularly linoleic and linolenic acids in plant are the preferred substrate for oxidation by lipoxygenases. Certain lipoxygenase is enzymes can

also catalyze the oxidation of unsaturated fatty acids when they are esterified in lipids. Presence of oxygen and light accelerates enzymatic activity [3, 10]. Lack of oxygen does not halt oxidation because some forms of lipoxygenases can oxidize fatty acids without the presence of oxygen, thus forming free radicals.

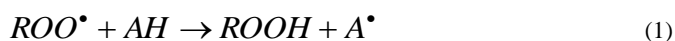
The presence of trace amounts of hydroperoxides accelerates the oxidation of unsaturated fatty acids by lipoxygenase, particularly under anaerobic conditions. Free radical formed from the decomposition of hydroperoxides can elevate further oxidation, which causes earlier than expected off-flavor formation and results in lower oil stability during storage [3].

The oxidation of oil is influenced by the fatty acid composition of the oil, oil processing, energy of heat or light, the concentration and type of oxygen, and free fatty acid, mono - and diacylglycerols, transition metals, peroxides, thermally oxidized compounds, pigments, and antioxidants. These factors interactively affect the oxidation of oil and differentiation of the individual effect of the factors is not easy [2].

Minor unsaponifiable compounds present in Oil

Edible oil consists of mostly triacylglycerols, but it also contains minor components such as free fatty acids, mono- and diacylglycerols, metals, phospholipids, peroxides, chlorophylls, carotenoids, phenolic compounds, and tocopherols. Some of them accelerate the oil oxidation and others act as antioxidants: phospholipids, color compounds, tocopherols, phenolic compounds. Free fatty acids act as prooxidants in edible oil [5].

Polyphenols and tocopherols are the main groups of phenolic compounds acting as primary antioxidants to inhibit oxidation in virgin olive oils. They mainly act as chain breakers by donating radical hydrogen to alkylperoxyl formed during the propagation step of lipid oxidation and subsequently forming a stable radical (A^*) through the well-known reaction [11]:



Most of the groups of minor compounds are reported to have either a beneficial or detrimental effect on oil stability although the positive contribution of the primary antioxidants present in the unsaponifiable fraction is the major determinant in the resistance of virgin olive oil to oxidation [11].

Probably the same thing occurs in walnuts oil, because according to studies [4, 10] oil nuts also contain minor unsaponifiable compounds. This minor substances thrives enzymes in walnut oil. Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity.

Buranasompob A. et al. [2] hypothesized that short time heat treatments inactivate lipoxygenase (LOX) or lipase enzymes and extend the shelf-lives of walnut and almond kernels (Figure 2 and 3).

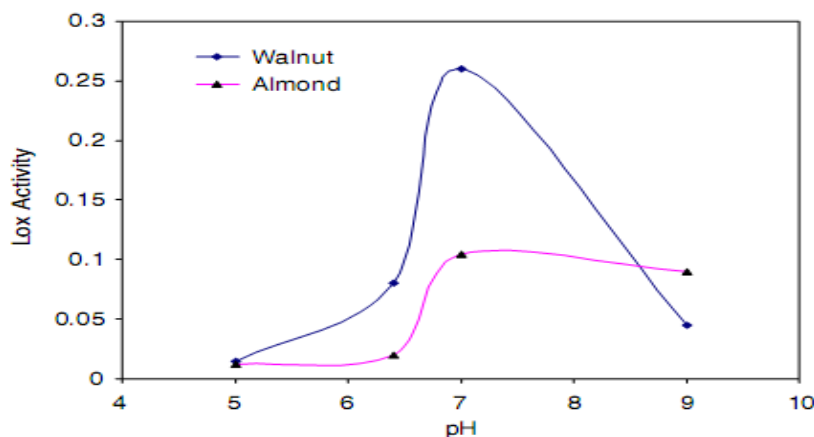


Fig. 2. Lipoxigenase activity (mMO₂/l s) in the homogenates of untreated almond of walnut kernels (1.0 g/ml) in pH range of (5.0–9.0)

(—●—) Walnut, (—▲—) Almond

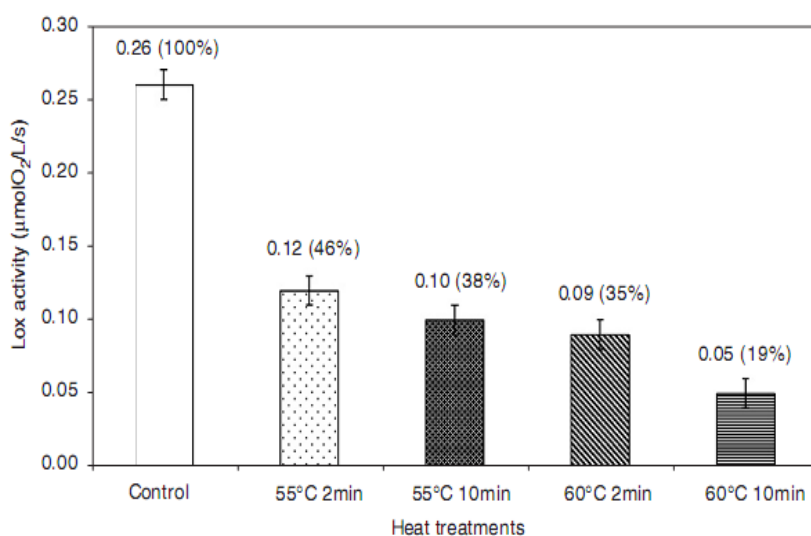


Fig. 3. Lipoxigenase activity in homogenates (0.1g/ml, pH 7.0) of control and heat treated walnut kernels

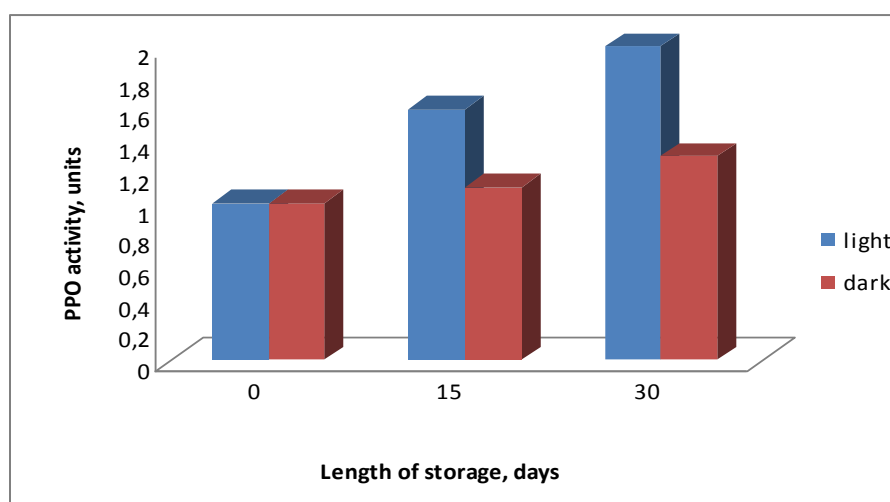
The enzyme polyphenol oxidase (PPO) is present in almost all plants, and catalyzes the oxidation of monophenols and o-diphenols to quinones. PPO in walnut (*Juglans regia* L.) has been little studied since the early 1900s [4, 5 and 10].

In Table 1 we present experimental results recorded on walnuts harvested in Moldova during the years 2010 and 2011, stored at ambient temperature, and walnut kernels packed in polietelen bags, stored in light and dark conditions. Following indicators were investigated: PPO activity, total acidity (TA) and water activity (Aw).

Table 1. Change indicators PPO, TA and Aw Walnut *Juglans regia* L during storage

Parameters investigated	Harvest year		During storage (days)	Storage conditions	
	2010	2011		light	dark
PPO activity, units of activity	2	1	0	1	1
			15	1.6	1.1
			30	2.0	1.3
TA, %	0.4	0.3	0	0.3	0.3
			15	0.46	0.4
			30	0.5	0.4
Aw, %	0.500	0.495	0	0.495	0.495
			15	0.481	0.477
			30	0.479	0.475

Figure 4 we present changes activities PPO in walnut kernels *Juglans regia* L. packed in polietelen bags, stored the dark and light (15 and 30 days).

**Fig. 4.** Changes in PPO activity of walnuts kernels samples during 30 days storage period

Light and darkness have a different effect on enzyme activity in storage. The flow of light increases enzyme activity. It is necessary to examine the factors that contribute to increased enzyme activity in order to minimize enzymatic degradation walnut oil. Thus we can partially solve the problem of keeping the oil.

CONCLUSION

This paper presents a bibliographic and experimental study of enzymes' impact on quality of walnuts (*Juglans regia* L.) and walnut oil. Quality and safety of walnuts and walnut oil, during the storage, depend largely on the enzyme content and their activity. Enzyme activity in walnuts depends on storage conditions: temperature, humidity, pH, light access, packaging.

Sunlight increases enzyme activity. The core of walnuts should be stored in waterproof packaging to oxygen, water vapor, light, to avoid chemical and enzymatic degradation of walnuts and walnut oil.

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MICROBIAL CONTAMINATION OF JUGLANS REGIA L. WALNUTS STORED IN MOLDOVA

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Abstract: This paper includes an analysis of microbial contamination of *Juglans regia* L. walnuts based on a bibliographic study of estimates made in the last 10 years by research workers from different countries. Existing studies show that nuts microbiota is quite diverse and is conditioned by many factors. If the conditions of fruit processing and storage are not respected, it leads to increasing the microbiological risk. This can affect the consumers health and can cause economic losses. The paper also presents an initial study of microbial contamination of walnuts grown in Moldova. The main objectives of the present research were to evaluate the mold, the total number of yeasts and other microorganisms of contamination in autochthonous *Juglans regia* L. walnuts, which were cultivated and stored at different stages of maturation. In the study (bibliographical and experimental), there are determined the factors which condition the microbiological risk, and are suggested ways to reduce microbial contamination of walnuts during storage.

Key words: microbial contamination, walnuts stored

INTRODUCTION

Various nuts are used as a raw material in many industries as well as for a direct consumer. Walnuts *Juglans regia* L. are rich sources of unsaturated fatty acids, proteins, vitamins, minerals etc. They contain an important amount of protein and fat and their products have wide acceptance as food throughout the world. Due to the extremely high fat, protein and low water content of various nuts such as hazelnut, almonds, walnuts, these products are quite refractory to spoilage by bacteria. Molds can grow upon them if they are stored under conditions that permit sufficient moisture for their propagation [7]. So the bibliographic and experimental study of interest across the microbial contamination walnuts stored in commercial markets.

Walnut trees are a habitat for a wide variety of fungal and (to a lesser extent) bacterial taxon's, some of which can pose a serious threat to plant health. Measures should be taken to further prevent the accumulation of microbial inoculum, e.g. removal of leaf debris [1]. Microbial biodiversity associated with the walnut *Juglans regia* L. is reported in several studies [1-11]. Study [1] includes microflora analysis on walnuts trees in South Tyrol (Italy). From 3.800 isolates obtained, belonged 3.742 (96,4%) to fungi, only 138 (3,6%) were bacteria. Fungal isolates were classified into 30 genera, *Alternaria*, *Penicillium*, *Phoma*, *Botryosphaeria*, *Fusarium*, *Cladosporium*, *Phyllosticta* and *Epicoccum* being the most taxa. More isolates were obtained from leaves 45.3% than from twigs 31.8% and fruit 23.0%.

Microbial growth depends on a variety of environmental parameters. Among them temperature and relative humidity play a predominant role [6, 7].

This study was conducted with the following aims:

- Analysis of microbial contamination of *Juglans regia* L walnuts based on a bibliographic study;
- Investigation of microbial contamination of walnuts grown in Moldova.

MATERIALS AND METHODS

Materials

The study was conducted on autochthonous walnuts which were stored for 1 month, 1 and 2 years. Nuts *Juglans regia* L. were harvested in Telenesti, Moldova, during harvest 2010 and 2011. Storage took place at ambient temperature.

Microbiological Analyses

A twenty-five g analytical unit taken from the 100g sample unit was used in aerobic bacterial count, mold count. Plating for total bacterial count was carried on standard nutriment agar and for molds on Seabourad dextrose agar [9, 13]. Isolated colonies after incubation period of 3 days at 28°C for bacterial count and 7 days at the same temperature for mold count were studied.

Also, total mold, yeast contamination and bacterial counts of the samples were determined using a dilution plate method. About 25g walnut samples (whole nuts, kernels) were suspended in 225ml of 0.1% peptone solution and homogenized. Homogenized samples were diluted with 0.1 % peptone solution to concentrations of 10^{-2} and 10^{-3} . Each dilution (100µl) was dispensed and spread onto sterile BA/ Seabourad in Petri plates, followed by incubation at 28°C for 3/5 days. Total mold and yeast counts were estimated by back-calculation based on observed mold for each dilution and was expressed as the number of colony forming units (CFU) per gram in each sample.

RESULTS AND DISCUSSION

Bibliographic study indicates that the microbiota nuts depend on variety, geographical conditions, climate, the collection, processing and storage [1-12]. The climatic parameters (temperature, relative humidity, precipitations and UV irradiation), the composition of leaf surface (senescence) and the availability of nutrients all change over the microbial colonization [1]. Molds of many genera may be found on examining nuts [6]. That mould genera, occurring most frequently in shelled and unshelled nuts, were, *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium*. *Aspergillus* and *Penicillium* species predominated in all the tested shelled nuts being (32-39%) *Aspergillus* and (25 to 31%) *Penicillium*. While *Aspergillus*, was dominant in all the unshelled nuts (41-50%). Other genera were found to be in low percentage (*Rhizopus*, *Mucor* and *Cladosporium*). The results [6] are in accordance with Smith and Arend [7] who stated that *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Cladosporium* represented the common genera in nuts.

Data reported by M.J. Sejny et al.[6] show the qualitative and quantitative distribution of different groups of bacteria present in the shelled and unshelled nuts. It was found that Gram positive spore forming bacilli, Gram positive Micrococci, Gram positive rods and Gram negative short rods were generally contaminating the samples. Their quantitative distribution varied through the tested nuts. The most dominant bacteria appearing in the unshelled nuts was Gram positive spore forming bacilli being 55 and 68% in shelled and unshelled walnuts respectively. While Gram positive Micrococcus were 24 and 29% in the respective value. The dominant groups of bacteria presented in shelled nuts showed nearly the same trend as those on unshelled nuts.

The distribution of Gram negative short rods were found very low in the nuts (2-10%), comparing with the other groups of bacteria. These results confirm the work of King et al [9] who reported that microorganisms associated with commercially shelled nuts were numerous and varied. They added that the genera of bacteria isolated from almonds included *Bacillus*, *Micrococcus*, *Streptococcus*, *Brevibacterium*, *Escherichia* and *Aerobacter*. These

results are also in harmony with Hall [10] and Chypley and Heaton [11] who found that of Gram negative short rods as *Pseudomonas*, *Escherichia*, *Leuconostoc*, *Proteus* and *Aerobacter* were found associated with commercially shelled nuts.

Microorganisms associated to Brown Apical Necrosis (BAN) of walnut [8]:

- *Xanthomonas arboricola* pv.juglands, isolated from all walnut fruit affected tissues in more than 36% fruits.
- *Fusarium* spp.(*F.moniliforme*, *F.solani*, ...) isolated from exocarp and sees in 4% fruits (depending on fruits tissue)
- *Alternaria* spp.only isolated from exocarp at the rate of 1% (more frequent in exocarp and mesocarp).

In Figure 1 we present incidence (Affected fruits) fruits walnuts *Juglans regia* L. under study [8].

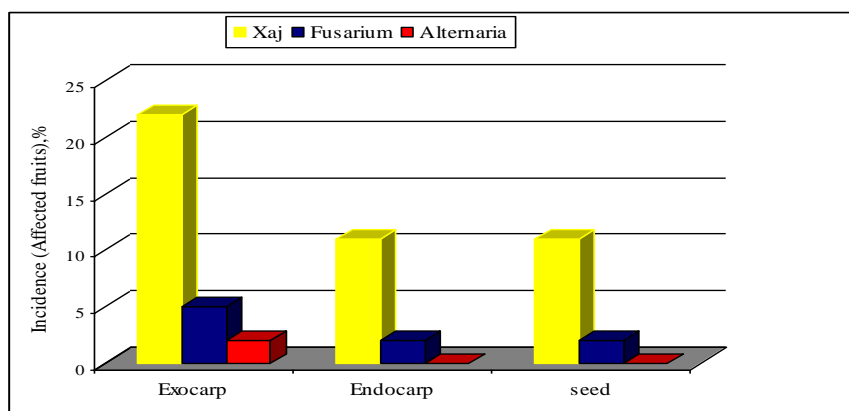


Fig. 1. Microorganism isolated from walnut fruits with BAN symptoms [8]

Infection with microorganism walnuts *Juglans regia* L. can occur in Stade growth, ripening and harvest. In Figure 2 we present incidence walnuts caused by microorganism associated with BAN (*Fusarium* and *Alternaria*).



Fig. 2. Seed infections in walnut fruits caused by microorganism associated with BAN [8]

In Table 1 we present experimental data obtained from analysis of walnut *Juglans regia* L, grown in different years and stored at ambient temperature, shelf life 10 and 22 months.

Table 1. Seasonal variation of fungi and bacteria of five experimental walnuts *Juglans regia* L in three different seasons

Harvest year	During storage, (months)	Sample, no.	This microorganisms		
			Fungi and yeasts	Bacteria	
				Gram positive	Gram negative
2010	22	1	++++	++	+++
		2	++++	++++	++
		3	+++	++++	-
		4	++++	+	-
		5	++	-	+++
		6	++++	+++	++
		7	+++	++	++++
		8	+	++++	+
		9	++++	++	-
		10	+++	-	++++
2011	10	1	++	+++	++++
		2	+	++++	++
		3	+++	+++	+
		4	++++	+	-
		5	++	-	-
		6	++	++	++
		7	+	++	-
		8	+	++	+
		9	++	++	-
		10	+++	-	++

(+) = minor infestation; (++) = average infestation; (+++) = major infestation; (+++++) = heavy infestation; (-) = lack of infestation.

The results of microbiological control of walnut *Juglans regia* L., grown in Moldova are the limits reported in bibliographic study. It was found that longer storage of nuts contributes to increased microbial infestation. The rate of infestation abundance of walnuts harvested in 2010 amounted to fungi and yeasts 50% and 30% Gram positive and Gram negative 20%. Nuts harvested in 2011 were less infested. Heavy infestation rate being only 10% for fungi, yeasts, Gram positive and Gram negative bacteria.

In Figure 3 we present some images of colonies of microorganisms found in walnuts *Juglans regia* L., collected in Moldova, stored 22 months at ambient temperature.



Fig. 3. Isolated colonies after incubation period of 7 days at 28°C

When storing nuts should be taken into account Code of Practice for the prevention and reduction of aflatoxin contamination in nuts (CAC/RCP 59-2005):

- Storage facilities should be clean and dry (if possible keep the relative humidity < 70%), well-vented structures that provide protection from rain, entry of rodents and birds, drainage of ground water and have minimum temperature and humidity fluctuations. If possible, temperature should be kept between 0°C and 10°C to minimize fungal growth during storage.
- Good storage practices should be implemented to minimize the levels of insects and fungi in storage facilities. This may include the use of suitable, registered insecticides and fungicides or appropriate alternative methods. Nuts stored in sacks should be placed on pallets which are positioned to allow good ventilation and access.
- Water activity, which varies with moisture content and temperature, should be carefully controlled during storage. *Aspergillus flavus*/A. *parasiticus* cannot grow or produce aflatoxins at water activities less than 0.7.

CONCLUSION

Bibliographic and experimental study shows that walnuts *Juglans regia* L. can be infected with fungi, yeasts and bacteria, which minimize their quality. Infection rate depends on climatic parameters (temperature, relative humidity, precipitations and UV irradiation), variety nuts and storage conditions. Good storage practices should be implemented to minimize the levels of insects and fungi in storage facilities.

To store walnuts should be taken into account Code of Practice for the prevention and reduction of aflatoxin contamination in nuts (CAC/RCP 59-2005).

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ESTIMATION OF APICAL NECROSIS IN JUNGLANS REGIA L. WALNUTS HARVESTED IN MOLDOVA

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Abstract: Blight caused by *Xanthomonas* pv. *Juglandis* (Xaj) is a bacterial disease of Persian *Juglans regia* L. walnut. This article refers to an international bibliographic study on microbial risk caused by this bacterium. Recent studies indicate that the bacterium *Xanthomonas arboricola* pv. *juglandis* is the most frequently microorganism associated with apical necrosis and can produce initial infections in young nuts. This bacterium was isolated on fruit orchards from Austria, Bulgaria, France, Germany, Greece, Portugal, and Romania. Also, in this work there are characterized and other microorganisms such as *Alternaria* spp. and *Fusarium* spp. isolated from „apical necrosis”. Apical necrosis was estimated in walnuts *Juglans regia* L. harvested in Moldova.

Key words: apical necrosis, walnuts, *Juglans regia* L., *Xanthomonas arboricola*, Brown Apical Necrosis (BAN)

INTRODUCTION

Xanthomonas arboricola pv. *Juglandis* is the causal agent of walnut blight, one of the most important and widespread diseases of Persian (English) walnut (*Juglans regia* L.), causing severe damage to leaves, twigs and nuts [7]. The apical necrosis was first observed in the late 90s following an intense early fruit drop and was found to affect the main walnut production in Spain [1, 2], France [4, 5], Italy [3], and Turkey [9]. Apical necrosis infections are more frequent and severe in the early fruit developmental stages. Initial infections occur after fruit set and in environmental conditions are favorable, infection spreads externally and internally through the walnut fruit tissues [9].

Environmental conditions favorable to apical necrosis are not yet clearly determined. However, local wet and warm conditions during the walnut fruit initial growing period enhance the disease incidence and severity. Preventive sprays with copper derive applied according to the schedule proposed for walnut blight control [8] contributes to reduce the incidence of apical necrosis. Removal of mummified nuts from the trees and dropped fruits from the orchard ground reduces infestation *Xanthomonas arboricola* pv. *Juglandis*, prevent further yield losses. Appropriate soil characteristics and walnut fertilization help to reduce the damage [6].

DISCUSSIONS AND APPROACHES

Global walnut (*Juglans regia* L.) production increases from year to year. Bacterial diseases are threatening these nut crops all over the World. *Xanthomonas arboricola* pv. *juglandis* (Xaj) is the agent of walnut bacteriosis, and has been associated also to brown apical necrosis. Several walnut genotypes have shown a range of tolerance to this disease in diverse countries. Deep bark canker (*Brenneria rubifaciens*) and Shallow bark canker (*Brenneria nigrifluens*) are the other bacterial diseases affecting walnut [5].

The main bacterial diseases that threaten both crops and possibilities to control them by using genetic resources are discussed in several studies [1-9].

Xanthomonas arboricola pv. *Juglandis* (Xaj) is the causal agent of walnut blight, the most important bacterial disease of *Juglans regia* and other *Juglans* species, which affects a high percentage of flowers and fruits, but does not kill bearing trees [1, 5, 7]. This bacterium has been also isolated from tissues affected by bacterial apical necrosis [3]. Blight has been recorded from Europe (Austria, Bulgaria, Denmark, France, Germany, Greece, Italy, Moldova, Netherlands, Poland, Portugal, Romania, Russia, southern Russia, Slovenia, Spain, Switzerland, UK, Ukraine and former Yugoslavia) Asia, [Azerbaijan, China, (Hubei, Henan, Jiangsu, Shaanxi, Shandong), Georgia, India, (Himachal Pradesh, Uttar Pradesh) etc [5].

To investigate the genomic variability of *X. Aboricola* pv.juglandis, 66 isolates obtained from different countries (England, France, Italy, The Netherlands, Romania, Spain, USA, and New Zealand) were analyzed using the Amplified Fragment Length Polymorphism (AFLP) technique. AFLP analysis proved to be reproductive, reliable, and sufficiently sensitive to reveal the individual genomic variability within *X. Arboricola* pv *juglans* isolates used in this study. Result indicates that geographic location could be partly responsible for the genomic heterogeneity [7].

Being polyphenols involved in tolerance to bacterial diseases, it has been shown that the gene jrPPO1 is the sole polyphenol oxidase PPO gene in walnut able to encode a jrPPO enzyme that is expressed in the leaves, hulls and flowers of walnut trees. It can also happen that some modifications in the anatomical traits of the epidermis of walnut progenies.

Figures 1-2 are apical necrosis of walnut fruit *Juglans regia* L, Figure 3 - colonies of *Xanthomonas arboricola* pv. *juglandis* isolated from apical necrosis lesions in walnut fruits, growing on modified Tween Medium [9]



Fig. 1. External symptoms of apical necrosis in young fruits collected from walnut tree (A). Internal symptoms of apical necrosis in fruits collected from walnut tree (B) or dropped to the soil (C) [9].



Fig. 2 External and internal extent of necrosis is not always correlated [9]



Fig.3. Colonies of *Xanthomonas arboricola* pv. *juglandis* isolated from apical necrosis lesions in walnut fruits, growing on modified Tween Medium [9]

Table 1 summarizes the information from different countries on tolerance/resistance/low susceptibility to *Xaj* in *J. regia*. *Polyphenols and Xaj*. It is known that polyphenol oxidase (PPO) catalyzes the oxidation of polyphenols to quinones, which are very reactive against disease agents [5].

Table 1. *Juglans regia* accessions resistant/tolerant to *Xaj*. found in native walnut population.

Country	Locality	Selections or cultivars	Resistance/tolerance	References
China	Taigu, Shanxi	Jinboxiang 3	<i>Xaj</i> resistance in the open	Tian et al., 2008
	Kunming, Yunnan	‘Yunxin Gaoyuan’, out of <i>J. sigillata</i> x <i>J. regia</i> ‘Yulin A7’, cross made in 1977	Resistant to <i>Xaj</i> in the open	Xi et al., 2007
	Kunming, Yunnan	‘Yunxin 90303’ derived from <i>J. sigillata</i> cv. ‘Santai’ x <i>J. regia</i> cv. ‘Xinzao No. 13’	Diseases resistant	Zhao et al., 2007
	Tai’an, Shandong	‘Luguo 4’, open pollinated	<i>Xaj</i> resistant in the open	Zhang et al., 2009

		native genotype		
	Ghengxian county, Gansu	'Longnan 15'	<i>Xaj</i> resistant in the open	Guo et al., 2009
Greece		'Amigo', 'Ashley', 'Serr', 'Eliaana', 'Grand Jean', 'EK-1', 'EH-28', 'EH-29'	The lower <i>Xaj</i> susceptible in the trial conditions	Tsiantos et al., 2007
Italy	Pignataro Maggiore, area of Caserta	Ex situ collection	All <i>Xaj</i> susceptible	Piccirillo, 2003 Piccirillo and Petriccione, 2006
Romania	Oltenia	550 genotypes out of native population	Several genotypes are <i>Xaj</i> tolerant	Botu et al., 2001
	Valcea	Valcor, Valmit and Valrex, out of native population	Texting in orchard for <i>Xaj</i> and other traits	Botu et al., 2007
	Valcea	'Portval' (syn. 'VL 26 B'), seedling rootstock	Good resistance to diseases, <i>Xaj</i> not specified	Achim et al., 2007
Spain	Galicia	>550 natural genotype tolerant in the open were evaluated for <i>Xaj</i> , susceptibility	'MBLu-20', 'MBLu-21' and 'MBC-45', did show low <i>Xaj</i> susceptibility in lab.	Aleta et al., 2001
	Cantabria	Native seedlings aged over 15-years-old	28 were selected as <i>Xaj</i> free symptoms. In lab all were susceptible	Arrieta and Diaz, 2007; Lopez et al., 2007
	Murcia	Ex situ collection	Segregation to <i>Xaj</i> tolerance were found in an ex situ collection	Frutos et al., 2008, 2009, 2010; Ruiz et al., 2010
Slovenia		24 genotypes preselected	Most promising (<i>Xaj</i> resistant) are late leafing genotypes 'Z-62', 'Krc' and 'Z-60'	Solar and Stampar, 2006

Turkey		19 genotypes	All genotypes <i>Xaj</i> resistant	Akca and Ozogun, 2004
		Sen 2, C440, 77 H-1, Kaplan 86, Tokat 1, Kaman 1 and Bilecik	All selections <i>Xaj</i> low susceptible	Özaktan et al., 2007
Yugoslavia		Nbs. 10/88, 40/92, 30/93, 28/94, 9/96, selected from native population	Highly resistant to diseases	Mitrovic, 2003
		Nbs. 10/88, 40/92, 30/93, 28/94, 9/96	All resistant to <i>Xaj</i> .	Mitrovic et al., 2007

Microorganisms associated to *Brown Apical necrosis* BAN of walnut:

- *Xanthomonas arboricola* pv. *juglandis*, isolated from all walnut fruit affected tissues in more than 36% fruits.
- *Fusarium* spp. (*F. moniliforme*, *F. solani*, etc.) isolated from exocarp and seed in 4% fruits (depending on fruit tissue)
- *Alternaria* spp. only isolated from exocarp at the rate of 1% (more frequent in exocarp and mesocarp).
- *Xaj* was isolated from external and internal symptoms of walnut fruits in sampling period and produced typical Ban symptoms on detached walnuts.
- Some *Fusarium* spp. Isolates were pathogenic on detached nuts.

CONCLUSION

This paper includes a bibliographic study of accidents apical necrosis of walnut fruit *Juglans regia* L from different regions. The incidence of walnut blight can vary markedly between cultivars, locations and years with the development of damaging epidemics when weather conditions are favorable. The most important bacterial diseases in walnut seem to be due to *Xaj*, have a worldwide distribution.

In Moldova we also observed incidence of apical necrosis walnuts. *Xanthomonas arboricola* pv. *Juglandis* is the causal agent of walnut blight, one of the most important and widespread diseases of Persian (English) walnut (*Juglans regia* L), causing severe damage to leaves, twigs and nuts. It is necessary to study this effect in order to minimize losses crop and reduce quality walnuts and nut products.

In this context this paper is a first step in this direction.

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INTENSIFICATION OF SURFACTANTS' SYNTHESIS ON FRIED OIL AND GLYCEROL CONTAINING MEDIUM

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The possibility of waste use for biosurfactant production was determined. Presence of fumarate and citrate intensified biosurfactant synthesis by 2.5-fold. It was shown that C₄-dicarboxylic acids enhanced activity of surfactant biosynthesis enzymes. Cultivation on mixture of substrates resulted in increased surfactants quantity.

Key words: biosurfactants, synthesis intensification, antimicrobial activity

Searching for the ways to process waste has become very topical today. Biotechnology is one of the most environmentally attractive methods, which has the ability to solve the problem of waste utilization and to produce the valuable microbial products, for example biosurfactants. Due to their physico-chemical properties, the use of microbial surfactants have been proposed for various industrial applications, as additives in foods, cosmetics and detergent formulations [1, p. 427, 4, p. 478]. In the food industry, the most useful property is the ability to form stable emulsions, which improves the texture and creaminess of dairy products. Biosurfactants are also used to retard staling, solubilise flavour oils and improve organoleptic properties in bakery and ice cream formulations and as fat stabilisers during cooking of fats.

Despite the outstanding properties and advantages of biosurfactants their price is still too high compared to the synthetic ones. One of the ways to reduce the cost of biosynthesis is with the use of low-cost growth substrates, such as wastes of other industries [2, p. 660].

The oil-oxidizing bacteria were isolated from the oil-polluted samples of soil and identified as *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vacinii* K-8. The ability of these strains to synthesize the metabolites with surface-active and emulsifying properties was determined during the cultivation in medium with hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (glucose, ethanol) substrates [3, p. 273].

The aim of present work – development of approaches for increasing biosurfactant synthesis by *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vacinii* K-8 on wastes.

Our experiments showed the possibility of cultivation of *R. erythropolis* IMV Ac-5017 on a medium with 1–2 vol. % of fried sunflower oil (after repeated use in public catering) and oil-containing wastes (soapstock), and indexes of biosurfactant synthesis were 1.2–2.6 times higher than in a medium containing *n*-hexadecane (traditional substrate). It was found that the addition of glucose (0.1 %) at the beginning of cultivation into the medium containing 2 vol. % of fried oil, accompanied by an increment of surfactant concentrations of 4-fold compared with the cultivation of *R. erythropolis* IMV Ac-5017 on a glucose-free medium.

The study of growth and the biosurfactant synthesis by *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vacinii* K-8 in the medium containing glycerol

(0.5–1.0 vol. %) showed that the strains were able to assimilate this substrate and synthesize metabolites with surface active and emulsifying properties. But conditional surfactant concentration (CSC*) was lower than in the case of *n*-hexadecane or ethanol as substrates. Due to the fact that the lowest rates of synthesis of surfactant have been observed in *N. vaccinii* K-8 (CSC* = 1.1), the aim of further experiments was to optimize the nutrient medium and cultivating conditions for the strain K-8. With single-factor experiments and mathematical methods of planning, the composition of nutrient medium for *N. vaccinii* K-8 was optimized (0.5 g/L of NaNO₃, 0.3 g/L of yeast autolysate and 1.5 vol. % of glycerol). It was established that after optimizing the composition of the medium, the amount of surfactant of *N. vaccinii* K-8 increased by 4 fold.

It was shown that the increase in the synthesis of *R. erythropolis* IMV Ac-5017 surfactant in the medium with ethanol or *n*-hexadecane observed in the case of simultaneous introduction of citrate and fumarate in concentrations of 0.1 and 0.2 % respectively in the early stationary phase of growth. Experiments have shown that the introduction of citrate and fumarate while cultivating *N. vaccinii* K-8 in the medium with glycerol was also accompanied by an increase of surfactant synthesis. The highest indexes – increase of the conditional surfactant concentration of 35–40 % and emulsification index of 20 % compared with the cultivation of strain K-8 in a medium without organic acids – were achieved by the simultaneous addition of fumarate and citrate (0.1–0.2 % of each). Similar studies conducted with strain IMV B-7241, showed that the simultaneous introduction of fumarate and citrate (0.01–0.02 %) made the concentration of exocellular surfactant increase twofold compared to bacteria growing in the medium without organic acids. Importantly, under these conditions we did not observe an increase in biomass. An increase in surfactant concentration of IMV B-7241 strain was the result of the simultaneous functioning of two anaplerotic pathways, also resulting in a 3–5 fold increase in activity of biosynthesis enzymes.

Further studies were devoted to the surfactant synthesis by *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on a mixture of *n*-hexadecane and glycerol. Cultivating on a mixture of glycerol and *n*-hexadecane (0.5–1.0 vol. %) led to a 1.5–3 fold increasing surfactant synthesis.

We showed that biosurfactant preparations of IMV B-7241 (0.15–0.22 mg/mL) and IMV Ac-5017 (0.61–2.1 mg/mL) strains were effective against *Escherichia coli* IEM-1 (67 % of cell loss), and vegetative (45–100 % of cell loss) and spore (75 % of spore loss) cells of *Bacillus subtilis* BT-2.

The effects of extracellular metabolites, including surfactants of *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* K-8 on some phytopathogenic bacteria was investigated. It was shown that after treatment (2 h) with surfactant preparation (0.15–0.4 mg/mL) of strains IMV Ac-5017 and IMV B-7241 survival of (10⁵–10⁷ CFU/mL) of pathogenic bacteria cells (genera *Pseudomonas* and *Xanthomonas*) was 0–33%. The quantity of cells of most studied pathogenic bacteria was reduced by 95–100 % in the presence of a surfactant and other extracellular metabolites preparations (0.085–0.85 mg/mL) of *N. vaccinii* K-8. These data show the promise of microbial surfactants for the development of environmentally safe products to control pathogenic bacteria.

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COMPARISON OF TWO IMMOBILE PHASES USED IN HPLC ANALYSIS OF ORGANIC ACIDS IN WINE

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Abstract: Organic acids are one of the most important substances in wine, which make major contributions to the composition, stability and organoleptic qualities of wines, responsible of wines' microbiological and physicochemical stability. For searching of optimal conditions for HPLC analysis of organic acids in wine there were investigated two immobile phases, such as: modified C18 type and column, in which separation of the analytes is based on ion exclusion and ion exchange effects. Thus NUCLEODUR C18 Pyramid (*Macherey-Nagel*) is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. The NUCLEOGEL SUGAR 810 H (*Macherey-Nagel*) is packed with a sulphonated spherical polymer of gel type in the H⁺ form. It is specifically designed for the separation of numerous polar organic compounds like organic acids, alcohols, sugar alcohols and sugars.

Key-words: HPLC, separation of polar compounds, organic acids, reversed phase

Introduction

The success of wines produced depends on careful attention in controlling the vinification process. Organic acids make major contributions to the composition, stability and organoleptic qualities of wines. Their preservative properties also enhance wines' microbiological and physicochemical stability [1].

Obviously, it's necessary to have a reliable and simple method of identification of some important wine's indexes, which will help to improve and intensify the control over the wines quality during the making process and storage.

Modern scientific methods of analysis and control are needed to achieve this purpose.

High performance liquid chromatography (HPLC) is an important analytical tool for separating and quantifying components in complex liquid mixtures. A variety of liquids and stationary phases can be used in liquid chromatographic systems. Thus by choosing the appropriate equipment (i.e. column and detector), this method is applicable to samples with components ranging from small organic and inorganic molecules and ions to polymers and proteins with high molecular weights [2].

Method and Materials

The popularity of chromatography as a method of quantitative analysis is due to the fact that it combines the 2 processes at once. Above all, this is a separation of a mixture of substances, and if the sensitivity of the detector is known, quantification of the separated in the column of individual substances. Thus, in contrast to other analytical methods, chromatography is not necessary that the method of detection was specific to the substance or the class of substances. This method allows to quantify the content of each component without any treatment or difficult pre-sampling process of the test mixture. The advantage of liquid chromatography consists in the fact that it allows to define the substance at

ambient temperature, while the gas-chromatography requires high temperatures at which some substances may be disintegrated, and also allows to determine non-volatile components.

For searching of optimal conditions for HPLC analysis of organic acids in wine there were investigated two immobile phases, such as: modified C18 type and column, in which separation of the analytes is based on ion exclusion and ion exchange effects.

Thus NUCLEODUR C18 Pyramid (*Macherey-Nagel*) is a silica phase with hydrophilic endcapping, designed especially for use in eluent systems of up to 100 % water. It has the next *technical characteristics*: 250x4 mm; special phase with polar endcapping; pore size 100Å; particle sizes 5 µm; carbon content 14 %; pH stability 1–9 [3].

NUCLEOGEL SUGAR 810H (*Macherey-Nagel*) is packed with a sulphonated spherical polymer of gel type in the H⁺ form with the next *technical characteristics*: 300x7.8 mm; strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form; particle sizes 7 µm. It is specifically designed for the separation of numerous polar organic compounds like organic acids, alcohols, sugar alcohols and sugars [4]. The separation of the analytes is based on a combination of different mechanism. Ion exclusion and ion exchange effects are dominated, due to the charge of sulfonic acid groups.

For searching of separating possibilities of these phases there was used liquid chromatograph LC-20AD by *Shimadzu* with spectrophotometer SPD-20AV on the wave length 210nm.

Results and Discussion

The columns NUCLEODUR C18 Pyramid (1) (*Macherey-Nagel*) and NUCLEOGEL SUGAR 810H (2) (*Macherey-Nagel*) were used for separating standard mixture of organic acids and real samples of wines.

For the first system there was used 0.2% solution of H₃PO₄ as eluent with flow rate 0.5ml/min. For the second system – 10mM H₂SO₄ with the same flow rate 0.5ml/min.

Chromatograms of standard solutions of the main organic acids are shown on the fig.1,2.

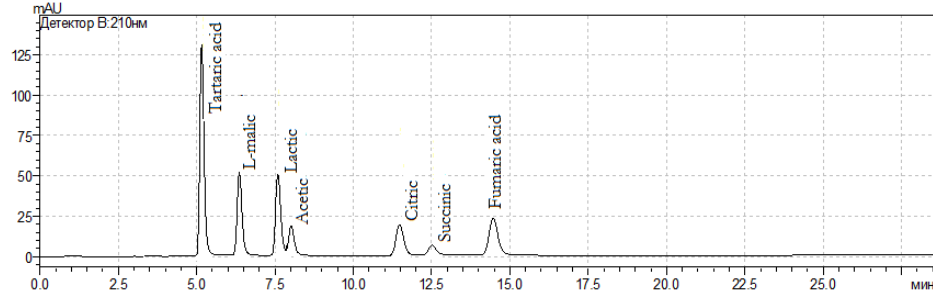


Fig. 1. Chromatogram of the standard solution of organic acids. Nucleodur C18 Pyramid, 250x4,0mm; 0.2% H₃PO₄; 0.5ml/min; 35°C; 210nm.

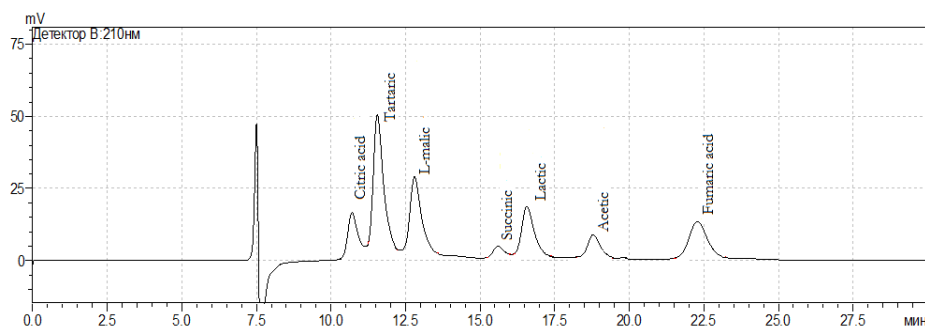


Fig. 2. Chromatogram of the standard solution of organic acids. Nucleogel SUGAR 810H, 300x7,8mm; 20mM H_2SO_4 ; 0.5ml/min; 40°C; 210nm.

In addition chromatographic column Nucleogel SUGAR 810H has good separating properties for “wine’s sugars” like fructose, glucose, sucrose.

Analysis of real samples of wines were done in the same conditions described above. Unfortunately column Nucleogel SUGAR 810H is not suitable for this purpose, especially for analysis of semisweet and sweet wines, because of the close retention times of *Fructose* and *L-malic* acid. In the case of Nucleodur C18 Pyramid *Fructose*’s peak has its signal earlier than main wine’s acids.

The comparative characteristics of signals obtained on these two immobile phases at the analysis of standard solutions of organic acids are shown in the Table 1. There are such characteristics like: ratio between height of the peak and its band width, resolution factor (R_s – fig.3).

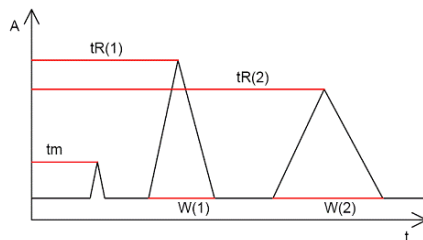


Fig. 3. Calculation scheme of resolution factor for two consecutive chromatographic peaks. $\Delta t = t_{R1} - t_{R2}$. $R_s = 2 \Delta t / (w_1 + w_2)$.

If the difference in retention times of two peaks is relatively large, and the width of the bases ($w_1 + w_2$) is small, then the resolution of the peaks is good. The two substances can be identified if their $R_s = 0.5$. For a satisfactory separation the R_s factor should be equal to 1.0. In the case of a quantitative analysis R_s need to be from 1.2 to 1.5 [5].

As it’s shown in chromatograms (fig.1 and 2) for column with modified C18 phase there are two close peaks (lactic and acetic acids) with resolution factor 1.0, when for the second chromatographic system there are two problem couples – citric acid plus tartaric acid and succinic acid with lactic acid with resolution factors respectively 0.9 and 1.0.

An important factor in the chromatography is an efficiency of the chromatographic system. Talking about the efficiency of chromatographic system it means the ability of this system to prevent the peaks broadening. Let the factor of efficiency (F) would be taken the

ratio of the peak height to its base width. It's obviously that if this factor is bigger, then the chromatographic system works effective.

Table 1. The comparative characteristics of signals obtained on two immobile phases at the analysis of standard solutions of organic acids.

Analyte	$F (F=h/w)$ (Nucleodur C18 Pyramid, 250x4.0mm)	$F (F=h/w)$ (Nucleogel SUGAR 810H, 300x7.8mm)
Tartaric acid	10,7	3,3
L-malic acid	4,3	1,8
Lactic acid	6,0	1,2
Acetic acid	2,0	0,7
Citric acid	1,7	1,2
Succinic acid	0,7	0,5
Fumaric acid	1,5	0,6

As it's shown in *Table 1* chromatographic system with column Nucleodur C18 Pyramid (*system 1*) is more effective than Nucleogel SUGAR 810H (*system 2*) for all analytes. For example for tartaric acid with concentration in aqueous solution 0.29g/dm^3 height of its chromatographic peak in *system 1* is 130mAU, when in *system 2* it's only 40.3mAU (3.25 times less).

Conclusions

Taking into account obtained data it may be noted that chromatographic system with column Nucleodur C18 Pyramid is more effective than compared Nucleogel SUGAR 810H. Thus peaks of the main organic acids of wine in the *system 1* have bigger value of their heights, while their base width are less than the same characteristics of peaks obtained in *system 2*. Besides, in *system 1* there is only one couple of chromatographic peaks with resolution factor 1,0, that talks about satisfactory separation of these peaks, but is not perfect for the quantitative analysis; when there are two problem couples of peaks in the *second system* with resolution factors 1,0 and less.

Consequently, chromatographic system with immobile phase Nucleodur C18 Pyramid in conditions: column parameters – 250x4.0mm, 100Å, 5 µm; eluent – 0.2% H₃PO₄; flow rate – 0.5ml/min; oven temperature – 35°C; detection – 210nm – this system is effective for HPLC analysis of organic acids in wine.

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STORING POULTRY IN VACUUM PACKAGES AND IN GAS ADJUSTED ENVIRONMENT

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Abstract: The aim of this research is the study of the changes of microbiota in the atmosphere packaged, vacuum packed and gas packed (GAE - CO₂ -40%, O₂-20%, N₂-70%) poultry, stored in a frozen state at 0-4°C. Before being packed, the poultry has been treated with antimicrobial solution 0.5% H₂O₂, and subsequently with UV rays. The studies have been performed in the SPIHFT laboratory of food biotechnology. After storing the samples for 5 days in a frozen state at 0+4°C, we discovered that the level of NAMFAG was constant - 10⁵ UFC per 1g product and didn't exceed the admissible level in neither of the used methods of packing and storage.

Keywords: poultry, fowl processing, storage conditions, processing method.

Introduction

In the last decade, Republic of Moldova recorded an increase of poultry grown in breeding farms, as well as in the industrial process, with the use of intensive breeding technologies. This increase of poultry production is determined by the possibility of growing poultry in a short term (broiler chicken gain the necessary weight for slaughter in 40 days).

Poultry is considered white meat; it has dietetic properties and can be used in the alimentation of children of all ages (older than 3 months), grown-ups and elderly persons. People all over the world like to eat poultry in natural state, which represents 80-85% of the bird's weight and only 10-12% are used to prepare chopped meat products [1, 2].

Meat processing represents a complex of processes, closely related to biology, chemistry, processing technology and engineering, marketing and trade.

Worldwide, the industry of meat processing develops at a very high rate. USA has an increased interest in meat processing technology and in the poultry export. In the same time, EU and CIS countries develop their technologies, in order to obtain natural poultry products in cold storage and frozen state [3, 4, 5].

The main problem is that different species of prokaryotes such as *Pseudomonas*, *Clostridium* bacillus, *Listeria monocytogenes*, *Streptococcus*, *Lactobacillus* and *Enterobacter* can be found at the surface of poultry in frozen state. Moreover, because poultry (broiler chicken, duck, goose, turkey) contain easily oxidizable fat, the shelf life of these products in frozen or refrigerated state is relatively short.

There are several methods of antimicrobial treatment for poultry: physical, chemical and combined methods. The most efficient are the chemical methods, which imply the treatment with aqueous solutions of chlorine, paracetic acid, hydrogen peroxide, functional additives, and UV rays.

Poultry and poultry products represent one of the most important elements of a person's rational nutrition. Poultry products are considered a good source of proteins,

vitamins, fatty acids, polyunsaturated fatty acids and other substances vital for the normal development of the human body [6].

Packing and storage methods for poultry

The competition on the poultry meat market forces the manufacturer to come up with new decisions and original ways to sell the product better. These decisions may imply creating new and original products or improving the already known products and packages, which may increase the shelf life and make them "familiar" to the consumers. The innovation in this area can increase the sale rate up to 30%.

The package is very important in the branding process of any product, including the natural semi-finished poultry products. Marketing studies [7, 8, 9] showed that the proportion of spontaneous food purchases is high, and consumers choose goods within 10 seconds. The publicity also influences the consumer's choice, but the final decision is made right in front of the rack, when the person sees the exterior design and the package of the product.

The package used for natural semi-finished poultry products has several purposes: to protect the product from environmental factors; to give the product a nice appearance; to maintain the internal conditions (t, pH, p, W) of the products; esthetical aspect.

The requirements of the package are: chemical and microbiological harmlessness; to be air-proof, compact, according to the customer's demands; to maintain the shape of the products and to be ecological.

The most common package for the natural semi-finished poultry products are plastic bags and plastic trays of different origin, so-called containers.

Packing meat in containers has already become a classical method. The bags can be air pumped, thermal sealed and tied. The downsides of this type of package - low rate of hygiene, short shelf life and unattractive design.

The polymeric materials that can be used for the containers are polypropylene (PP), polystyrene (PS), polyamide (PA), oriented polyamide (OPA), polymer ethylene with vinyl alcohol (EVOH) [5, 7].

Each of these polymers has its own benefit, but their combination will increase the storage period of poultry products, while ensuring all the necessary protection.

Poultry products belong to the group of alimentary products that "breathe" during the storage [8]. The storing process limits the oxygen's access to the package and maintains the uniform elimination of the breathing products - CO₂.

Packing poultry products in the protection gas atmosphere prevents the penetration of steam and foreign gas into the package. The benefits of gas packing method are: the increase of the shelf life and the impossibility of the package to touch the product's surface. The gas adjusted environment is composed of oxygen, carbon dioxide and nitrogen. The nitrogen is the one that provides volume. The high content of oxygen is used to maintain the color of the meat stable. The gas with high content of CO₂ prevents the reproduction of aerobic micro flora.

Storing the fresh meat in CO₂ atmosphere enhances the storage period with 15-40%, the conservation effect being directly proportional to the concentration of CO₂. The action of CO₂ has an impact on fungi, *Pseudomonas* and *Achromobacter* bacteria, while the yeast and *Lactobacillus* are less affected. The meat of the broiler chicken requires storage of CO₂ and nitrogen atmosphere, at 50:50 or 30:70 ratios. Still, nowadays most of the manufacturers pack poultry in bags or containers of different polymeric material and the

vacuum or inert gas environment pressurization is less used. One plausible explanation can be that the last two methods make the price higher and this is characteristic not only for Moldova, but also for Russia, Ukraine and European countries. These methods are popular in USA, where 90% of products are cooked and ready for direct consumption.

Results and discussions:

Before proceeding with the lab testing, we have purchased anatomical parts of broiler-chicken (wings, legs, mesh, and thigh) from “Vispas and Co” LLC, in Hirbovat village, Anenii-Noi, Republic of Moldova. The fresh refrigerated meat has been technologically treated in different conditions. The primary procedures of cleaning and rinsing with ice water, ice water + 0.5% H₂O₂; 0.1% H₂O₂ and treatment with UV rays and the dosage in polymeric propylene containers were held at the laboratory of food biotechnology in SPIHFT.

The final procedures of packing, pressurization in atmospheric and gas adjusted conditions (CO₂ -40%, O₂-20%, N₂-70%) were performed in the packaging department of the “Harvit-Pro” company, in Cricova, Chisinau.

The prepared samples have been stored in the refrigerated state at the 0+4°C in the laboratory fridge.

The following microbiological parameters have been determined during the storage:

NAMFAG (number of Aerobic mesophilic and facultative anaerobic germs) - GOST 10444.15, GOST 9958;

- coliform bacteria - GOST 30518, GOST 4288, GOST 9958;

- pathogenic microorganisms, including Salmonella - GOST 30519, GOST 9958, GOST 4288;

- sulfate-reducing Clostridia - GOST 10444.9 – 88;

- Staphylococcus aureus - GOST 10444.2;

Table 1 Microbiologic indices of the fresh broiler meat after the primary treatment

Microbiological indices	Treating method		
	Ice water	Ice water + H ₂ O ₂	H ₂ O ₂ +UV
NAMFAG, UFC per 1g product	1.2x10 ²	2x10 ²	1.5x10 ²
Coliform bacteria per 0.001g product	Detected	Detected	Detected
Pathogenic microorganisms, including Salmonella per 25g	Not detected	Not detected	Not detected
Sulfate-reducing Clostridia per 0.1g and 0.01 g product	Not detected	Not detected	Not detected
Staphylococcus aureus per 1.0 g product	Not detected	Not detected	Not detected

The microbiological analysis of the fresh broiler meat revealed that the NAMFAG is in admissible limits 1x10³ UFC per 1g of wings and thigh, 1x10⁴ UFC per 1g of legs and

mesh (SanPiN 2-3-2-1078-01 stipulates that the admissible limit of NAMFAG for the natural semi-finished poultry products with bones is 1×10^5 UFC per 1g).

Pathogenic microorganisms, such as *Salmonella*, sulfate-reducing *Clostridia* and *Staphylococcus aureus* have not been found in neither of the initial samples.

Coliform bacteria, which are not limited by the SanPiN and the Rules of the microbiological criteria for the alimentary products, approved on 16.03.2009 by the Decision nr.221 of the Government of Republic of Moldova (Monitorul Oficial nr. 59-61, art. 272 on 24.03.2009), have been found in all of the initial samples.

We consider that the causes of the presence of coliform bacteria in the initial samples are the unsatisfactory conditions of preparation and industrial transportation of the researched raw material.

Table 1 reveals that all 3 methods of primary treatment: ice water; ice water + 0,5% H_2O_2 ; 0,1% H_2O_2 and subsequent treatment with UV rays reduces the level of NAMFAG in semi-finished products such as mesh and thigh from 1×10^3 UFC per 1g to 1×10^2 UFC per 1g of product.

Even after the initial treatment, the coliform bacteria have been detected in 0.01g of product, same as in the fresh samples. This proves that the treatment measures do not kill them. Other groups of microorganisms have not been detected.

According to the data from table 2, the storage of semi-finished tested products in the refrigerated state at the $0+4^\circ\text{C}$ for 5 days allows us to maintain the level of NAMFAG in the legs and wings samples in the admissible limits for all 3 methods of storage and pasteurization: atmospheric conditions, vacuum and gas adjusted environment (CO_2 -40%, O_2 -20%, N_2 -70%), which correspond to the level 10^5 UFC per 1g product. 6 days of storage in the same conditions caused the increase of the total number of germs up to 10^6 UFC per 1g product, which exceeds all the admissible limits.

It is worth mentioning that the result of sensory evaluation of the samples stored for 3 days is satisfying: the appearance, color and smell of the samples correspond to the characteristics of the refrigerated poultry meat. After 6 days of storage in the conditions named above the sensorial characteristics of the products are poor: the wings, legs and thigh emanated an inadmissible smell for an alimentary product, the surface of the refrigerated products was sticky, which means that the process of alteration has already begun.

The physico-chemical characteristics revealed the increase of the acidity and peroxide index, as a result of the process of fats oxidation. This correlates with the decrease of the mass of fat in the legs and wings samples, packed in atmosphere, vacuum and gas adjusted environment.

Table 2 The microbiological changes of the semi-finished poultry products during the storage at the temperature 0+4°C (3-6 days of storage) A – simple atmosphere, V – vacuum, GAE – gas adjusted environment

Microbiological indices	Legs						Wings			
	A		V		GAE		V		GAE	
	5 days	6 days	5 days	6 days	5 days	6 days	5 days	6 days	5 days	6 days
NAGFAG	2,5x10 ⁵	3,1x10 ⁶	1,3x10 ⁵	3,3x10 ⁶	3,0x10 ⁵	4x10 ⁶	0,5x10 ⁵	1,5x10 ⁶	1,5x10 ⁵	2x10 ⁶
Coliform bacteria per 0.001g of product	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected	Detected
Pathogenic microorganisms, including Salmonella per 25g	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Sulfate-reducing Clostridia per 0.1g and 0.01 g product	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Staphylococcus aureus per 1.0g product	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

Conclusions

The technological tests performed in the laboratory of food biotechnology, where the anatomic parts of chicken-broiler have been treated with ice water, ice water + 0.5% H₂O₂; 0,1% H₂O₂ and subsequently treated with UV, showed that the level of NAMFAG decreases from 1x10³ to 1x10² UFC per 1g of product.

Packing the natural semi-finished poultry products in containers with vacuum and gas adjusted environment (CO₂ -40%, O₂-20%, N₂-70%) makes possible their storage for 5 days in the admissible limits of the microbiological characteristics.

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INFLUENCE OF TECHNOLOGICAL TREATMENTS ON PHENOLIC COMPLEX AND COLORS INDICES IN RAW RED WINES

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Abstract: The present work was performed study of the influence different strains of yeast from the National Collection of Microorganisms for Wine Industry, the physico-chemical parameters, content of phenolic substances and color indices of red sparkling wines.

Saccharomyces yeasts affect the content of phenolic substances and anthocyanins during alcoholic fermentation, which causes polyphenolic complex content reduction. Concomitantly it was established that yeasts are responsible for the enzymatic medium that catalyzes the oxidative condensation of phenolic substances.

Keywords: local yeast, dry raw red wine, phenolic substances, sparkling wine

Introduction

In the work are presented results of technological appreciation of different yeast strains from Collection of Microorganism for Wine Production Industry (CMWPI) of Scientific-Practical Institute of Horticulture and Food Technologies (SPIHFT). In order to highlight the influence of yeast strains on the content of phenolic substances in the production of red wines with moderate amounts of phenolic substances. Were studied following important technological properties: fermentation activity, biomass accumulation, yeasts influence on physical and chemical indices and phenolic complex.

It is known that the sorption of phenol connections slows diffusion of nutrients through the cell membrane of yeast, inhibits their physiological activity. Therefore, the high content of phenolic substances impressive influence on the kinetics of fermentation of must and reduce the growth of yeasts.[3]

Also *Saccharomyces* yeasts influence the content substances of phenolic and anthocyanins during fermentation, absorbing them into membrane leads to the reduction of the polyphenolic complex after sedimentation of yeast, and they respond of the enzyme environment that forms during fermentation which can lead to oxidation of different phenolic complex components, especially antiochians.

Therefore, for the production of red wines with moderate amounts of phenolic substances, it is necessary to use special yeast strain capable of maintaining the optimum fermentation and ensure production of quality red wines, with minimal impact on the phenolic complex.[3]

As result of researches were studied from 5 strains of yeast from CMWPI and are differentiated by their influence on fermentation activity and physicochemical content for the production red wines with moderate amounts of phenolic substances.

Given the aforementioned study and selection of local yeast strains for the production of quality red wines is actual also present scientific and practical interest in the Republic of Moldova.

Materials and methods

The research was conducted in the laboratory of "Sparkling wines and Microbiology" section "Micro winemaking" Scientific and Practical Institute of Horticulture and Food Technology in 2010-2011. As research objects were used dry red wine made from Merlot vine. Yeast strains were studied from Collection of Microorganism for Wine Production Industry of SPIHFT. When performing research were applied physico-chemical analysis methods recommended by the International Organisation of Vine and Wine and those modified in the SPIHFT [2].

Table 1. Quality indices of red grapes processed to produce raw material for sparkling red wines. (harvest year 2011)

Grape variety	Mass concentration:		Technological potential	
	of sugars, g/dm ³	titratable acidity, g/dm ³	Amount of phenolic compounds, mg/dm ³	concentration of anthocyanes, mg/dm ³
Merlot	214	7,45	2568	426

To produce raw material for sparkling red wines under "Micro winemaking" (SPIHFT) was used Merlot grape variety and local yeast strains № 64, № 88, № 9, № 81 and № 29.

Results and discussions

Based on the obtained results that are shown in Table 2 has been shown that yeast strains have a major influence on the basic physico-chemical parameters in the process of fermentation and maceration for the production of raw material red wines.

Table 2. Influence of yeast strain on the physico-chemical parameters of raw materials red wines made from Merlot grapes (y.h. 2011)

Grape variety	Experience	Description of experience Fermentation and maceration with	Alcoholic concentration, % vol.	Mass concentration of		pH	Potential OR, mV
				titratable acidity, g/dm ³	volatile acidity, g/dm ³		
Merlot	1	Yeast strain № 64	12,49	6,6	0.46	3,39	199
	2	Yeast strain № 88	12,22	7	0.3	3,33	203
	3	Yeast strain № 9	12,77	7,1	0.46	3,32	204
	4	Yeast strain № 81	12,02	7,3	0.3	3,33	202
	5	Yeast strain № 29	12,06	7	0.6	3,32	203

Volatile acidity is between range of 0.3-0.6 g/dm³ with a maximum of 0.6 g/dm³, recorded at 5 experience using strain № 29, the pH is constant only experience 1 (using strain № 64) has a higher value 3.39. Redox potential varies between 202 ± 2, the minimum

value is also recorded by yeast strain № 64. Titratable acidity varies depending on the yeast strain used, with a gap of up to 0.9 mg/dm³, maximum of 7.3 mg/dm³ being recorded at yeast strain № 81 and the minimum was obtained from the sample obtained by using strain yeast № 64. These disparities are obtained due to the specific fermentation particularities of yeasts strain.

To highlight the influence of yeast strain on alcoholic concentration data was shown in fig.1

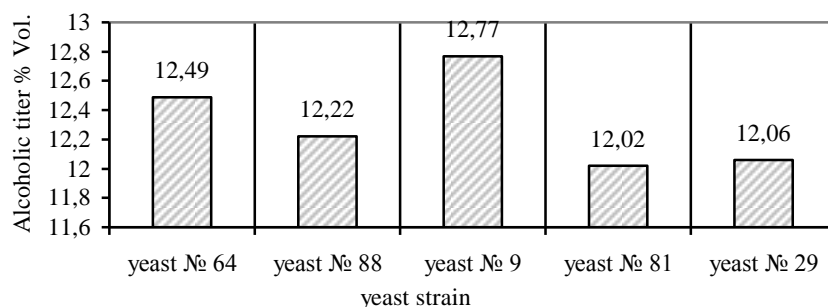


Fig. 1. Comparative analysis of alcoholic titre in the red wines raw materials obtained by using different yeast strains , h. y. 2011.

Analyzing obtained data of alcoholic concentration stands out yeast strain № 9 with maximum of 12.77% Vol alcohol and the minimum was recorded in the wines obtained using yeast strains № 81, 29 with concentrations from 12.02 to 12.06% Vol . therefore № 9 of yeast strain, 64 had the highest yield of fermentation of glucose to ethanol fermentation and higher resistance of studied environmental conditions.

The wines studied were determined content of phenolic substances, anthocyanes, color and organoleptic indices. The results are shown in table 3.

Analyzing the table, it can be shown that strains of yeast used in fermentation and major influence on color indices. Deviating color density to 6 points maximum being recorded at the wine fermented with yeast strain № 88 reaching 14.6 points followed by wines fermented by yeast strains № 64, 81. The lowest values being recorded in Ex. № 3,5 (used yeast strains 9;29), therefore we conclude that these two strains of yeast have major influence on the color indices diminishing this important parameter. Color hue is within the allowable limits characteristic for young wines.

The data represented in Figure 2 demonstrates that yeast strain № 88 favored extraction and keeping of phenolic substances in the studied sample registering the maximum amount of phenolic substances 1355 mg/dm³ and comparative high concentration of anthocyanins 247 mg/dm³ mg/dm³. Wine obtained with use of yeast strain № 64 also showed advanced phenolic substances content (1262 mg/dm³) but and having the lowest content of anthocyanes (202 mg/dm³), this indicates that this strain of yeast facilitates reduction of anthocyanins during fermentation. Yeast strains № 81 and 29 have almost identical results with a low content of phenolic substances (1106 to 1071 mg/dm³) and moderate amounts of anthocyanes.

Table 3. Phenolic substances content, color and organoleptic indices of raw material wines obtained with use of different strains of yeast (it 2011)

Grape variety	Experience	Description of experience Fermentation and maceration with	Amount of phenolic compounds ,mg/ dm ³	Antociane lor concentra tion,mg/d m ³	Color density (Cd=A420+ A520+A620)	Color hue (Ch=A420n m/ A520nm)	Note organoleptic Points
Merlot	1	Yeast strain № 64	1263	202	12,50	0,45	7,9
	2	Yeast strain № 88	1355	247	14,64	0,48	8,0
	3	Yeast strain № 9	1083	254	9,36	0,54	7,85
	4	Yeast strain № 81	1106	238	11,73	0,47	7,9
	5	Yeast strain № 29	1071	232	8,57	0,61	8,0

In Figure 2 is shown comparative analysis of concentration of phenolic substances and anthocyanes in raw materials red wines obtained by use of different yeast strains.

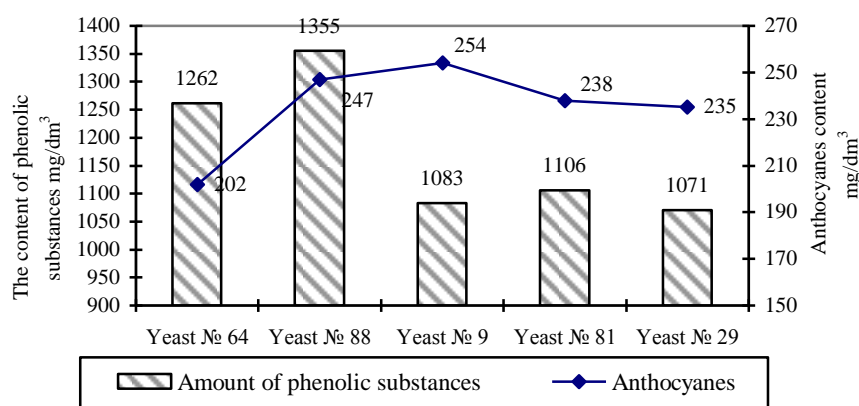


Fig. 2. The content of phenolic substances and anthocyanes in the red wines raw material obtained with the use of various yeast strains..

Should be mentioned that yeast strain № 9 is of particular interest because of its specificity: having a lower content of phenolic substances compared with the other samples (1083 mg/dm³) while in the sample is registered highest concentration of anthocyanes (254 mg/dm³) which in turn to be the main parameter that is responsible for the color of the wine and therefore report (anthocyanes / phenolic substances) is the most advanced compared to other yeast strains.

The studied wines were subjected to organoleptical analysis within the “Sparkling Wines and Microbiology” laboratory of SPIHFT, the results are shown in Fig. 3.

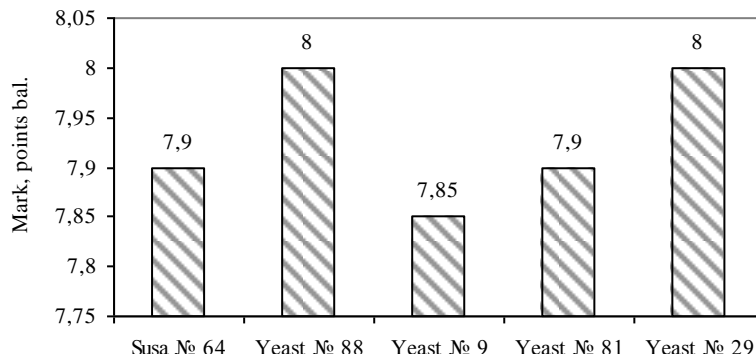


Fig. 3. Organoleptic analysis of raw material red wines obtained with the use of various yeast strains

Organoleptic analysis have showed that yeast strains № 88 and 29 were appreciated with to the highest mark of 8.0 points having in the taste, complex red fruit flavor, velvety ruby red color. Wines obtained with the use strains 81 and 64 were also obtained satisfactory mark 7.9 points, the lowest score it obtained the wine yeast strain obtained by 9 to 7.85 points.

CONCLUSIONS

1. Yeast strains have a major influence on fermentation and maceration process for the production of raw material red wines. Analyzing alcoholic concentration data where highlighted yeast strain № 9 and 64. And therefore best adapted to the specific conditions of experience and recorded to the highest yield of fermentation of glucose to alcohol. .
2. Yeast strain selected for fermentation and maceration has a major influence on extraction and keeping of phenolic substances and anthocyanes, And also was highlighted influence on the concentration of these parameters with using yeast strain № 88 which have the highest concentration of phenolic substances and major level of anthocyanes. At yeast strain № 64 concentration relation of concentration of (anthocyanes / phenolic substances) have the lowest values of this parameter, and the yeast strain № 9 have highest ratio, therefore careful selection of yeast strain is a valuable winemaker tool for regulation of phenolic substances and anthocyanes content in production of raw material red wines.
3. Following laboratory testing the highest quality parameters were recorded in the red wines obtained with the use of yeast strain № 88 recorded high content of phenolic substances, anthocyanes and accumulated the highest score in organoleptical taste, which why yeast strain № 88, with the highest technological potential, it is recommended for primary fermentation of sparkling raw material red wines.

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STUDY OF YEAST STRAINS *SACCHAROMYCES VINI* FROM WINE CENTRE "CRICOVA" ON THE TEMPERATURE FACTOR

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Abstract: Temperatura mediului este un factor important de existență a sușelor de levuri, care sunt folosite în oenologie și poate juca un rol decisiv în procesul de selecție și evidențiere a culturilor cu proprietăți tehnologice avansate. Intervalul de termotoleranță pentru diferite sușe de levuri este individuală, dar majoritatea dintre ele se pot dezvolta într-un interval destul de larg: de la 0...5 până la 30...40 °C, cu o valoare optimă între 20...30 °C. Din gama largă de indici biochimici și fiziologici, care caracterizează activitatea levurilor, temperatura este un factor cheie, studiul căruia are o importanță deosebită teoretică și practică.

Keywords: selection, wine yeast, temperature, fermentation

Introduction

The temperature of the natural environment is an important factor which determine the existence of the yeast and capable to influence on their natural selection. Usually microorganisms are divided into three categories according to their behavior in different temperature, which reflects the adaptability and stability of their enzymatic apparatus to this factor. Microorganisms can be psychrophilic, mesophilic or thermophilic, optimal development of which occurs at temperatures below 20 °C, between 20 and 45 and above 45 °C, respectively. Yeast belong to the first two categories.

Temperature is an important factor of life of all the yeast, and for each of the different functions of yeast cells: respiration, fermentation, growth - there are different optimal temperature conditions. In fact, these limits depend on the type of yeast, and even race, as well as aeration, medium composition, especially the presence of alcohol, and thus determine a more or less broad zone where possible reproduction of yeast and fermentation of sugars.

Aim of study - to test the effect of temperature on strains yeast *Saccharomyces vini*, isolated from the stum in a wine center «Cricova».

Materials and methods

We used six strains of yeasts isolated from the must in winemaking center «Cricova» (Table 1).

To establish the response of investigated yeast cells on the temperature, a 2-3- day cultures grown on grape must at 28 ± 1 °C and inoculated on a solid nutrient medium *grape must- agar* in Petri dishes and incubated 2-4 weeks within 1 ... 43 °C.

Table 1. Studied yeast strains

Nº	Title
1	CNMN-Y-26
2	Cricova Chardonnay(3)
3	Cricova Chardonnay(4)
4	1S
5	1VT
6	3VT

*The name of the yeast strains is conditional

Besides the ability to assimilate carbohydrates at different temperatures fermenting energy of yeast were studied. To do this, they were cultured on grape must, with a concentration of sugars 198 g / l in the range 15-40 ° C. The study was carried out in a glass container with a capacity of 200 cm³, equipped with a clog device consisting of a rubber tube with a drilled hole where the tube is inserted a few centimeters long with a finely drawn-out end of promoting the creation of anaerobic conditions. In 150 cm³ of must 3% of two-day yeast suspension were entered. Appreciation of the rate of fermentation was carried out by the quantity of released carbon dioxide. The quantity of gas was monitored by weighing.

Results and discussion

In assessing the response of investigated yeast cells on the temperature began to note the growth of the biomass at 5 ° C in the 10-12th day, depending on the strain. As the temperature of the medium in all variants of the experiment increased earlier and abundant growth of cells was recorded (for example, at 10 ° C, it was observed on day 5, and at 20 ° C and above is on the 2nd day). The maximum temperature of reproduction of yeast cells in which scant stroke was 39 ° C.

Table 2. Optimum temperature for the studied yeast strain

Name strain	CNMN-Y-26	Cricova Chardonnay(3)	Cricova Chardonnay(4)	1S	1VT	3VT
Nº	1	2	3	4	5	6
t, °C	5-39	10-35	10-35	10-35	6-36	8-38

Microscopic examination of yeast, cultured on agar medium at different temperatures showed that when the temperature changes, the elliptical shape of the cells, characteristic *S.vini*, is rounded, almost to the spherical (Fig. 1).

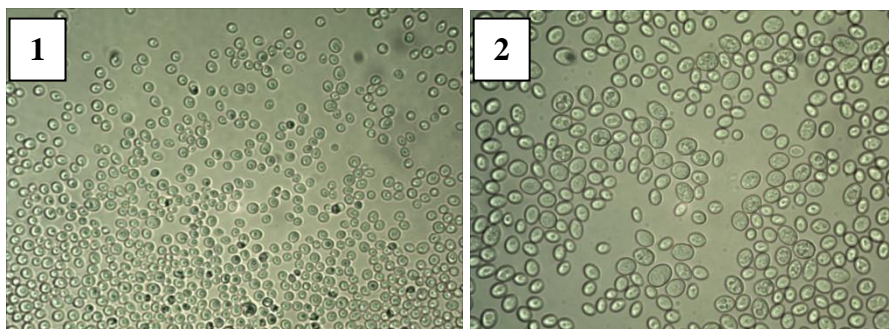


Fig. 1. Cells of strain CNMN-Y-26, grown in a medium grape must- agar at 15 (1) and 35 °C (2).

Table 3 presents the results of the investigation of the fermentation, the yeast strains isolated at different environmental temperatures.

Table 3. The rate of fermentation for different yeast depending on the temperature G CO₂ per 24 hours)

Fermentation temperature, °C	Name of yeast strains					
	CNMN-Y-26	Cricova Chardonnay(3)	Cricova Chardonnay(4)	1S	1VT	3VT
15	5,5	3,1	3,4	3,8	5,4	5,1
20	7,6	5,8	5,7	6,0	7,7	6,8
25	9,8	7,6	7,7	7,3	9,8	7,3
30	10,2	8,4	8,5	8,2	10,3	8,5
35	11,7	9,0	9,1	9,0	11,5	9,8
37	10,5	8,1	7,9	8,1	10,8	8,8

Analysis of the data shows that the studied wine yeast are able to exhibit a high fermentative activity in the temperature range of 25-35°C.

The quantity of gassing carbon dioxide for the research strain is different and has a minimum of 3.1 g / L for strain Cricova Chardonnay (3), and a maximum of 11.7 g/L for strain CNMN-Y-26, but there is still rule that when the temperature raises the quantity of emitted gas increases too, and at maximum temperature its mass decreases. Mass concentration of sugars decreases with increasing temperature, and after 20 days for all the studied strains was at the level of 0.5-1 g / L at the temperature of fermentation - 35 °C and above 37 °C sugars are not fully fermented - 7-10 g / L.

If there are more favorable conditions for yeast metabolism, free access of oxygen and an increase in inoculated biomass, allowed to ferment at 37 °C for about 90-100% of sugars.

Conclusions

Thus, the study of the relationship of yeast *S. vini* to temperature, isolated in nature Wine Centre «Cricova», showed their tolerance in the range of 5 to 37 ° C.

The rate of fermentation of yeast increases with temperature up to 30 ° C, but at temperatures above 35 ° C, the rate of fermentation decreases.

Optimum temperature fermentation of sugars varies depending on the type of yeast, and ranges from 25 to 35 ° C. The ability of yeast fermentation to 90-100% of sugars at high temperatures suggests that it arises as a selective advantage due to temperature environment.

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TECHNOLOGICAL APPRECIATION OF YEAST STRAINS FROM THE WINE CENTER "CRICOVA"

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Abstract: Pentru producerea vinurilor albe seci este necesară selecția de sușe de levuri capabile să fermenteze în condiții specifice: la temperaturi joase; în timp scurt; cu capacitatea de a flocula precipitatul, ceea ce nu necesită tehnologii suplimentare de limpezire sau filtrarea vinului; cu capacitatea de a forma spumă maximală sau mijlocie ca un indicator de prezență a substanțelor superficiale active și a puterii fermentative. În lucrarea dată a fost efectuată aprecierea tehnologică a sușelor de levuri locale, evidențiate din centrul vitivinicol „Cricova”, destinate producerii vinurilor albe seci.

Keywords: local yeast, dry white wine, technological evaluation

Introduction

Yeasts responsible for alcoholic fermentation in wine, usually penetrate the surface of must grapes, used equipment or direct administration of specific yeasts .

The fermentation process can be conducted both natural - without inoculation of selected yeasts, and the administration in must the yeast form or active dry yeast. Currently active dry yeasts are widespread in many countries, and using their excellent results are obtained, but, ultimately, higher quality wine is achieved when it is done with the use of indigenous yeasts .

In recent years, increased interest in using local yeast strains identified in fermented must, which have some specific metabolic characteristics and can positively influence the quality of the product .

Although commercial yeasts are widely used for must fermenting, is considered using local yeasts can be more effective, so it is assumed that they will be more competitive. This proves that they have a dominant potential in the wine fermentation process. In addition, using local yeasts needs to ensure typical sensory properties for wines produced in some centers.

Diversity of yeasts present in wine is a useful tool for selecting new strains dominate during the fermentation process and to increase expression in wine organoleptic characteristics. Purpose of research is assessing technological local yeast strains, shown in the center of wine "Cricova" for dry white wines.

Materials and methods

Strains of yeast.

In the present paper were studied local yeast strains (Cricova Ch (2), Cricova Ch (3), Cricova Ch (4), 1S, 1VT, 3VT), shown in the center of wine "Cricova". Studies on its morphological, cultural and physiological-biochemical allowed, using the identifier after Kudreavțeva, to establish that yeast strains identified belong to the species *Saccharomyces vini*.

Ability of flocculent precipitate.

Ability of flocculent precipitate was determined visually in YPG liquid medium, used as control flocculant and non-flocculant yeasts.

Killer factor.

To determine the phenotype was used method (Sangorin, 2001).

Foaming.

Foam height was measured daily during alcoholic fermentation. According to Martínez-Rodríguez yeast strains were classified into three categories, depending on the height of foam: low = 2 mm foam, foam middle between 2 and 4 mm and abundant foam, more 4mm.

Resistance to SO₂ was determined on solid agar medium on must with different doses of SO₂ (100-150 mg / L), distributed in Petri dishes, incubating yeast with constant temperature of 27°C. Sowing biological material fermented mash (2 days) depletion loop method revealed that the cultures have grown faster.

Criotolerance.

To determine the physiological properties by studying resistance in cold fermenting yeast strains was analyzed dynamic fermentation must at a temperature 10°C.

Determination of sugars in musts was made with the densimeter, according to GOST 13192-73;

Determination of sugars in wine was performed by indirect titration method according to GOST 13192-73;

Results and discussions

It was found that the behavior of yeast strains to low temperature and SO₂ varies with the strain of yeast. It was also established that the ability to form foam and the capacity to flocculent precipitate, different, depending on the strain of yeast used. Results of these investigations are presented in Table 1.

In the results, the yeast strains studied can be divided into several groups.

Consistent resistance at low temperatures, in three groups:

- Resistance – low temperature (+ + +): Cricova Chardonnay (2), 1VT.
- Relatively strong (+ +): Cricova Chardonnay (3), Cricova Chardonnay (4), 3VT.
- Less resistant (+): 1S

Consistent resistance to SO₂ into two groups:

- Resistant (+ + +): Cricova Chardonnay (2), 1VT, 3VT.
- Relatively strong (+ +): Cricova Chardonnay (3), Cricova Chardonnay (4), 1S.

After foaming, the three groups:

- The maximum formation of foam: 3VT
- The middle foam formation: 1VT, Cricova Chardonnay (2)
- The minimum formation of foam: Cricova Chardonnay (3), Cricova Chardonnay (4), 1S.

After the flocculant capacity, the two groups :

- flocculation: Cricova Chardonnay (2), 1S, 1VT, 3VT
- Non-flocculation: Cricova Chardonnay (3), Cricova Chardonnay (4)

Our study showed that all strains of yeasts are highlighted Neutral phenotype, not disappear in the presence of killer phenotype strains and strains not suppress the activity of sensitive phenotype.

Thus, for dry white wines need to select strains of yeast able to ferment in specific circumstances, for this purpose were selected these criteria following local yeast strains: Cricova Chardonnay (2) and 1VT.

Table 1. Assessment of technological indices of local yeast strains.

Denumirea sușei de levuri	^(a) Foaming , mm	^(b) esistance at low temperatures	^(c) flocculant capacity	^(d) resistance to SO ₂	phenotyp e
Cricova Chardonnay (2)	++	+++	+	+++	Neutru
Cricova Chardonnay (3)	+	++	–	++	Neutru
Cricova Chardonnay (4)	+	++	–	++	Neutru
1S	+	+	+	++	Neutru
1VT	++	+++	+	+++	Neutru
3VT	+++	++	+	+++	Neutru

Legendă: (a) +++ maximum foaminess, ++ medium, +minimum

(b) +++resistant, ++ relatively resistant, + less resistant

(c) + flocculation, – Non-flocculation

(d) +++ resistant to SO₂, ++ relatively resistant

Conclusions

1. Technology assessment is the first step to select the most competitive local yeast strains for producing dry white wines.
2. Additional information is necessary to know about secondary compounds of alcoholic fermentation, which have a very important value informing the wine.
3. Experimental results have practical value, so that there is real opportunity to improve the quality of dry white wines.

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AROMATIC POTENTIAL AS AN ALTERNATIVE METHOD FOR CHARACTERIZATION OF YEAST STRAINS

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Abstract: În ultimul timp, consumatorii consideră că aromă și gustul vinului sunt principalele caracteristici care determină calitatea și valoarea produsului. Aroma vinului este un amestec unic de compuși volatili provenit din strugurii inițiali (arome varietale), produse secundare formate în timpul fermentației mustului (arome fermentative) și de maturare (arome post-fermentative).

În scopul studierii influenței sușelor de levuri asupra potențialului aromatic al vinurilor albe seci au fost determinate unele substanțe volatile, prin metoda cromatografiei gazoase. Rezultatele obținute demonstrează că conținutul substanțelor volatile a vinurilor albe seci variază în dependență de sușa de levuri utilizată.

Keywords: aroma of wine, yeast strain, volatile substances

Introduction

Previous studies have shown that yeast strains have a large impact on wines chemical complex and found that the volatile composition could be an alternative method for characterization of yeasts used in wine production .

Some authors use synthesis of different amounts of acetylmethylcarbinol, 2,3-butadiene or acetic acid as a base for studying the genetic strains variability of the genus *Saccharomyces*, which can serve as a way to improve the quality of wine or fermentative properties of yeasts.

During alcoholic fermentation yeasts form and other alcohols, except ethanol, so-called higher alcohols, mainly represented by n-propanol, isobutanol, isopentanol etc. They may derive directly from the corresponding amino acids or sugars in the environment. Each strain of yeast *Saccharomyces* genus has its own capacity to produce these secondary compounds of fermentation. Yeast strains producing of higher alcohols behave differently depending on musts studied.

Esters formation in wine is made in two ways: biological esterification occurs during alcoholic fermentation, malolactic and / or esterification of acetic by chemical or enzymatic reactions that occurs very slowly during storage / aging of wine. Both ways may occur depending on the technological and almost equal participation in the formation of esters in wine.

Materials and methods

Strains of yeast. In the present paper were studied local yeast strains (Cricova Ch (2), Cricova Ch (3), Cricova Ch (4), 1S, 1VT, 3VT), allocated in the wine center "Cricova". Studies performed on the morphological, cultural and physiological-biochemical properties permitted, using identifier by Kudreavțeva, establishing that yeast strains identified belong to the species *Saccharomyces vini*. As a control were studied industrial dry active yeast: *LitoLevureChardonnay* (France), yeast selected from the National Collection of Microorganisms for the Wine Industry: strain no. 29.

As a raw material was used grape must of the variety Chardonnay harvest of 2011. Initial physico-chemical characteristics of the grape are presented in Table 1.

Table 1. Physico-chemical characteristics of the grape (harvest 2011)

grape variety and conditions	sugar, g/L	Titrateable acidity, g/L tartaric acid	pH	Potential OR, mV
Chardonnay (microvinification)	195	8,8	3,09	216,9

Determination of volatile substances was performed by gas chromatography method.

Results and discussions

Comparative analysis of aromatic content of Chardonnay dry white wine (harvest 2011) achieved by classical technology using different yeast strains allowed the establishment of significant differences. The results obtained are shown in Table 2.

Table 2. Content of volatile substances in fermented dry white wine different strains of yeast (mg / L).

Substance	Yeast strain							
	Cricova Ch(2)	Cricova Ch(3)	Cricova Ch(4)	IS	IVT	3VT	Nr.29	LittoLevure (France)
Acetic aldehyde	4,8	3,4	2,5	2,7	3,4	18,9	2,1	15,6
ethyl acetate	24,9	23,4	20,4	22,6	19,8	23,7	25,8	16,7
isoamyl acetate	0,53	0,42	0,38	0,40	0,55	0,45	0,47	0,38
methyl alcohol, g/L	0,02	0,02	0,02	0,02	0,015	0,02	0,01	0,02
Higher alcohols								
2-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
n-propanol	15,1	9,9	10,0	12,9	10,1	7,4	6,0	10,1
Isobutanol	20,3	20,9	32,4	31,3	23,0	30,4	30,9	23,8
n-butanol	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5	<0,5
Isopentanol	141,1	171,9	155,6	171,1	148,0	169,2	167,0	187,0
sum of higher alcohols	177,5	203,7	199,0	216,3	182,1	208,0	204,9	186,6

The results presented in Table 2 demonstrate that the content of volatile substances in dry white wines Chardonnay (harvest 2011) varies depending on the strain of yeast used.

Acetic aldehyde concentration limit values vary from 2.1 up to 18.9 mg / L. Obviously yeast strain has a significant influence on the content of acetic aldehyde, which can be explained by the specific characteristics of each yeast strain to eliminate relatively large or small quantities of this substance.

For example: the use of yeast strain No.29 acetic aldehyde concentration is 2.1 mg / L (minimum), and the use of yeast strain 3VT acetic aldehyde concentration is 18.9 mg / L (maximum).

A less significant influence yeasts have studied the content of n-butanol and 2-butanol, where the determined values were below 0.5 mg / L.

Isobutanol concentration in dry white wines vary depending on the strain type of yeast used and the variation range of values is quite wide and is up from 20.3 to 32.4 mg / L. Maximal concentrations of isobutanol have been established in yeast strain used CricovaChardonnay (4).

Changes in concentrations of n-propanol in wine raw material is within the range 6.0 to 15.1 mg / L. Isopentanol concentration in dry white wines studied is about 60% of the sum of the higher alcohols, and the difference value is 45.9 mg / L. The highest concentration of isopentanol was found in wine achieved with dry active yeast strain (LittoLevure).

Isoamyl acetate content, that gives a hint of banana, varies slightly between 0.38 to 0.55 mg / L.

Another important component that forms the must fermentation is ethyl acetate, which directly influence the organoleptic properties of wine obtained.

It is known that ethyl acetate is part of the group mean fatty acid esters, and most of the ester group enanthic assign a strong sense of fruit wine.

Therefore, ethyl acetate directly participates in the formation of wine aroma obtained. In addition, ethyl acetate affect the taste of wine. At concentrations higher than the olfactory perception, he gives a stringent flavor. All wines contain healthy ethyl acetate, formed during fermentation, up to 160 mg / L.

In our case, values of ethyl acetate are in the range of 16.7 to 25.8 mg / L, respectively lowest concentration was found in wine obtained by using active dry of yeasts(LittoLevure) and highest in wine obtained by using yeast strain No. 29, but this difference is insignificant in this period.

The analysis of complex volatile dry white wines studied, we can conclude that in all wines the methyl alcohol content is about 0.02 mg/dm³, which proves that nature of yeast does not affect methyl alcohol concentration.

Conclusions

1. Higher alcohols, esters, aldehydes, volatile acids and other substances formed during fermentation of must in the manufacture of dry white wine contribute to the formation of complex flavors.

2. Specified yeast strains are able to positively or negatively influence the aromatic content of wines.

3. Our results show that knowledge of the biochemical properties of yeast strain used for producing dry white wines can have a decisive role.

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LIPID COMPOSITION OF FLAX SEED OIL

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Abstract: The composition of seven varieties of flax seeds was investigated. 34.2 - 44.4% wt vegetable oil in the seeds was found to be. Fatty acids composition of the triacylglycerols was determined by capillary gas chromatography. Linolenic (35.3 - 42.0%) and linoleic (14.9 - 19.0 %) acids are the main unsaturated fatty acids. Palmitic acid (8.7 - 12.1%) predominated in fraction of saturated acids, followed by stearic acid. The quantity of tocopherols determined by HPLC with fluorescence detection was 602 - 788 mg/kg. Phospholipids were isolated by column chromatography and quantified spectrophotometrically at 700 nm. Their percentages in the oil were found to be 0.1 - 1.0% wt. Total sterol content determined by gas chromatography was 0.2-0.4%.

Keywords: *Linum usitatissimum* L.; glyceride seed oil; phospholipids; sterols; tocopherols; fatty acids.

Introduction

The flax plant (*Linum usitatissimum* L.), fam. *Linaceae* is not a new crop and native to West Asia and the Mediterranean. As the source of linen fibre, flax has been cultivated since at least 5000 BC. Today it is grown for its glyceride oil and for fibres. Linseed (Flax) seed oil is a kind of high quality edible oil and is used in cosmetic, pharmaceutical industry and as food additive. It is rich in α -linolenic acid and various unsaturated fatty acids. It plays an important role in the promotion of human intelligence, physical brain, preventing cardiovascular disease, and the suppression of disease genes (10). On the other hand the presence of polyunsaturated acids makes the oil unstable to oxidation. In view of this reason fatty acid profile of the oil is an important parameter in evaluating the quality of the flax oil. The oxidative stability of the oils is greatly affected and by minor components such as tocopherols, sterols and phospholipids.

This study focuses on the determination of the total content of glyceride oil in the 7 varieties of flaxseeds (*Linum usitatissimum* L.) cultivated in Bulgaria, the general content of biologically active substances as sterols, phospholipids and tocopherols in the oils. Fatty acid composition of triacylglycerols was investigated too.

Materials and methods

All solvents and reagents were analytical grade and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka (Chemie GmbH, Switzerland). Reference tocopherol isomers and individual sterols were purchased from Merck (Darmstadt, Germany). TLC plates were prepared in the laboratory using Silica gel 60 (Merck, Darmstadt, Germany).

Samples.

The Flaxseeds were grown and obtained from the Institute of Plant Genetic Resources, Sadovo, Bulgaria, crop 2010.

Isolation of glyceride oil and determination of oil content

The seeds (50g sample) were air-dried and the oil was extracted with n-hexane in Soxhlet unit for 8 h. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content in the seeds [1].

Phospholipids.

Another part (10 g) of air-dried seeds was subjected to Folch extraction according to Christie [8]. Polar lipids were isolated from the total lipids by column chromatography [8]. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm. Etalon – 10 µl/cm³ water solution of KH₂PO₄ as P. Content of phospholipids in the sample – 1-125 mkg/kg as P.

Sterols.

The oil was hydrolyzed with ethanolic KOH [8], sterols were extracted with light petroleum ether and purified by silica gel TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany), impregnated by 0.2% NaOH water solution with mobile phase n-hexane: diethyl ether 1:1 (by volume). Sterol content was determined by Gas chromatography [3] on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 m x 0.25 mm (I.D.) capillary DB-5 column, Hewlett Packard GmbH, Austria) and a FID.

Tocopherols.

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a "Merck-Hitachi" (Merck (Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck (Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000 [2].

Fatty acids.

The total fatty acid composition was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50°C according to Christie [8]. Fatty acid methyl esters (FAME) were purified by silica gel TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane:acetone 100:8 (by volume). GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 m x 0.25 mm (I.D.) capillary InnoWax column (cross-linked PEG, Hewlett Packard GmbH, Austria) and a FID.

Results and discussion***General characteristics of the seeds and oils***

General characteristics of the seeds were determined such as: oil content in dry seeds, content of total phospholipids, sterols and tocopherols in the oils. The results are shown in Table 1.

Table 1 Content of glyceride oil in *Linum usitatissimum* L. and phospholipids, sterols and tocopherols in the oils*

Compounds	Varieties <i>Linum usitatissimum</i> L.						
	A900012	A900013	A900014	A900015	A900016	A900017	A900018
Oil content, %	33.8	37.3	38.0	41.6	37.1	36.2	44.5
Tocopherols, mg/kg	731	766	768	770	788	775	602
Phospholipids, %	0.7	0.9	1.0	0.4	0.8	1.0	0.7
Sterols, %	0.3	0.4	0.3	0.3	0.3	0.3	0.2

*Average of three determinations

The content of glyceride oil in the seeds of all cultivars varied in limits of 33.8 - 44.5%. The seeds of A900018 and A900015 were richest in the oil - 44.5% and 41.6%. Those quantities are higher than the results reported earlier by *Beltagy* – 36.0-39.0% [5] and *Bozan* -33.6% [7].

The quantity of phospholipids in the oils (0.4-1.0%) was closed to percentages of other vegetable oils as sunflower and rapeseed (0.7-0.9 % and 0.7-1.0 % respectively (*Gunstone, Zlatanov et al.*)[10,3].

Total content of tocopherols in the glyceride oils (602-788 mg/kg) were in closed quantities and higher than date reported by *Przybylski* (347 mg/kg) [12] and *Gunston* (440-588 mg/kg) [10], but lower than percentages established by *Oomah et al.*–845-972 mg/kg [11]. γ -tocopherol predominated in the tocopherol fraction, followed by γ -tocotrienol and α -tocopherol.

The content of sterols was found to be 0.2-0.4%. Those values were in agreement to data reported by *Przybylski* and *Gunston* (0.23% and 0.42% respectively) [12; 10].

Qualitative fatty acid profile of triacylglycerols was showed in Table 2. All of investigated triacylglycerol fractions contain a significant number of fatty acid, but the qualitative composition was found to be similar. The predominant constituents in the oils were unsaturated fatty acids as linolenic, linoleic and oleic (81.8-87.6%). Linolenic acid is the main unsaturated component (35.3-42.0%), followed by linoleic and oleic acids. This lower content was at the expense of higher quantity of monounsaturated oleic acid (26.0-33.5%). Palmitic and stearic acid, the main saturated components, were detected in reasonable amounts.

Table 2 Fatty acid composition of seven varieties of *Linum usitatissimum* L.*

Fatty acids,%	Varieties <i>Linum usitatissimum</i> L.						
	A900012	A900013	A900014	A900015	A900016	A900017	A900018
C 14:0 Myristic	0.2	0.2	0.2	0.1	0.1	0.1	0.2
C 14:1 Myristoleic	0.1	0.1	0.2	0.3	0.1	0.1	0.3
C 15:0 Pentadecenoic	0.1	-	0.1	-	-	0.1	0.1
C 16:0 Palmitic	12.1	9.0	10.1	10.7	8.7	9.0	10.5
C 16:1 Palmitoleic	0.2	0.1	0.1	0.2	0.1	0.1	0.2
C 17:0 Margaric	0.1	0.1	0.1	0.1	0.1	0.1	0.1
C 18:0 Stearic	5.7	4.8	4.3	4.2	4.3	3.1	4.0
C 18:1 Oleic	31.1	30.6	26.0	29.1	33.5	30.3	27.4
C 18:2 Linoleic	15.1	17.5	19.0	18.9	15.8	16.4	14.9
C 18:3 Linolenic	35.3	37.6	39.6	36.2	37.3	40.7	42.0
C 20:0 Arachidic	-	-	0.1	0.1	-	-	0.1
C 20:2 Eicosadienoic	-	-	0.2	0.1	-	-	0.2
SFA**	18.2	14.1	14.9	15.2	13.2	12.4	15
UFA***	81.8	85.9	85.1	84.8	86.8	87.6	85.0
MUFA****	31.4	30.8	26.3	29.6	33.7	30.5	27.9
PUFA*****	50.4	55.1	58.9	55.2	53.1	57.1	57.1

*Average of three determinations

** Saturated fatty acids

*** Unsaturated fatty acids

**** Monounsaturated fatty acids

***** Polyunsaturated fatty acids

These data are significantly different to fatty acid composition reported earlier by Bhatti [6], Gunston [10], Choo et al. [9], Bozan [7] and Przybylski [12] according them the quantity of linolenic acid was found to be higher - 46.0-58.3%. The results might be explained by the different agrometeorological conditions (mainly temperature and humidity) for cultivations of the plants.

Conclusion

The seeds of *Linum usitatissimum* L. cultivated in Bulgaria contain glyceride oils rich in biologically active substances as sterols, phospholipids and tocopherols. Fatty acid composition of triacylglycerols was specific and was characterized with lower content of linolenic acid at the expense of oleic acid.

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STUDIES REGARDING THE INCIDENCE OF BACTERIA FROM LISTERIA GENUS IN FISH MEAT

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Abstract: *Listeria* is a bacterial genus whose species are Gram positive bacilli and it contains seven representatives. Joseph Lister was an English surgeon who was one of the first in the field of sterile surgery and the name *Listeria* is given in his honor. Sources of *Listeria* are soil, contaminated water, animals and vegetables. Because bacteria from *Listeria monocytogenes* specie are very dangerous for human health, with a mortality rate of 20% for infected patients, a serious attention has to be accorded in consumption of those foods able to transmit microorganisms. This article presents a study about the incidence of *Listeria* in two species of fish which were tested to prove presence or absence of *Listeria monocytogenes*, because fish meat is one of the foods susceptible to transmit bacteria and to induce grave intoxication. From fresh water fish Carp was selected and as a representative of ocean fish, Mackerel respectively. Different methods for detection were described and discussed. Those methods are legally accepted in European and Romanian normative for food consumption security as Food Microbiology and Specific Rules for Microbiological Analyzes. OXFORD, PALCAM and CAMP are test used for detection and confirmation of *Listeria* species of bacteria. Also API-*Listeria* tests were used for confirmation the presence or absence of different *Listeria* species in tested samples. Agar with sheep blood medium was inseminated with samples of fish meat because this is the proper medium for bacterial colonies development. The reason is the fact that bacteria colonies formed dark colored rings rounded by a black haloes, because in their metabolic process bacteria produce hydrolysis of aesculin (a glycoside contained in agar medium). The results of analyzes for those two species of fish meat do not confirm the presence of *Listeria monocytogenes*, but other species of *Listeria* (*Listeria welshimeri* and *Listeria innocua*), fortunately not pathogenic, were present.

Keywords: pathogenicity, *Listeria monocytogenes*, fish meat, infection

1. Introduction

Bacteria from *Listeria* genus and *Listeria monocytogenes* especially provide a high level of pathogenicity, being responsible by a lot of alimentary toxic-infections and other dangerous diseases, even female abortion. Many sources of *Listeria monocytogenes* exist in nature, like environment, animals, people [1-7].

From environment, *Listeria monocytogenes* was mainly isolated from soil, water and vegetables in decomposition. Weis detected *Listeria monocytogenes* in 21% of samples (from 779 samples of soil and plants). The high capacity of bacteria to survive in difficult conditions permits to explain infections which occur from sources contaminated long time before the reference moment. It was noted that in wastewater dispersed on farm land, bacteria remain active till eight weeks, what could explain human infections in New Scotland – Canada in 1981. The pathogenicity of *Listeria monocytogenes* is proved by the fact that almost 20% of infected patients died. Even the reported cases of listeriosis diminished in developed countries, researchers found varied modes of transmission of bacteria through diverse foods [1,2,5,7,8].

2. Materials and methods

There were selected two species of fish, a carp representing a fresh water fish and a mackerel representing an ocean fish respectively. The samples were analyzed in The Laboratory for Food Safety of DSVSA Suceava.

From fresh water fish Carp was selected and as a representative of ocean fish, Mackerel respectively. Different methods for detection were described and discussed. Those methods are legally accepted in European and Romanian normative for food consumption security as Food Microbiology and Specific Rules for Microbiological Analyzes [15-20].

Preparing of samples followed SR EN ISO 6887-4/2005 regarding Food Microbiology and Specific Rules for Microbiological Analyzes and initial suspensions and decimal dilutions preparing followed SR EN ISO 6887-1/2002.

Fish samples investigation was performed through the method of analyze for detection and confirmation of *Listeria monocytogenes* implemented and actualized in Romania by SR EN ISO 11290-1/2000 with amendment A1/2005 regarding isolation media and hemolysis test.

Analyzes evolved following next stages:

- **Primary enrichment.** In 225 mL liquid media with low concentration of selective agents of semi-Fraser bouillon, insemination of 25 mL of sample followed by 24 h incubation at 30°C was performed.
- **Secondary enrichment.** From obtained culture 0.1 mL were transferred in each test tube with 10 mL Fraser bouillon, followed by incubation for 48 h at 35°C or 37°C.
- **Corrugation and identification.** From obtained cultures samples were passed in two isolation media (agar OXFORD and agar PALCAM), with 24 – 48 h incubation at 35°C. In parallel, plates inseminated with positive control tell-tale (reference stem of *Listeria monocytogenes* ATCC 13932) were incubated in similar conditions [3,5,8].
- **Confirmation.**

3. Results and discussions

Examination of final obtained cultures confirmed that OXFORD and PALCAM media were highly selective for inhibition of associated flora [3,5,8].

On control tell-tale *Listeria monocytogenes* formed dark colored colonies, rounded by a black haloes, because in their metabolic process bacteria produce hydrolysis of aesculin (contained in agar medium) (see figures 1 and 2).

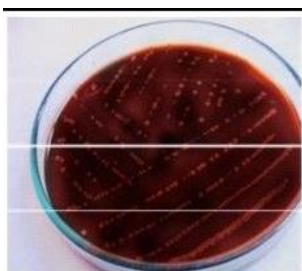


Fig. 1. Aspect of colonies of *Listeria monocytogenes* from positive control tell-tale sample on PALCAM selective medium, after 24 hours in thermostatic conditions

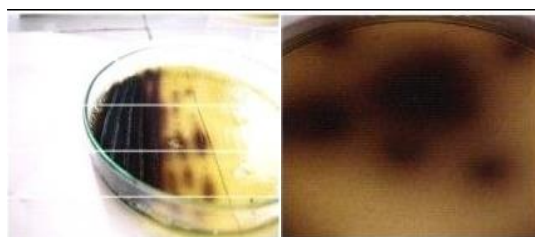


Fig. 2. Aspect of colonies of *Listeria monocytogenes* on OXFORD selective medium, after 24 hours in thermostatic conditions

Confirmation for *Listeria monocytogenes* was realized by hemolysis test and CAMP tests. Because *Listeria* generates hemolysis on agar with sheep blood, this could be used to confirm the presence or absence of bacteria. So, *Listeria monocytogenes* stem from control tell-tale sample produced small colonies rounded by small but clear zone of hemolysis, which is characteristic.

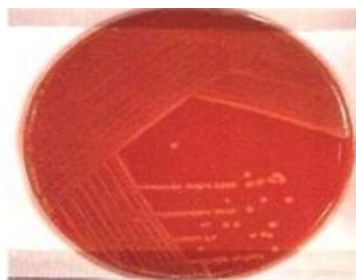


Fig. 3. Aspect of colonies of *Listeria monocytogenes* on an agar with sheep blood medium

Another reaction, gentle or accentuated could be highlighted with a CAMP test. This test consists in insemination of agar surface with *Staphylococcus aureus* and *Rhodococcus equi* on vertical parallel lines (knurling). Then were knurled horizontal parallel lines with *Listeria monocytogenes*, *Listeria innocua* and *Listeria ivanovii*, but horizontal lines did not intersect vertical lines, a distance of 1 – 2 mm remaining. Incubation time was 24 h at 37°C. Image in figure 6 presents a positive reaction, consisting in an arrow form at virtual intersection of the lines.



Fig. 6. CAMP test – control tell-tale plate (+)

The test results show the absence of *Listeria monocytogenes* and *Listeria ivanovii*, but a possible contamination with *Listeria innocua* or other species of *Listeria* genus.

Another confirmation was achieved using Api-Listeria tests, containing dehydrated substrates. Inoculated and incubated for 18 – 24 h at 35 – 37°C, the color change could signalize the presence or absence of bacteria. So, *Listeria innocua* and *Listeria welshimeri* respectively were confirmed for analyzed samples.

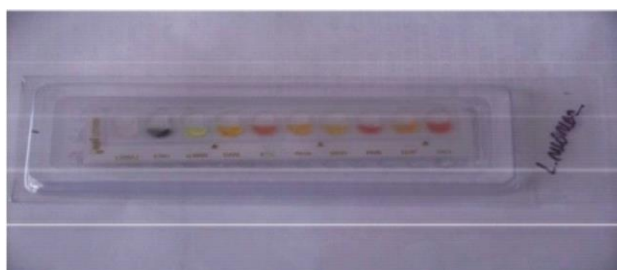


Fig 7. *Listeria monocytogenes* identified on API-LISTERIA galleries



Fig. 8. *Listeria welshimeri* identified on API-LISTERIA galleries



Fig. 9. *Listeria innocua* identified on API-LISTERIA galleries

All results of tests are summarized in table 1.

Table 1. Distribution of species of *Listeria* genus bacteria

Examined product	Number of samples	<i>Listeria monocytogenes</i>	<i>Listeria innocua</i>	<i>Listeria welshimeri</i>
Probe 1. Fish meat from <i>Carp</i> specie	5	absent	absent	2
Probe 2. Fish meat from <i>Mackerel</i> specie	5	absent	2	1

4. Conclusions

Analyzes of those two species of fish with five samples each infirm the presence of *Listeria monocytogenes*. Carp fish meat contain *Listeria welshimeri* at two samples from five and Mackerel fish meat contain *Listeria innocua* at two samples and *Listeria welshimeri* at one sample from five, respectively. Because *Listeria innocua* and *Listeria welshimeri* are not pathogenic, conclusion is that tested fish could be approved for consumption.

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3D-METAL COORDINATION COMPOUNDS - BIOSTIMULATORS GROWTH

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Abstract. The paper presents some aspects of coordination of transitive metals (cobalt (II), copper (II), nickel (II), oxovanadium(IV), manganese (II), zinc, iron (II) with dihydrazide of semicarbaziddiacetic acid (Dig) $\text{H}_2\text{N}-\text{C}(\text{O})-\text{NH}-\text{N}(\text{CH}_2\text{CONHNH}_2)_2$ (Dig) studied by elemental analysis and physicochemical methods. based on the data we obtained a structural diversity of combinations and possibilities of coordination of ligands. Study various biological properties showed that synthesized salts can serve as stimulators of extracellular amylolytic enzymes biosynthesis and increase the efficiency of obtaining biomass.

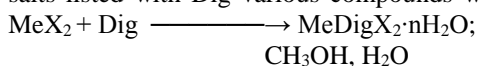
Keywords: metals transitive, dihydrazide semicarbaziddiacetic acid, stimulate biosynthetic

1. Introduction

Coordination of transition metal compounds with polyfunctional polydentate ligands and form a field of study of theoretical and applied chemistry. Among them are the metal chelates with organic ligands containing atoms of oxygen, sulfur, nitrogen - electron donor and acceptor energy. In particular it advises making complex salts with special importance for various branches of the national economy (agriculture, mechanics, pharmaceutical, etc.). Interest of the study was to ligands containing several coordination centers capable of forming different combinations so complex composition, structure, properties as well as chemical, biological potential, such as thio- and semicarbaziddiacetic acids

2. Material and methods

For the synthesis of coordination compounds have been taken that neutral salts of nickel (II), oxovanadium(IV), manganese (II), zinc, iron (II) and dihydrazide of semicarbazid- diacetic acid $\text{H}_2\text{N}-\text{C}(\text{O})-\text{NH}-\text{N}(\text{CH}_2\text{CONHNH}_2)_2$ (Dig) [1]. Synthesis was performed in solutions of alcohol and / or methyl. Direct interaction of metal salts listed with Dig various compounds were obtained according to the general scheme:



$\text{CH}_3\text{OH}, \text{H}_2\text{O}$

where $\text{Me} - \text{Co}^{2+}, \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Ni}^{2+}, \text{Mn}^{2+}$ ($\text{X} = \text{Cl}^-$); $\text{Me} - \text{Fe}^{2+}, \text{VO}^{2+}$ ($\text{X} = \text{SO}_4^{2-}$); $n = 1, 2$

Composition and structure of substances was determined by elemental analysis, various physico-chemical and physical methods (IR Spectroscopy, thermogravimetry, X-ray spectra) has determined that combinations achieved. It was researched and their biological activity on intracellular hydrolases biosynthesis of various fungal species: *Penicillium viride* (Producent pectinases), *Aspergillus niger* 33 (Producent amylase), *Rhizopus arrhizus* (Producent lipases). The tests have been performed with the solutions of different concentrations of the suggested substances in individual selected conditions for each strain through known methods in enzymology.

Combinations of cobalt (II) and copper (II) showed good growth regulators, namely action "retard" germinated corn on immutable processes of photosynthesis.

3. Results and Discussion

The utilization of complex combinations of the transition metals with polyfunctional, polydentate ligands in the biosynthetic process in biology, medicine, animal breeding, agriculture is just present-day problem.

It also regulates the most important biochemical processes occurring in living organisms. Attempts microbiological research showed that complex substances can be used as stimulating biosynthesis of many biologically active substances: proteins, enzymes, vitamins etc [2,3].

Evaluation of biological properties of substances taken have shown that these influences differently the biosynthesis and activity hidralaze different species, depending on source fermentative system (STAM-producent).

On the basis of the achieved tests were found, that some obtained coordination compounds manifested weak inhibitory properties ($\text{ZnDig}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$), and others - stimulators of biosynthesis intracellular amylolytics ferments

Therefore, for micromycete *Aspergillus niger* 33 – producent of amylases the best stimulator has been proved to be the complex combination $\text{VODigSO}_4 \cdot 2\text{H}_2\text{O}$, and $\text{Co}(\text{Dig})(\text{NCS})_2 \cdot 2\text{H}_2\text{O}$ (tab.1).

Table 1. Dihydrazids influence of 3d metals on *Aspergillus niger* 33 fungal biomass yield

The Substance used	Biomass, g/L			% of control		
	1	5	10	1	5	10
$\text{Co}(\text{Dig})(\text{NCS})_2 \cdot 2\text{H}_2\text{O}$	11,85	16,01	13,45	93,30	124,12	94,38
$\text{Co}(\text{Dig})\text{Cl}_2 \cdot 4\text{H}_2\text{O}$	11,65	14,00	13,10	91,70	108,52	91,92
$\text{VODigSO}_4 \cdot 2\text{H}_2\text{O}$	11,08	14,75	16,30	87,20	121,60	114,38
$\text{ZnDig}(\text{NO}_3)_2 \cdot \text{H}_2\text{O}$	9,73	11,55	13,15	76,57	89,53	92,28
Control	12,70	12,90	14,25	100,0	100,0	100,0

Based on the investigation it was found that more effective biostimulation by dihydrazides proposed solution concentration is 5 g /l [4].

4. Conclusions

The accumulated results give the possibility to describe thoroughly the mechanism and the properties of influence of the proposed complexes upon the biosynthetic processes of the microbiological systems.

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RESEARCH HYALURONIC ACID COMPLEXATION WITH BIOACTIVE METAL IONS

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Abstract: The paper contains some results for obtaining complex salts (associates) of some 3d-metal ions (Co^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+}) with hyaluronic acid (HA) or sodium salt HA, which can be used in pharmaceutical, cosmetic, and food industry. Substances were studied by IR spectroscopy.

Keywords: hyaluronic acid, complex compounds, bioactive metal ions, importance

1. Introduction

Ever-increasing demands of hyaluronic acid (HA) for pharmaceutical, cosmetic and food industry imposes researches to study and investigate its properties; seek local sources of hyaluronic acid; develop cost-effective methods of production of hyaluronic acid; search new chemical derivatives, medicinal and cosmetic preparations on its base.

Hyaluronic acid gaining more and more popularity in medicine and cosmetics. Many cosmetic products contain hyaluronic acid, due to its ability to moisturize and smooth the skin. Clinical studies show that hyaluronic acid speeds healing of wounds and promotes the disappearance of old and new scars. Hyaluronic acid injections do less visible wrinkles and blemishes caused by acne or injuries.

One of the important properties of HA is that its macromolecules can be conjugated with bioactive compounds for pharmaceutical industry. So, hyaluronic acid can be a vehicle for topical medicines, which drives their absorption. Interesting properties have sodium, potassium, iron, zinc, copper and other salts of HA, which are successfully used in cosmetic and pharmaceutical industry.

2. The properties of hyaluronic acid and its complex – compounds with metal ions

Hyaluronic acid was discovered about 70 years ago in vitreous of bovine eyes. It is a natural biopolymer, class proteoglycans, whose molecule is formed from the remains of β -D-glucuronic acid and N-acetyl- β -D-glucosamine linked by β -glucosidic bonds (1–3) - and β -(1–4), the long unbranched chains (Fig.1.) [1].

From the primary structure of the macromolecule it can be seen that HA has a repeatable disaccharide unit in which glucuronic acid is linked to glucosamine with β -glycosidic link between atoms C_1 and C_3 , respectively. Basic structural unit is repeated in macromolecular chain is a very rigid chain segment with a length of 11.98 Å. Spatial distribution of anionic groups and their degree of ionization contribute to the conformation of the molecule due to mutual electrostatic rejection of negative charges along the polysaccharide backbone. Tertiary structure of HA in concentrated solutions, gels and solid, is influenced by the large number of hydrogen bonds inter- and intramolecular polar groups of abundant, such as: $-\text{OH}$, $-\text{COC}-$, $>\text{C}=\text{O}$, $-\text{NH}-$, $-\text{COO}-$. Structure can be influenced also by hydrophobic interactions of protein fragments. The degree of polymerization and molecular weight varies by tissue type.

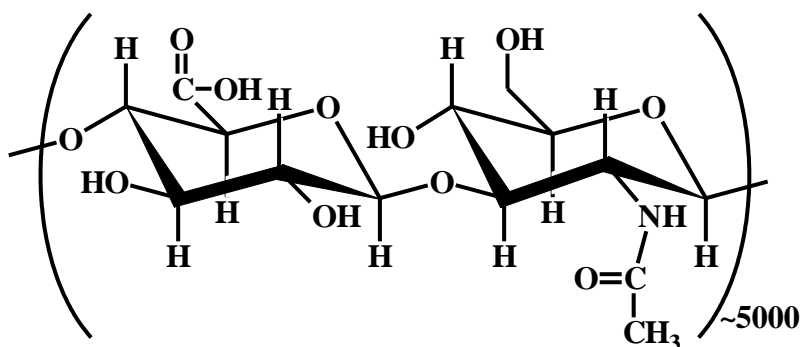


Fig. 1. Structure of hyaluronic acid

HA content in the human body is an important factor on which the physiological process of aging and immunity of the body depends upon. To strengthen the immune system and prevent various diseases including cancer various "food additives" are prepared in which HA is used as an ingredient or as compound. HA is called "star" of cosmetology, "hope" of rejuvenation and "pledge" of beauty. Benefits of HA supplementation – Cosmetic effect: skin hydration from the inside out, correcting in this way wrinkles. – Anti-arthritic: lubricate joints, especially knees and hips ones. – Rejuvenate, anti-aging effect: for men and women between 30 and 40 who are beginning to see signs of aging mirror. The effects are felt quickly after first supplementation with hyaluronic acid.

HA is present in all tissues of the body and performs several important functions. HA is involved in the transfer of nutrients into cells and in the removing toxins from the cells. Important role in the detoxification of the body belongs to d-metal ions, such as Co^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , Zn^{2+} participating in the various enzymes. To ensure the cells micronutrients are necessary such forms of transport, which prevent non-specific absorption of ions of these elements and selectively forward them to the cells. A mode of transport is hyaluronic acid, which easily penetrates into the cell by endocytosis.

Interesting properties complex-compounds of hyaluronic acid with d-metal ions are described in the paper [2, 3].

3. Complexation of some 3d-metal ions with hyaluronic acid

Complexes of hyaluronic acid (HA) with some d-metals increases resistance to chemical irritants and toxins. Moreover, in case of intoxications with heavy metals, the use of HA will facilitate the removal of these metals from the body, due to its ability to bind the metals in stable complexes. This causes a high interest in the study and use such complexes.

In this paper we investigate the complexation of HA with ions of some 3d-metals, such as Co^{2+} , Cu^{2+} , Fe^{2+} , Fe^{3+} . HA for the study was extracted from rooster combs [4]. Complexes were formed with 1N solutions of chlorides respective metals and 1% solutions of HA. The reaction products were washed with water, then with 96% ethanol and dried.

The compounds were studied by IR spectral analysis (Fig. 2).

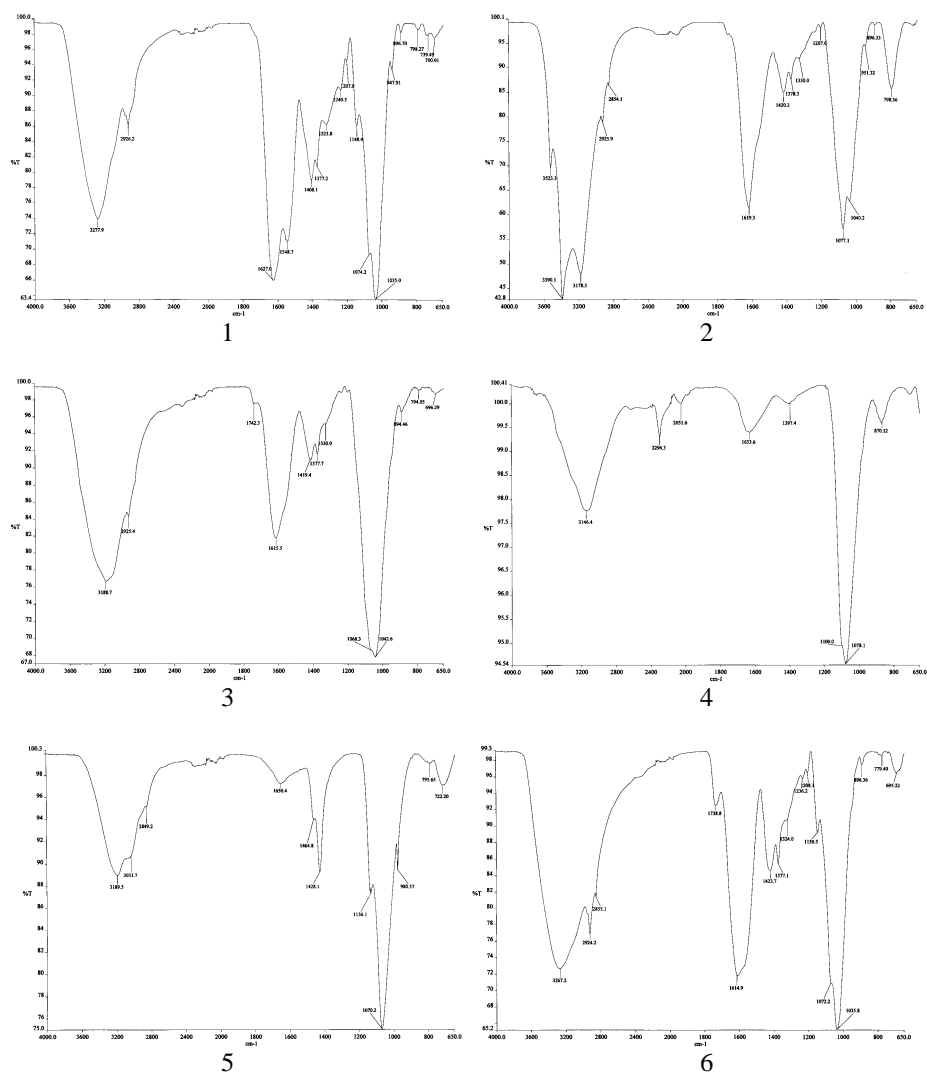


Fig. 2. IR spectra of HA (1) and associates of HA: with Co^{2+} (2), with Cu^{2+} (3), with Cu^{2+} (it never was washed with ethanol) (4), with Fe^{2+} (5), with Fe^{3+} (6).

IR spectra of hyaluronic acid and its associates with the metal ions show that the formation of associates accompanied by a shift of the absorption bands in the region $1740\text{--}900\text{ cm}^{-1}$. This suggests that the formation of associates is due to the interaction of metal ions with electron donor atoms (O, N) of hyaluronic acid.

According to IR spectroscopy of the products, the ions Cu^{2+} and Fe^{2+} form stable complexes with the amide and the carboxylic group, whereas Fe^{3+} , Co^{2+} form less stable complexes.

The study of complex HA with metal ions continues.

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INVESTIGATION OF THE EXTRACTION PROCESS OF HYALURONIC ACID FROM NATURAL SOURCES

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Abstract: Properties and methods for obtaining hyaluronic acid and its derivatives from raw material of animal origin are reviewed. The importance and practical application of hyaluronic acid in various fields are discussed.

Keywords: hyaluronic acid, obtaining methods, natural sources.

Introduction

The production and usage of hyaluronic acid (HA) is growing worldwide. In Moldova, hyaluronic acid is less known as pharmaceutical and cosmetic component.

HA is a precious natural biomaterial, biocompatible, safe and non-allergic. For these reasons HA is one of the most "agreeable" cosmetic ingredient. Emulsions based on it have a smooth soft consistency. Products of HA are compatible with human skin and do not cause allergic reactions. Some properties have also sodium, potassium, calcium, zinc, copper and other salts of HA, which are successfully used in cosmetic industry.

In our country we have enough cheap sources that can be used for hyaluronic acid preparation. The problem is to develop economically efficient methods of obtaining and purification of hyaluronic acid and its derivatives in quantities sufficient to be sold and used in the production of other bioactive compounds based on it.

The properties of hyaluronic acid

The name of HA reflects its transparent nature (the Greek word for glass is hyalos) and the content of one of uronic acids (glucuronic acid).

Hyaluronic acid is a compound present in the human body. It is one of the main constituents of the extracellular matrix of connective tissue and it is concentrated in synovial fluids, heart valves, eyes, cartilage.

In pure form HA is a white odorless powder soluble in water and insoluble in organic solvents with high viscosity. Specific rotation in aqueous solutions is $-(70-80)^\circ$. HA is a polyelectrolyte which $pK_{HA} = 3.21$. A molecule of HA is able to retain about 200–500 molecules of H_2O . It has a high specific hydrodynamic volume. The molecule form depends on the pH and ionic strength of the solution. It was found that as the result of electrophoresis for an ionic force of 0.12 only 14% of ionized groups are effective, and for an ionic force of 0.02 already 80% of these groups are effective [1].

Chemical and spatial structure of the macromolecule, high molecular weight, high viscosity of the solution and hydrophilic qualities are important biological properties of HA. Due to these properties, HA has various biological functions and roles in animal bodies such as: participation in embryogenesis and morphogenesis processes, intercellular relationships and communication, mechanical strength of tissues, reducing friction in biomechanical systems, formation and proper function of cartilages, formation and

maintenance of transparent structures of the eye, the permeability of biological membranes including vascular walls, water retention [2].

Biological researches have shown no toxic properties, irritating, allergenic. A comparative study was conducted on biocompatibility and safety of HA obtained from three natural sources: umbilical cord, cockscomb and bovine vitreous [3].

The importance and usage areas of hyaluronic acid

Due to its properties, HA is one of the most attractive biomaterials for pharmaceutical and cosmetic industry [4].

Hyaluronic acid provides lubrication and hydration of connective tissues, including those of the skin. In the absence of lubrication and hydration of tissue and lose elasticity if skin leads to wrinkles and creases.

Due to its structure, HA form a film on the skin invisible, transparent and elastic, while acting in depth in the tissue, cartilages and joints. Its role is to keep the most important characteristics of young and healthy skin: suppleness, elasticity and tone. HA capacity to restore the interstitial matrix and liquid skin turgor alterations and wrinkles is successfully used for "rejuvenating" skin.

HA is a glycosaminoglycan, an essential component of extra cellular space, in which the collagen and elastin fibers are suspended. HA has an increased capacity to retain water, like a "sponge" that allows maintaining hydration, elasticity, skin firmness. Unfortunately, body's ability to produce hyaluronic acid decreases with age, and so the skin tissue becomes dehydrated, wrinkled and tonicity diminishes. HA injections are used more than 12 years in Europe and gradually replaced the injection of bovine collagen, which is sometimes complicated and required tests prior allergic reactions. Being a natural filler, it has a very low rate of allergic reactions - 0.06% vs. 3% for collagen.

HA is used to fill wrinkles and lip augmentation. Today the area of its application and usage in aesthetic medicine has expanded greatly, it is frequently used for volumization, nonsurgical facelift, correct dark circles and breast augmentation.

Also, HA is an important component of cartilage. In this role it dampens shocks in the joints, has lubrication effect and protects joints for chronic inflammation (eg arthritis). It is used to successfully heal stretching of ligaments. HA is a common ingredient in anti-osteoarthritis preparations and is frequently injected into joints, being a very effective treatment.

HA helps the immune system, acting as an antioxidant, it increases water retention in tissues, increases lubrication of heart valves, and serves as an adjunct to anti-infection treatment.

In the 1990s, hyaluronic acid began to be used in ophthalmology to treat corneal trauma.

Besides keeping joints lubricated, hyaluronic acid helps water retention in other tissues of the body, providing hydration of collagen and elastin. Interest in the use of HA as bioactive ingredient in skin care products came with the discovery that the volume of him in the skin decreases with age.

Clinically have been proven extraordinary efficiency of hyaluronic acid to fill wrinkles and smoothing. All clinical trials have shown that hyaluronic acid helps heal wounds faster and fading scars.

Currently procedures to inject cross-linked hyaluronic acid to fill wrinkles and lip augmentation, so-called fillers, are used [5]. For this purpose fits and are generally used

products that are marketed as Amalian (Sweden), Perfectha (France) Remake (Italy), Aphrodite Gold (Germany) etc.

Unlike many other biologically active substances, HA shows all its valuable properties at very low concentrations (0.01 to 0.1%), which allows to create effective cosmetics, whose prices will fit producers as well as consumers. This refers to high molecular weight HA, which are now part of moisturizing creams, lipsticks and lip balms, cellulite cream, sunscreen lotions, anti-inflammatory lotion and wound healing [6, 7].

HA content in the human body is an important factor on which the physiological process of aging and immunity of the body depends upon. To strengthen the immune system and prevent various diseases including cancer various "food additives" are prepared in which HA is used as an ingredient or as compound [8, 9]. HA is called "star" of cosmetology, "hope" of rejuvenation and "pledge" of beauty. Benefits of Hyaluronic Acid supplementation – Cosmetic effect: Skin Hydration from the inside out, correcting in this way wrinkles. – Anti-arthritis: lubricate joints, especially knees and hips ones. – Rejuvenate, anti-aging effect: for men and women between 30 and 40 who are beginning to see signs of aging mirror. The effects are felt quickly after first supplementation with hyaluronic acid.

Obtaining hyaluronic acid

Properties of hyaluronic acid are determined by molecular mass, extraction mode, traces of proteins and other proteoglycans that can contaminate preparations obtained [10].

In research conducted hyaluronic acid was obtained from several natural sources of raw material: crest of hens (CH), cockscomb (CC), bovine vitreous body (BVB), bovine umbilical cord (BUC) [11,12]. Obtain preparations of hyaluronic acid was performed according to the scheme of Fig. 1.

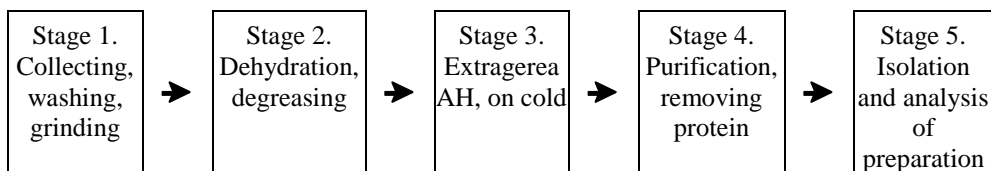


Fig. 1. Process scheme for obtaining hyaluronic acid

In the proposed method the degreasing of raw material is carried out fully before extraction of HA, along with dehydration in Soxhlet apparatus with acetone for 2 hours. HA extraction is performed with NaCl solution only at cold (4–10 °C). From the obtained extract in cold, HA settles with 96% ethanol or acetone. The product is redissolved and proteins are removed by heating-cooling at pH 7 and pH 5 to 5.5 in CHCl₃ processing. From the obtained solution is settled and isolated the sodium hyaluronate, HA respectively, with corresponding efficiency.

Characteristics of obtained preparations are vary depending on source of raw materials and obtaining method. For obtained samples was determined the mass part (ω) of hyaluronic acid in the raw material which was subjected to extraction, the protein mass (Lowry method) [13], relative viscosity (Ostwald method) [14] (Tab. 1.).

Table 1. Feature of HA preparations obtained from various sources of raw material

	CH	CC	BVB	BUC
ω (%)	0,5 - 0,7	1,1 - 1,5	0,1 - 0,2	1,4 - 1,8
Proteins (%)	3 - 5	1 - 3	1 - 3	2 - 4
Relative viscosity (η)	11 - 12	12 - 13	5 - 7	12 - 13

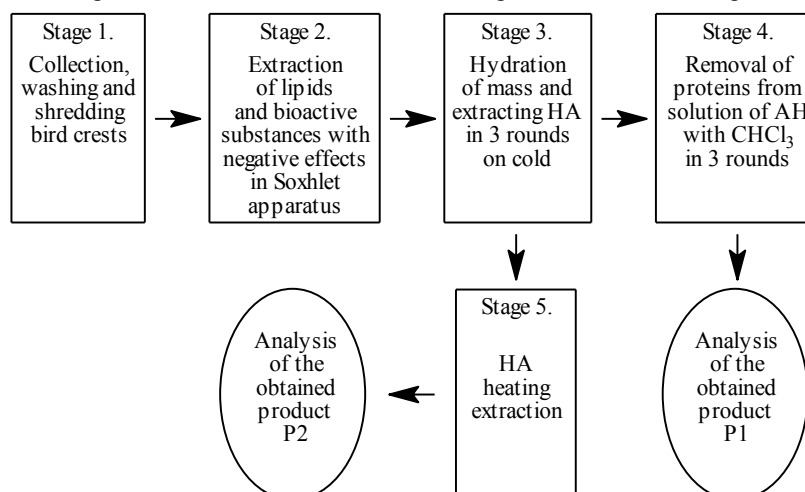
Identification of HA in the obtained preparations was made based on the infrared absorption spectra.

Mentioned raw material sources of HA have a different accessibility. Our investigation shows that hen combs and cock combs is preferred.

Taking into consideration the results of research and availability of raw material sources we have agreed to obtain hyaluronic acid from the hen and cock combs collected from local poultry companies [15].

Research result is the issuance process for obtaining HA, increasing the extraction, cost reduction, improving the quality of the final product suitable for use in medical, cosmetic and food.

Obtaining of HA was carried out in several stages as illustrated in Fig. 2.

**Fig. 2.** Scheme process of obtaining and purification of hyaluronic acid, sodium hyaluronate and protein complex of hyaluronic acid

The 1% HA (Product P1) aqueous solution obtained by the proposed method is a viscous liquid, eculent hard, transparent or slightly opalescent, colorless, odorless. Relative viscosity of the solution of 0.1% HA Ostwald Viscometers measured at a temperature of 18 °C was equal to 12.

1% solution absorbance measured at 257 nm HA (absorption maximum nucleotide) and 280 nm (absorption maximum of protein) – is less than 0.1 a ($l = 10$ mm).

Obtained hyaluronic acid is kept in 96% ethanol, in closed bottles protected from light at 0–4 °C temperature.

The 1% solution of the product P2 (hyaluronic acid-protein complex) presents ultraviolet absorption at a wavelength 266.5 nm equal to 0.609 a. ($l = 10$ mm).

The resulting products are used in further studies.

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MODERN STUDY OF BEHAVIOR SUCROSE

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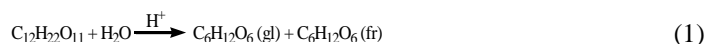
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Abstract: The disaccharides called sucrose not hydrolyze in aqueous solution; the reaction is known as sucrose inversion, is catalyzed by mineral acids, and can be studied by the polarimetric method. Treaties chemical kinetics fit this kind of reaction order $n = 1$, [1- 4]; the reaction rate depends on the concentration of sucrose, being considered a chemical reaction of zero order towards water. Specialty papers mention that the order of reaction might be determined by graphical integral method; plot, towards values of reaction times, four functions of sucrose concentration (c_t , $\ln c_t$, $1/c_t$, $1/2c_t^2$). This paper proposes a modern kinetic study on acid hydrolysis of sucrose, without mechanical agitation of reactant mixture. The multiple measurements were made with automatic polarimeter, and objective experimental results were processed with a computer; the software offers additional information on the dispersion of experimental data, and the results are outstanding.

Keywords: sucrose, hydrolyze, acid medium, optical activity, kinetic parameter

1. Introduction

We start from the belief that the study results mentioned reaction kinetics are dependent on: the behavior of reactant, limits quantitative method of investigation and mathematical processing device limits of the experimental results. The literature includes transformation by acid hydrolysis of sucrose - known as sucrose inversion - in the category of chemical reactions of order $n = 1$. Sucrose and its hydrolysis products – glucose and fructose - are optically active substances. Considering that, the hydrolysis reaction is irreversible



The authors considered that the transformation reaction mixture can be studied by optical business value; value of the optical activity of the mixture should fall to negative values due to fructose. At any time t , molar concentration of sucrose, c_t is calculated with

$$c_t = \alpha_t - \alpha_f \quad (2)$$

where [5]:

- α_t - value of optical activity reactant mixture, in any moment, when hydrolyzed part of the amount of sucrose,
- α_f - optical activity value at the end of hydrolysis process; we found that this value is not reproducible.

To conduct a study polarimeter reactant mixture - between measurements is introduced in a thermostatic chamber and using a classically polarimeter - is found that:

1. The experimental values have a normal evolution, but some are surprising (much higher or much lower).
2. By plotting the concentration functions - only for integer values of the order of reaction - value pairs defined in the plan only curves.

In this case, we considered that the experimental assembly is designed poorly, and is not the best processing experimental values. The two previous findings have led to the improvement of experimental assembly and method of processing and interpretation of experimental values, as follows:

- study of the reactant mixture molar concentration of sucrose is very small, so that the mixture density is very close to that of the solvent (distilled water);
- avoid mechanical stirring reaction mixture (the polarimeter tube is maintained and thermostated in working position);
- using an automatic polarimeter;
- the automatic polarimeter is associated with a Windows operating system to take over and processing of experimental results [6];
- a method of calculating the molar concentration of sucrose, taking into account the contributions of all asymmetric chemical species that are present in the reactant mixture;
- determination of kinetic parameters of reaction by differential graphically method.

By using the proposed experimental assembly and processing of the experimental values are obtained interesting conclusions.

2. Materials and experimental methods

For the kinetic study, prepare reagents, glassware and laboratory equipment, as follows.

Reagents: double distilled water and two solutions, ($1g_{\text{sucr}}/100\text{ mL}$) one stock solution of sucrose and 1N HCl, reagents Merck booth.

The glassware and laboratory equipment: pipettes (5, 10, 25 mL), flasks (25, 100 and 500 mL), glasses, analytical balance, computer with operating system Windows, automatic polarimeter (Atago, type AP-300, $\lambda = 589\text{nm}$, the polarimeter tube has length $l = 200\text{mm}$, with RCS-232 interface to Windows; the proper intern software is HyperTerminal), thermostat liquid unity with recirculation (20-80°C, type Pye Unicam) and tool temperature control (Digital thermometer, tip Optronic, Krüss).

2.1 Experimental conditions

Polarimeter tube was placed inside a cylindrically thermostatic unity (connected in fluid circuit) and working temperatures were strictly controlled, 20°C. Polarimeter tube filled with reactant mixture is always in working position.

2.2 Experimental measurements

The reactant mixture comprises: 5 mL stock solution of sucrose, 10 mL solution of hydrochloric acid and 10 mL double distilled water; the initial concentration of sucrose is $c_0 = 0.2\text{g}/100\text{mL}$. Was obtained 1750 experimental values of $(\alpha_t)^\circ$. The automatically measured values are obtained successively at intervals of one minute. The software retrieves and stores the α_t values. By Excel, these values are converted into molar concentrations of sucrose c_t and then processed.

2.3 The transformation of α_t values in c_t values

To calculate the specific rotation of asymmetric substances, expressed in sexagesimal degree, $^{\circ}$, the polarimeter AP-300 us the formula:

$$[\alpha^{\circ}]_D^{20} = \frac{10000 \times \alpha}{l \times c \text{ (g/100 mL)}} \quad (3)$$

where: (α°) is the optical activity of a solution of concentration c (g/100 mL) measured in a polarimeter tube size l (mm).

The specific rotation values of the three saccharides – sucrose, glucose and fructose – are: $[\alpha_{\text{sucr}}] = +66.5^{\circ}$, $[\alpha_{\text{gl}}] = +52.5^{\circ}$ and $[\alpha_{\text{fr}}] = -133^{\circ}$. In the initial moment, $t = 0$, the experimental values describing the sucrose's contribution is in relation,

$$66.5^{\circ} = \frac{10000 \times \alpha_0}{200 \text{ mm} \times (0.2 \text{ g}_{\text{sucr}} / 100 \text{ mL})} \quad (4)$$

so as to $\alpha_0 = 0.266^{\circ}$.

The sucrose hydrolysis, equation (1), involves transformation into equimolar mixture of glucose and fructose. We suppose that after t minutes, x_t g sucrose were hydrolyzed, so that the reaction mixture contains:

$$m_{\text{sucr}} = (0.2 - x_t) \text{ g}; \quad m_{\text{gl}} = m_{\text{fr}} = 0.5263 \times x_t \text{ g} \quad (5)$$

The total optical activity of reactant mixture α_t , is the sum of optical activities of all sugars present

$$\alpha_t = \alpha_{\text{sucr}(t)} + \alpha_{\text{gl}(t)} + \alpha_{\text{fr}(t)} \quad (6)$$

The sucrose contribution may be calculated, knowing that:

$$[66.5^{\circ}] = \frac{10000 \times \alpha_{\text{sucr}(t)}}{200 \times (0.2 - x_t)} \quad (7)$$

thus

$$\alpha_{\text{sucr}(t)} = (0.266 - 1.33 \times x_t) \quad (8)$$

The glucose contribution is

$$[52.5^{\circ}] = \frac{10000 \times \alpha_{\text{gl}(t)}}{200 \times 0.5263 \times x_t} \quad (9)$$

$$\alpha_{\text{gl}(t)} = 0.5526 \times x_t \quad (10)$$

The fructose contribution is

$$[-133^{\circ}] = \frac{10000 \times \alpha_{\text{fr}(t)}}{200 \times 0.5263 \times x_t} \quad (11)$$

$$\alpha_{\text{fr}(t)} = -1.399958 \times x_t \quad (12)$$

The algebraic sum of the three contributions is:

$$\alpha_t = 0.266 - 1.33 \times x_t + 0.5526 \times x_t - 1.399958 \times x_t \quad (13)$$

$$\alpha_t = 0.266 - 2.177358 \times x_t \quad (14)$$

and may calculate the amount of sucrose consumed

$$x_t = \frac{0.266 - \alpha_t}{2.177358} \text{ g}_{\text{sucr}} / 100 \text{ mL} \quad (15)$$

Using x_t value is calculated the amount of unhydrolyzed sucrose per 100 mL solution and its molar concentration

$$c_t = \frac{(0.2 - x_t) \times 10}{342} = (0.2 - x_t) \times 0.02923 \text{ mol/L} \quad (16)$$

The c_t values obtained are used in kinetic study.

2.4 The verification of experimental assembly

The polarimeter tube was charged with a solution of sucrose, $c = 0.2 \text{ g sucrose}/100 \text{ mL}$, and for 165 minutes, were measured optical activity; the calculated value is $\alpha_0 = 0.266^\circ$. The values $(\alpha_t)^\circ$ was plotted and calculated values of the statistical parameters of normal distributions, fig 1 and table 1.

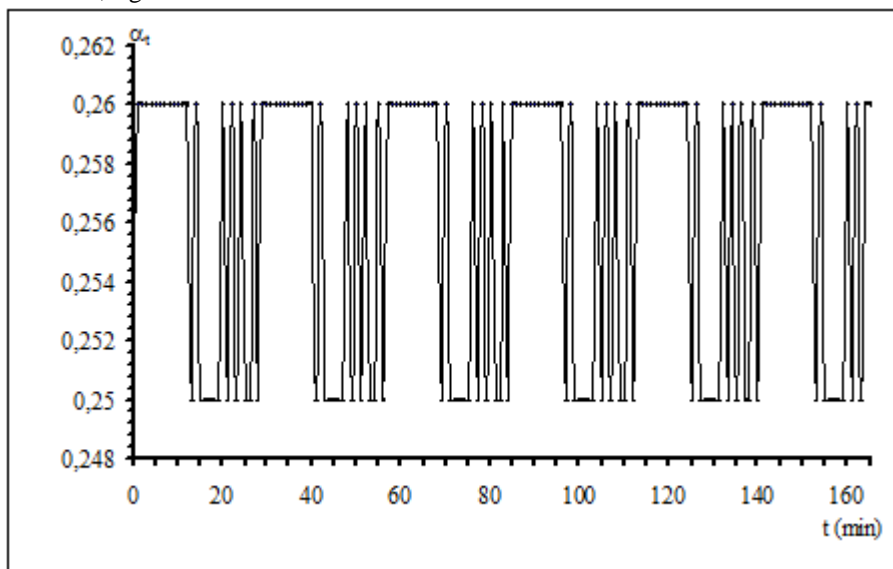


Fig. 1. The graphical representation of the pairs of values $\alpha_t = f(t)$, $t=0-165 \text{ min}$

According to figure 1, the population consists only of two experimental values are taken incidentally. According to table 1, are observed very close values of the statistical parameters of trend and very small values of statistical parameters of scattering. The graphical representation and the content of table confirm the stability of sucrose in solution and stability proposed experimental assembly.

Table 1. The values of the statistical parameters of normal distributions for population α_t

The values of the statistical parameters			
Statistical parameters of trend		Statistical parameters of scattering	
arithmetic mean	0.256145	standard deviation	0.003963
median	0.26	dispersion	0.000015
module	0.26	amplitude	0.01
central value	0.255	-	

According to figure 1, the population consists only of two experimental values are taken incidentally. According to table 1, are observed very close values of the statistical parameters of trend and very small values of statistical parameters of scattering. The graphical representation and the content of table confirm the stability of sucrose in solution and stability proposed experimental assembly.

3. Interpretation of results

To study the behavior of sucrose acid hydrolysis reaction must be interpreted the 1750 pairs of values ($c_t = f(t)$); figure 2 shows the diagram that describes the evolution of the value of the molar concentration of sucrose to entire domain of time.

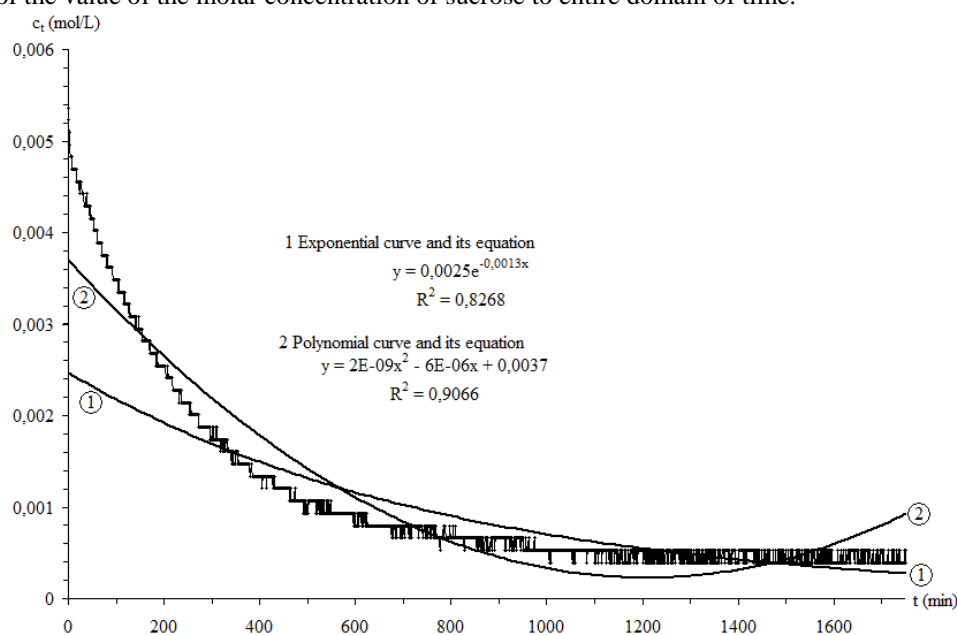


Fig. 2. Graphical representation of the 1750 values of molar concentration of sucrose with curves that describe the exponential and polynomial trend disposition thereof

Noting layout plan of the 1750 pairs of experimental values with exponential and polynomial trend, we can formulate the following four general conclusions.

1. Molar sucrose concentration value decreases. The reduction in molar concentration of sucrose is done in steps, not continuous. Figure 2 shows the successive intervals of time where the molar concentration of sucrose remains constant (successive levels of concentration); in timing units, the amplitude of these intervals increases over

time. It may be considered that in some point of time, hydrolyze a group of molecules sucrose. The penultimate concentration level is recorded at $t = 975$ min, characterized by the following experimental values: $\alpha_{975} = -0.13^\circ$ and $c_{975} = 5.3 \times 10^{-4}$ mol/L. The last recorded concentration level is at $t = 1142$ min, characterized by the following experimental values: $\alpha_{1142} = -0.14^\circ$ and $c_{1142} = 3.9 \times 10^{-4}$ mol/L. After this moment the reactant mixture begin a process of chemical equilibrium, when the molar concentration of sucrose oscillating between two values, $\alpha_t = -0.13^\circ$ and $\alpha_{975} = -0.14^\circ$. The $t = 975$ min value became a reference value. According the reference value, the experimental population values are distributed in two domains, as follow: the kinetic domain, containing experimental results values with $t < 975$ min and the domain of reversible equilibrium, characterized by values $t = 976-1750$ min. Reduction steps molar concentration of sucrose suggests that acid hydrolysis reaction adopt a specific mechanism; the associated sucrose molecules adopt a specific orientation.

To support the design on the association of sucrose molecules, we compare the diagrams $c_t = f(t)$ obtained in three different stages. Thus, fig 3 present the diagram obtained in the first stage considered. The diagram describes the behavior of molecules of sucrose in concentrated solution.

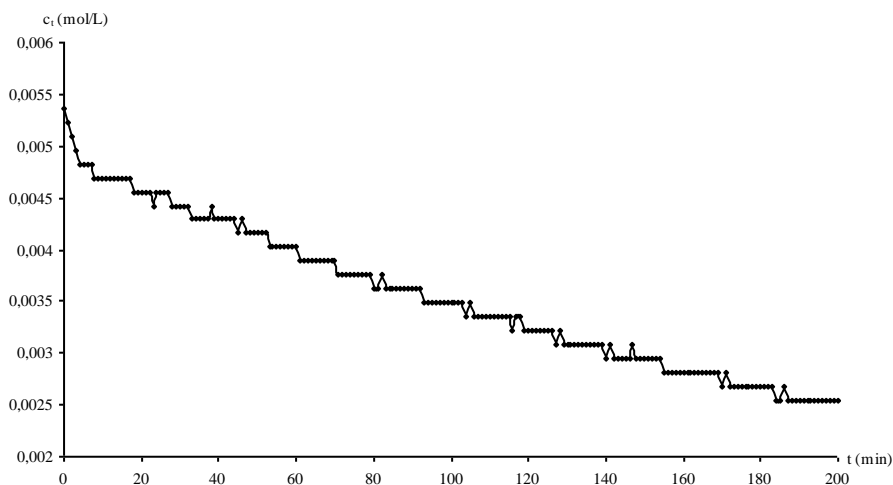


Fig. 3. The diagram higher molar concentration of sucrose in the range $t = 0-200$ min

Switching from a higher level of concentration to a lower level is made only after sucrose and water molecules were associated appropriate; molecules in favorable association hydrolyze, reducing the concentration of sucrose molar. The unfavorable molecular associations are destroyed, so that - in some cases - reactant mixture returns to the previous concentration level. Any crossing molar concentration value between two adjacent levels can be considered as an unstable chemical equilibrium.

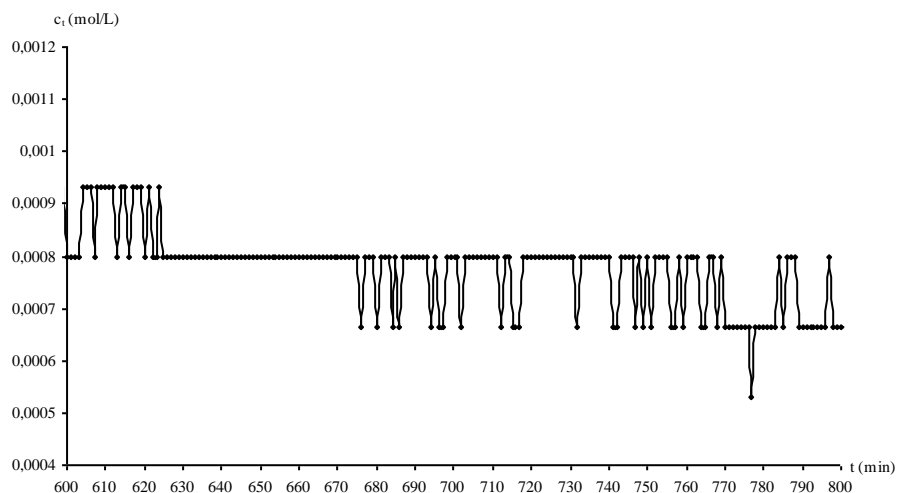


Fig. 4. The diagram medium molar concentration of sucrose in the range $t = 600\text{-}800$ min

The figure 4 contains a diagram that describes the behavior of molecules in the reaction mixture with medium level of sucrose concentration. In the diagram are present only three levels of concentration, but chemically unstable equilibrium frequency becomes high; per unit of volume, decreases the number of favorable molecular association's for hydrolysis process.

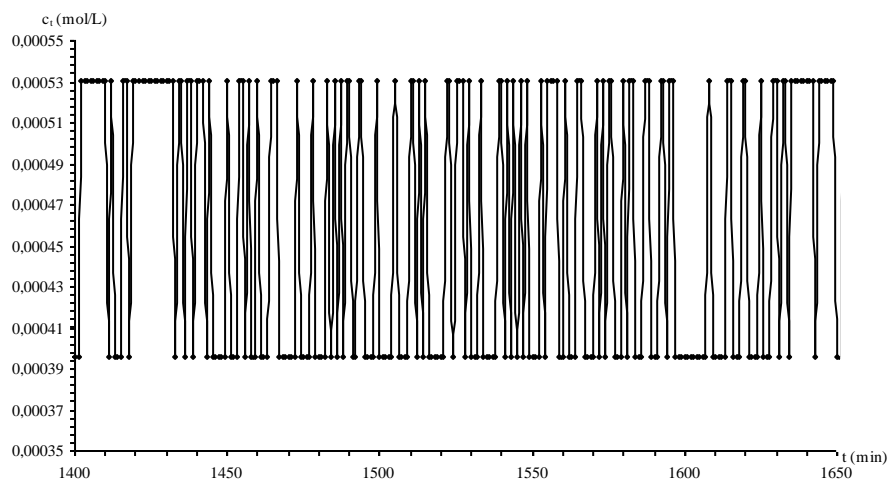


Fig. 5. The diagram lower molar concentration of sucrose in the range $t = 1400\text{-}1650$ min

Observing the diagram of figure 5, we say that lower molar concentration of sucrose is associated with high frequency of unstable chemical equilibrium; due to their high frequency, reactant mixture entered the classical chemical steady state.

2. The trend of experimental pair's values to be distribute on a downward exponential curve is expressed by the equation

$$c_t = 0,0025 \times e^{-0,0013 \times t} \quad (17)$$

$R^2 = 0,8262$, the value for the regression factor is small; this equation can not be used to carry out a complete kinetic study.

3. The trend of experimental pair's values to be distribute on a downward polynomial curve is expressed by the equation

$$c_t = 2 \times 10^{-09} \times t^2 - 6 \times 10^{-06} \times t + 0,0037 \quad (18)$$

which has a high value of regression factor, $R^2 = 0,9066$; it can be used to characterize the time evolution of reactant mixture. According the polynomial curve, in the reactant mixture, the molar concentration of sucrose decreases exponentially and then begin to take a slight upward trend.

4. Operating model allows to calculate the degree of hydrolysis at any time; it is noted that the degree of hydrolysis falls in

$$\alpha_{h_t} = 10.56-93.23\% \quad (19)$$

The sucrose hydrolysis is partial; reactant mixture adopts steady state value when

$$\alpha_{h_t} = 90.93\% \quad (20)$$

4. Conclusions

The content of this paper highlights the following contributions:

1. The superiority of analytical assembly which functions automatically and rigorously controlled experimental conditions, without mechanically stirring.
2. The superiority of processing and interpretation mode of experimental values, providing objective and continuous characterization of reactant mixture. This paper highlights the stage where the reaction is direct and the stage when it becomes reversible.
3. We obtain information which opens the prospect of a kinetic study on the mechanism (highlighting molecular associations), on kinetic and energy parameters.
4. This paper highlights the maximum degree of acid hydrolysis of sucrose.

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Section IV

Economics and Management in the Food Industry



BRANDING MOLDAVIAN WINES

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Abstract: The concept of Wine Brand Image is the national wine entity's meaning, or, in other words, "what the national wine entity stands for" in the mind of consumers. According to the brand image model, brand image might be determined by the following elements: features, attributes, benefits, emotional imagery, experience imagery and consumer imagery. All of the above are distilled into a short phrase that conveys the national wine entity's distinctive meaning, or else, its "competitive positioning". Moldovan wine industry recognizes the need to have a much better and consistent promotion on the target markets. Since the Russian market is not permanently accessible, the producers think about approaching other target markets while using a more classical approach. This article contains some basic ideas of a possible scenario for Moldova to develop and promote wine industry country brand. These ideas are collected from the experience of other countries and from expert's opinion about the possible scenario.

Keywords: Wine, brand, branding, marketing

Introduction

Everyone recognizes that for successful sales on modern market a quality product and aggressive marketing is needed. After a period of consistent and correct communication accompanied by a competitive offer of quality wines, Moldova shall get a positive image and shall be associated with good wines. From that point in time the industry and the country will obtain benefits from its branding efforts.

A positive image should be based on 3 equally important principles: consistent product quality; original and attractive visual identity; strong and correct brand management.

In order to realize this result a series of activities should be undertaken and all those activities should be consulted and approved by the private and public sector players. This public-private process and the success depend on the abilities of both sides to tackle complex issues and cooperate in new policies implementation.

Positioning and quality standards

The objective of this component is get a clear vision about which Moldovan wines we want to promote and what are the differentiating factors of Moldovan wines in the world competition. We need to be clear about the (quality&price) positioning of our wines on the market and also set the minimum quality standards of wine awarded the right to use the MARK. Moldova produces a large variety of wines in terms of type, varieties, style and in terms of quality as well. In the worldwide wine offer Moldova plays a minor role, traditionally having a more important one in the regional market (Eastern Europe). The style and quality of Moldovan wines is a result of many factors such as climate conditions, technologic & technical aspects of the grape-growing and wine production, education of the workers and specialists and some others. Moreover due to insufficient funds for active promotion of target markets Moldovan producers sell wines at lower prices and the wines

position is quite low in terms of shelf price. In consequence the producers try to reduce production costs to reach the requested price and the quality of wines is decreasing even more.

Based on the above mentioned it is necessary to choose a segment of Moldovan wines that could not be largely representative today, but is reachable by the majority of wine producers in the following 2-3 years. Many experts consider that today Moldovan wines have to be positioned at EU retail price of 4,99 Euro and higher with a minimum ex works price of 1,3 Euro per bottle. For some markets with lower margins, the ex-cellar price could be higher. Higher quality exclusive and limited edition wines can obviously go higher in shelf price. The 4,99 segment represents apr. 40 % of the EU wine market. It's an average price and can stimulate the new consumer to buy and try our wines. In order to benchmark the quality and desired positioning of Moldovan wines it is better to orient towards wines from Spain and Italy (under 5 Euro retail price) and (5-10 Euro for higher level wines).

French wines have a different concept and even 5-10 Euro retail price level do not possess smoothness and maturation. Australia sells very good wines at a low price in Germany. E.g. Linda Lee Merlot 2009 and Shiraz 2010 sold in Germany at 3,99 Euro. Wines are kept in steel tanks with wooden chips.

Moldovan wines of higher level (2-4 Euro ex-cellar price) are very similar in style with Californian wines which are sold at 30-50 USD retails price in USA, e.g. Shafer Vineyards Merlot (48 USD retail price) and Robert Mondavi Winery Cabernet Sauvignon (45 USD retail price). Moldova wines shall compete with wines from Spain, Australia and Chile and not California, South Africa and France. In addition to that Moldova needs to find its differentiating as countries have. For example: France worldwide awareness and prestige (Bordeaux, Champagne, New World countries rich fruity flavor and taste, intense color (Malbec, Pinotage, Zimfandele etc, Germany fresh and light (Riesling). Some ideas for differentiation of Moldova would be: a) to produce and promote Rose wines (very popular in summer spring); b) to make blends with very aromatic varieties (Traminer, Muscat etc.).

In any case the right to use the Mark will be awarded only to the wines that possess a certain level of quality. And this level will be defined by two exercises:

1. Survey of the existing wines in Moldova. The WG will need to do an analysis of the entire commercialized / bottled wine stock of Moldova and to come up with a clear picture about the volumes, types of wines, vintages, varieties and all major characteristics such as alcohol level, acidity, etc. Would be good to have an organoleptic tasting of those wines with an unified system, but that might be too difficult, instead random sample can be drawn and a large tasting organized.
2. Tasting and chemical analyses of direct competitors of Moldovan wines (Benchmark wines). Since information from the market indicates that Moldovan wines need to be prices minimum at 1,3 Euro/bottle ex-works and reach about 5 Euro retail price on EU market , the WG will select the same segment competing wines (including best sellers) and will make a chemical analysis and also a organoleptic testing. In order to be sure about the selected price segment, samples of wines from a higher and from lower segments will be analyzed as well. Based on the collected data a draft technical specification will be developed.

Having the clear picture about the existing wines and the target segment best sellers the WG will agree the minimum quality standards for Moldovan wines under the new Mark. The consultants suppose that about 50-60 % of actual Moldovan wines will be able

to use the Mark. Those are “healthy wines with correct flavor and taste, with malic-lactic fermentation processed (for reds). The red and white wines will not have oxidative signs and will possess a minimum alcohol level of 11%.

Logo and the visual identity

A brand consists of the visual identity (1%) and the associations in consumers’ mind (99%) about the characteristics of a product. In order to create a brand, a product shall be in place and its characteristics have to be communicated in a very consistent way. A brand is the result of a consistent and permanent communication along with a consistent product quality.

A comprehensive and diversified communication strategy will be developed in order to promote the new brand and the products associated with it. For this the brand needs a strong background. The best is to show the long history of winemaking in this country. Moldova can promote its history of Roman Empire culture of 3000 years, where winemaking was performing, but Georgia already promotes in an aggressive way its “7000 years of winemaking” and Moldova will not be original then.

Moldovan could promote its legend about the white stork carrying grapes for defenders of a fortress during the 15th century wars with Ottoman Empire. This legend and the developed logo about it has been used before by Moldovan wine industry, but might be necessary to get a “face-lifting” and a new modern design.

Since Moldova will be promoted a wine producing country together with its wines, the new Mark has to contain a name and a slogan. It could be “Wines of Moldova / Vin de Moldova” and slogan might be developed. Preliminary options are “Try something different” or “Quality Driven” or some others. Develop a story and strong visual identity with a message like “try something different” that could be changes after a few year periods to a stronger one like “Quality driven”.

Marketing and promotion

Developing and Implementing a promotion strategy at national level requires an integrated policy and all stakeholders must possess the ability to act jointly and repeatedly communicate the same message. National branding initiative is not an advertising campaign or marketing effort in the short term. This is a long-term strategic effort which will show some benefits over five years, and the greatest benefits may arise during the 15-20 years. Major benefits of the branding and promotion are:

- The increased awareness of the Moldovan wines in the target markets.
- Moldovan wines will have a clear picture and will be association with medium and higher quality wines from an Eastern European country and have some specific characteristics.
- The consumer will get an additional insurance of the quality of Wines of Moldova.
- The joint promotional efforts create strong impact and obtain visibility.
- After a period of successful promotion the entire industry will benefit of the image while entering the market pr expanding in the target market.

The strategy may include:

- Creating stories and messages around the brand and key statements to inform journalists and society to ensure consistent use of the brand.
- Application of logo on wine bottle after an established process for approving these wines

- Production of promotional and informational materials and equipment of all embassies and representations in international communication materials.
- Production of short film (30 seconds) with the introduction of the brand's logotype that can be subsequently broadcasted on TV, as well as during certain events.
- Production of souvenirs, insignia, key-rigs etc., which may be marketed and used as gifts for officials, tourists, participants in certain forums, etc.
- Promotion of the brand in the municipal infrastructure in Moldova. The ultimate goal is that after you receive a greater notoriety in Moldova will be easier to brand promoted by each citizen individually.
- Creation of a web-page on the brand that incorporates all formats and contents related to the brand.
- Ensuring the placement of articles about Republic of Moldova in the international media.
- Establishment and use of the title of Moldovan Wine Ambassador
- Promotion of the brand within international in Moldova events such as: Economic Forums and sport events (international football matches, etc.)
- Motivating the Moldovan diplomats to draw attention to the country, including through the brand promotion techniques.

The marketing and communication strategy will be oriented towards the most appropriate target countries and can be different for certain markets, depending on consumer's culture and habits. Specifically for wines the efforts will be oriented to Western and Eastern Europe, North America and Asia. Actually does not make sense to invest in promotion in Russia, since the image of Moldovan wine is still strongly negative.

Funding and finance administration

A primary advantage of the newly established body is the commitment of the government to channel through it all state funds dedicated to wine promotion. It will allow the sector to implement and coordinate a single policy. The state money will not be the only source of funding. The other two sources can be the levies paid by the participating companies (or even all companies). The level of levies has to be decided, and will be set by each company depending on the number of bottles sold under the new brand. Generally, the experts propose to use several options of funding that depends on the selected institution's form:

Option1.

- The funding members will pay fixed membership fees;
- The producers will pay levies depending on the volume or value of wine sold;
- The collection method is still to be decided.

Option 2

- The funding members will pay fixed membership fees;
- All producers will pay levies depending on the volume or value of wine and brandy sold;
- Ministry of finance will collect the levied through its fiscal system;
- The sources will be directed to Wine Export Promo Fund and will be administered by a PP council;
- The system will be similar to the "viticulture revitalization tax";
- Suggested tax 0,2 MLD/dl of wine and other products.

In any cases the funds have to be administered by a council with equal representation of private and public sector.

Institution and management control

The institution with functions of management and control over the Moldovan wine country brand need to respond to the following requirements:

- Sustainability: sustainable management and institution will maintain and contribute to wine country brand success;
- Professionalism: the management of wine brands requires professional skills and marketing capacities;
- Adaptability and flexibility: promotion activities and instruments should respond to the market developments and changes;
- Transparency and visibility: all stakeholders should have access to information, budget use, rule defining and decision making.
- Impartiality and objectivity: The institution's control function requires a high level of confidence from stakeholders.

For the management of the wine country brand, three options can be considered:

I. Non-Governmental Organization "Association Wine of Moldova", (AWM) instituted accordingly to the Law on associations nr. 837/1996*:

- All interested wine associations and companies can be members to the NGO. The structure: General Assembly, Administration Council, Executive Director and a President.
- The AWM will register the trademark "Wine of Moldova" and agree on the technical specifications for wine sold under the brand. Or the MAFI could register the trademark and transmit it for use to AWM.
- The procedures of respect of the technical specifications will be adopted by common agreement by AWM members and controlled internally.
- AWM fund will be constituted by: membership fees, taxes for services, other private and public funds.
- Based on AWM's promotion plan/strategy/actions the Ministry of Agriculture and Food Industry (MAFI) could allocate public resources for wine promotion.

*This model inspired of NZ, WOSA, Pro Chile presents issues of sustainability. MAFI could ignore at a moment this NGO, in case of appearance of other associations. The same role could be assumed by the already existing Moldovan Wine Guild.

II. Inter-professional Wine Organization (IWO) "Wine of Moldova" – created accordingly to the Law 257/2006 on Organization and Functioning of Agricultural and Food Product Markets**:

- All wine associations, wine companies could be members of the IWO. The proposed structure: General Assembly, Administration Council, Executive Director and a President.
- Accordingly to the Law 257/2006, MAFI will recognize the IWO as the representative association of the wine industry.
- IWO will represent the "unique voice" of the wine sector. One of the components of functional structure will be the "Wine of Moldova" Council, being actually the Council for Promotion.

- IWO will be founded by membership fees, taxes for services, other private and public funds.
- MAFI could create a National Promotion Fund for Moldovan Wines, from levies.
- The brand “Wine of Moldova” could be registered by the MAFI and contracted to IWO for use.

**The Inter-profession functions are broader than promotion. The Inter-profession is a complex structure and requires a good management. MAFI should make some precisions in legislation concerning what is to be considered to be “the representative Inter-professional organization” (i.e. 30% of the sector by volume and members). MAFI should engage to recognize and work only with the representative Inter-profession.

III Public-private institution “Wine of Moldova”(PPI) – created by a decision of the Government or by Law. PPI will have the statute of a public institution, with financial autonomy and have competencies in wine sector***:

- The structure of PPI: an executive director appointed by the MAFI, a council of directors from private sector, a president named by the council of directors. The executive director will be responsible to MAFI and shall coordinate the activities with the council of directors.
- MAFI will allocate the country brand and Promotion Fund to be managed by the PPI.
- The PPI could have broader functions than promotion.

***This model presents more public influence. There isn't any other institution alike in Moldova, so some legal problems could interfere.

Options for ownership of the country brand:

- a) MAFI registers the brand and lease it to the specialized institution of promotion.
- b) The institution for promotion registers the wine country brand.

Intellectual Property right type:

- Individual trademark: verbal and figurative mark will be registered, without any conditions of use. The condition of use will be regulated by a normative act or an internal regulation of use.
- Collective trademark: can be registered by associations of producers from wine sector, and also public entities. The regulation of collective marks' use is presented to AGEPI and published in AGEPI gazette.
- Certification trademark: can be registered only by certification authorities authorized to certify the compliance of goods or services in accordance with Law No. 186-XV on the Evaluation of Compliance of Goods of April 24, 2003. The regulation of use will be published.

Regulation of utilization

The country brand isn't just a logo or a trademark; it is a complex program of promotion. Therefore, a Program of wine promotion through the country brand “Wine of Moldova” should be elaborated and approved, as a major activity of the national strategy of promotion of Moldovan wines. The Program “Wine of Moldova” will contain the major activities to which the registered companies will be entitled to participate, promotional and marketing materials available, conditions of use of the country brand (logo and attributes) on products. In order to have the right to benefit from the Program, the companies should register to the Program and pay a membership fee annually.

The right to use the brand on products will be regulated by Decision of Government (technical regulation) or by a regulation of utilization as per collective or certification trademark. The regulation of utilization of collective or certification trademark should stipulate: conditions of use, procedures of certification, Royalties paid for use of the brand, sanctions, and ways of control over the utilization of the trademark.

The procedure of certification of the wine with rights to use the country brand will be designed accordingly to the type of the trademark. The compliance of wines to the standards of "Wine of Moldova" will include physic-chemical and organoleptic exams. In case of a certification trademark the control of the compliance with the regulation of use will be attested by the certification body. In case of a collective or individual trademark, the certification to "Wines of Moldova" standards of wines will be decided by the IWO/PPI based on the physic-chemical tests realized by the attested laboratories and organoleptic notes given by the Tasting commission of the institution which will administer the country brand.

The wines having the appropriate authorization of GI/DPO use will be exempted from the certification accordingly to "Wine of Moldova" standard, but still will have to be registered to the "Wine of Moldova Program".

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INCREASING OF ECO-EFFICIENCY IN FOOD INDUSTRY BY PROCESSES REENGINEERING

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Abstract: Contemporary, dynamic and global world created a series of modern challenges for economics and management of the food industry enterprises. The relationship between aspiration for bigger and faster profits, values of the subject, usefulness and safety of the food products becomes more dynamic. The balance in world economics is already broken and it is not in interests in food quality and safety, community and environment. In such a situation, with exceptionally high business organizations, burden is the application of processes reengineering. It is because the subject values are redefined by the models, systems and instruments of reengineering methodology. The focus of company activities is directed towards the community and environment benefits for solving the environmental problems related to food industry.

Keywords: food industry, eco-efficiency, management, reengineering, sustainability

Introduction

What decision to take the creative entrepreneur in the food industry now is converted to a main question? The investigations reveal that the choice is directly related to processes reengineering. The complex reengineering of business organizations holds out to entrepreneurs and managers four possible action fields – competition, value innovation, divergence and convergence [4]. Characteristics and potential of every one of these fields are shown in many previous researches [4, 5, 6].

Processing industry, including food processing, plays prominent part of Bulgarian economy. Increasing of production rates leads to strengthening of harmful environmental impact. Ecological problems of food industry are related to generation of specific and various wastewaters, big quantity of organic wastes, great raw materials, water and energy resources consumption, using of various packing materials and generation of packing wastes [8, 10].

The idea of eco-efficiency is relevant to production of food and services by the use of minimum natural resources with minimum environmental impacts (e.g. creating more with less). This is one practical and systematic approach which the business can adapt to its action in order to increase benefits for the environment and for the business organizations. Applying of eco-efficiency conception and methods for its assessment is relevant to sustainable development of society. There are many assessment approaches for evaluation of eco-efficiency described in the literature [2]. However, the uniform approach is missing. This is because of two reasons. The first one concerns the environmental requirements which are set on the input and are in any case more or less populist and/or contradictory to the subject's values. The second reason includes data which are used on the output but concern mostly economic values for the production instead of benefits for the environment and society as a whole. According to complex reengineering concept eco-efficiency of the production is a function of values for the society, environment and business organizations and also the values as a result from strategic eco-orientation, integration of environmental

effects, and investments for realization of definite production scenario of separate or complex environmental activities. In conformity with the reengineering philosophy efficiency of the separate environmental activity has to be measured by degree of increasing of incomes and values for the environment and society received from production eco-intervention divided to costs of this intervention [5].

The European Eco-efficiency Initiative and initiative of the World Business Council for Sustainable Development in collaboration with European Commission set the goal for integration of eco-efficiency to European business and to industrial economic policies of the EU. In 2011 has been introduced the resource-efficient Europe – Flagship initiative under the Europe 2020 Strategy.

The necessity of entire binding of environmental and economic thinking in the separate companies including from the food industry is clearly outlined in order to passing to greater energy and resources efficiency. In this relation on the base of concept “4E” (Equity, Efficiency, Environment, Ethics) eco-efficiency model for corporative management of Bulgarian business organizations is developed. This model includes implementation of value system, system for resource planning, sustainable and balancing system of indicators for efficiency assessment, codex of corporative sustainability, reengineering of strategy and making of “4E” decisions [1].

The idea for processes reengineering as a tool for realization of great benefits for the business and for the environment is developed with exceptionally intensive rates during the last ten years. It is considered that this approach is not useful for the companies which want only 10 % of growth but for these which strive for ten times more growth. There are different methodologies for reengineering of business processes reported in the literature. In 1999 consolidated methodology was presented which can be applied successfully from the business [9]. During the period from 1996 to 2012 in Bulgaria have been published a lots of researches related to reengineering approach. It starts to reveal the interdependence between the processes of strategic orientation, efficiency of the strategic management, internationalization, management of the innovations and of the risk and eco-efficiency [4, 5, 6].

All mentioned above show the necessity of reorganization and restructuring of the business processes in the food industry starting from the input flows across basic production processes and finally to distribution and marketing of the food product.

The main goal of this study is investigation of the possibilities for application of the processes reengineering concept in the food industry enterprises in order to increase their eco-efficiency.

Framework model for reengineering in the food industry

Relation of indexes “industrialization-environment” requires solution of series of problems connected with environmental management of enterprises. First of all these are the problems related to values and values program of the subject. Food production is developed in the frame of three main indexes: value system of the subject, striving for bigger and faster profits and food usefulness and safety. According to complex reengineering the point of intersection of inventories and the situation as combination of energy and information defines strategic orientation of each of these activities and variants in the space of business and consumption. Interaction of these activities and variants of business forms the image of the contemporary market economy in the food industry, which defines variety of relationships, interests, goods and food products. This interaction is

unthinkable without reengineering of the ecology in food industry by which more persistently is searched for answers of the following questions: What is the role of energetic for eco-efficiency in the food industry? Which are the sources and possibilities for energy production and what is the correlation between energy supply and demand? To whom, what type is the energy and how to produce it? How to ensure healthy and safety food? Which are the basic sources of environmental pollution? What are the reasons for environmental pollution, mainly by organic substances and solid particles? Which are the problems of the environmental management of global chain for supply and demand of food products?

According to data from Bulgarian National Statistics Institute part of mining and processing industries towards the total gross domestic product for 2011 is 21,2 % and part of the food industry is 2,6 %. Part of generated food processing wastewaters towards total industrial wastewaters is 10,73 % and towards total discharged wastewaters for the country is 3,99 %. Generated solid wastes from the food industry towards total wastes is 0,17 %. In the same time total emissions of industrial toxic substances are about 4016284 t, from which 88,7 % are CO₂ emissions. Energy consumption for receiving of raw materials in Bulgarian food industry per unit product is about 1,8 times more than in Turkey and Greece and about 1,5 times more than the average consumption in EU [3]. The main reasons for it are disunion of agricultural massif and farms, high fuel consumption because of old and non-effective equipment used, non-effective logistics, bad infrastructure and non-effective management. All of these data show that the basic ecological problems of food industry in Bulgaria are energy consumption, generation and discharging of wastewaters. The next are generation of solid wastes, fresh water consumption and etc.

Our investigations show that wastewaters from different branches of the food industry in Bulgaria are polluted mainly by different organic substances and suspended solids. In table 1 are presented average results for the basic indexes which define degree of wastewater pollution from some food industry enterprises. The obtained data are in accordance with results published before [10].

According to methodology of reengineering the fragmental approach for improving of environmental management in food industry is non-effective. It is necessary to apply a complex approach where the environmental management is the basic factor for development of food industry. That's why we recommend the use of the complex reengineering model, shown on figure 1. This model ensures that it is necessary to work on the values and values program of the subjects. It is because if some defects have arose in the value orientation of the subject related to realization of benefits for the society and for the environment the following question will be asked: How will be produced the necessary usefulness and safety food and beverages?

Table 1. Characteristics of wastewaters discharged from some industrial food enterprises

Index	Type of enterprise				Standard, mg/dm ³
	Cannery	Slaughterhouse	Meat processing	Milk processing	
COD, mg/dm ³	154.8	354.8	108.0	602.3	70
BOD ₅ , mg/dm ³	67.8	134.6	28.3	312.5	15
SS, mg/dm ³	30.0	90.8	37.0	182.2	50
FOG, mg/dm ³	8.0	4.5	8.0	10.6	3

COD – chemical oxygen demand; BOD₅ – biological oxygen demand for 5 days; SS – suspended solids; FOG – fats, oils and greases.

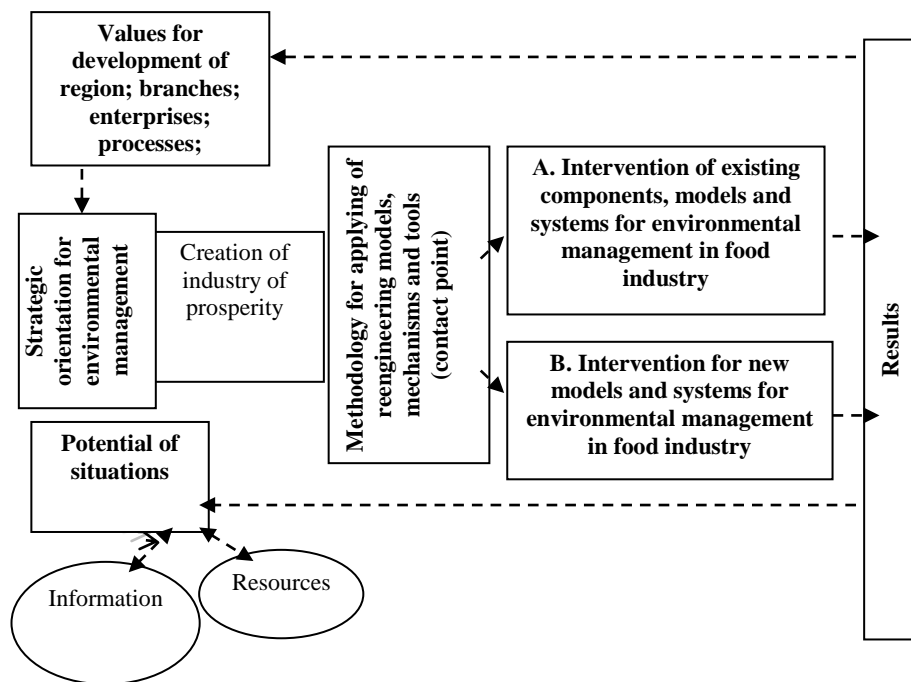


Fig. 1. Framework model of reengineering for development of environmental management in the food industry

That's why the requirements standing in front of business organizations have not to be only considered as well known environmental and food safety standards. The reorientation of the subject values is needed. It is necessary to provide upbringing and education in order to form an environmental culture, necessity and convictions relevant to environmental protection, sustainable development and natural lifestyle. Excluding the values, an important condition for development of the environmental management of food industry is resources and mostly the energy as a key resource. Energy is an essential input to ensure that processes function properly and that food and beverages are safe and can be preserved and stored under controlled condition. While the "hot" processes (e.g. drying, cooking, frying, evaporation, pasteurization, sterilization) rely on natural gas, petroleum derivatives and electricity to a lesser extent, the "cold" processes (e.g. freezing and refrigeration) are almost completely dependent on electricity [8].

It is out of doubt that the energetic is more closely bound to environmental problems, growing consumption, energy resources limitedness, costs for yield and delivery under an increasing of difficulties. Today human civilization depends entirely on electrical energy and fuels. But it still not recognized that the energy is integrated profit which people and organization in which he works obtain continuously from the nature but they do nothing for its increasing. Combustion of classic energy sources (coals, petroleum and

natural gas) emitted more than 20 milliard tones per year CO₂ into the atmosphere which is the reason for greenhouse effect and for global warming. According to some authors [5, 6], toward 1990 in Russia are used up energy resources per unit revenue 11 times more than in Japan, 7,5 times more than in Germany, 6 times more than in Great Britain and 4 times more than in USA. Moreover, it is known that the natural resources usage for productive activities is not efficient. For instance, in U.S. economy, only 7 % of natural resource extracted becomes product. Out of 7 %, only 1 % becomes durable and 6 % will become waste in the first use by customers. In addition, by considering these 1 % of durable materials, only 0,02 % of them are recycled [7]. World food providing and development of food industry provoke increasing of the energy demand while energy production by classic methods is limited. This reveals the most serious problem of the global “supply-demand” chain of energy. Really, increasing the energy price is irreversible and the main load is for consumers by purchasing of food. The rate of incomes growth per household is about 2 times less than the rate of price growth of qualitative and safety food. In order to exist solvent demand manufacturers often produce food with additives which are not nutritive for the humans. This compromise in food production leads to move away from the natural lifestyle and creates problems through the entire food chain. In such a situation one of the ways is production of “green” energy and searching for the possibilities of limiting and saving of energy throughout the global food chain. Undoubtedly, reengineering of the business processes concerning wind and solar energy, remanufacturing and recycling of the wastes in food industry will be the flavour of the next 10 years. It will ensure optimal and effective environmental management of business organizations. The interaction between human and nature will be improved too and the risk of environmental pollution will decrease significantly.

Our study reveals that the energy consumption and energy factors are the keys for movement of two main flows in human civilization: market needs and business needs. These are decisive for the complex index for influence on the consumption rate of food products and services (CPS). That's why we recommend the next formula and scheme (fig. 2) for reengineering expert in food industry.

$$CPS = \sum_{i=1}^n V_i \cdot I_i \cdot E_i \quad (1)$$

where: V_i – key factors of values for the subject;

I_i – key information and technological factors for the business;

E_i – key energy factors for business in food industry.

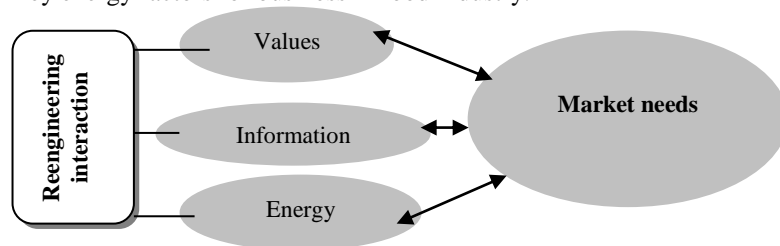


Fig. 2. Scheme for processes reengineering in food industry

In a short-time with the highest load for the business in food industry will be the consequences from energy factors impact and the organization of reengineering.

Conclusion

It is recognized that eco-efficiency in food industry is about inputs (materials and energy) and outputs (product, wastes, emissions) characterizing a production process and whole life-cycle of product. By optimizing these characteristics considerable reductions can be achieved in environmental impacts and costs. It is clear that in order to reach creative development of food industry have to be used reengineering technologies for “green” energy, optimizing the processes, improving of wastewater management, utilization of wastes, reusing and recycling, introducing of eco-efficiency indicators and implementation of environmental management standards such as series of ISO 14000, GS1, ISO 27001 and etc. This study is presenting a suitable model for business processes reengineering in food industry.

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PERSPECTIVES OF INNOVATION DEVELOPMENT OF UKRAINE

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Abstract: An analytical review of the development of innovative production of fat-containing products in Ukraine was research.

Keywords: Development of innovative, fat-containing products.

Introduction

Market economy of the states stimulates development of food industry, extending the assortment of the produced foods and ingredients, reducing energy- and resources consumption, and also reducing prime cost foods. The feature of the post-Soviet independent states is privatizing of food industry enterprises, except the spirit industry enterprises. Privatizing and market from one side awakes interest of proprietors to invest facilities in expansion of high quality food staff production, and from the other side is called to minimize material production inputs. In the market conditions the production of foods is determined by demand. Taking into account that 80 % of Ukrainian populations have minimum profits now, demand for cheap food increases [1].

We will consider development of production of food staffs on the example of fat-containing foods: dairy butter and vegetable oil.

The tendency of dairy farm reduction is preserved in Ukraine, and also the milk production is being reduced and as a result the production of dairy butter is being reduced. Milk has maximal production volumes in May-July, although the tendency to the declination of volumes on the whole is saved. Also marked influence is rendered by the state of affairs of dairies world market, in particular price declining for fat free dried milk and casein, as a result of which profitability of dairy butter production is being reduced.

Results and Discussion

In 2008 84,8 thousand t of dairy butter was produced in Ukraine, in 2009 - 74,7 thousand t, and in 2010 - 79,5 thousand t. Cost of ton of butter today is about 45 thousand UAH.

Milk is processed on productions directions as follows:

- whole milk products: pasteurized or sterilized drinking milk, baked milk kefir, curdled milk, yoghurt.
- dairy butter, casein, fat free dried milk, cream, sour cream;
- cheeses: curd, cheese, melted cheese.

As the for dairy butter price grows, and production of its volumes reduce steadily, then producers willingly substitute part of animal fats by vegetable oil:sunflower-seed refined oil, refined palm-oil, hydrated fat [2]. The price of ton of the refined sunflower-seed oil is about 14 thousand UAH. the Usage of hydrated fats in compounding of fat-containing product, consisting of mixture of fat and oil which called spread, allows to reproduce

organoleptic properties of natural dairy butter, even if in a product it is only 5 % fat of animal origin (natural dairy butter). Such replacement of fats can bring high profitability to the producers and to stimulate falsification of natural dairy butter. Educing the fact of falsification is possible, finding out the presence of nickel in a product which is used as a catalyst at hydrogenizing. Raw material for the production of hydrated fat is the refined vegetable oil mainly sunflower or rape. In Ukraine basic oil-bearing cultures for the production of vegetable oils are a sunflower, rape and soy. A sunflower occupies leading position, having the middle productivity of 15,0 c/ha. Rape is the most productive culture (20,8 c/ha), but the presence of limitations on chemical fatty-acid composition of oil and agro technical terms of growing slows his industrial till and processing. Soy, having the productivity of 16,8 c/ha, demonstrates the absolute increase of sowing areas and volumes of processing on a soy oil and feed schrot.

The major part of sunflower seed is processed in Ukraine, while a half the taken crop of soy and almost all seed of rape are exported. Processing of sunflower into vegetable oil with every year grows and market saturations foods of production are not yet reached. From 2008 power of oil-bearing cultures processing enterprises is being intensively increasing. In the south of Ukraine in Mykolaiv, Odesa, Zaporizhzhya, Poltava and Vinnytsya regions, foreign investors extend processing of oil-bearing cultures and build new oil-extracting plants.

Major part of produced sunflower-seed oil is exported in countries the CIS and countries of distant foreignness. Part of vegetable oil, which remains for an internal consumption, is being sent for hydrogenizing and margarine producing, and a remaining amount enters retail network and goes to the production of mayonnaise. Negligible quantity – 17-25 % from produced - margarine export. The basic component of margarine is hydrated fat which in margarine is about 98 %. Mainly margarine and hydrated fat is used by pastry, bakery, milk processing enterprises and enterprises of public food consumption and restaurant business. A spread is mixture of fat and oil, namely dairy butter and hydrated fat or margarine - appeared at the market in the beginning of 90th in the imported execution under the name easy oil, not containing cholesterol. Spreads had and have the marketing niche, as profits of population are low. Increase of average monthly available profit calculating on one man in 2008 and 2009 years became slower, that it is constrained, presumably, with a world crisis. Diminishing of profits a population entailed increase of demand on cheap food staffs.

In 2009 in a suburb South to the Odesa region (Novi Belyaru) there was built and put into an operation plant for processing of tropical oils on an investment from Malaysia and India. Through the Yuzhnii port in 2010 was imported 280 thousand t of palm-oil which is refined and fractionate on liquid olein faction and hard stearin. Stearin faction is used as a substitute of dairy butter in fat-containing foods. Educing such falsification of dairy butter is difficult, as a nickel in such product is not present, but fatty-acid composition substantially differs from a natural dairy butter. An import to Ukraine and export from Ukraine of tropical fats is presented on a fig. 1. Ukraine exports factions of palm-oil also, thus 80,8 % Russia buys in from a general volume. In 2011 the import of tropical oils shorted, especially palm-oil. It, presumably, it is related to the increase of prices for tropical oils and building of similar enterprises in Russia, in particular in Krasnodar.

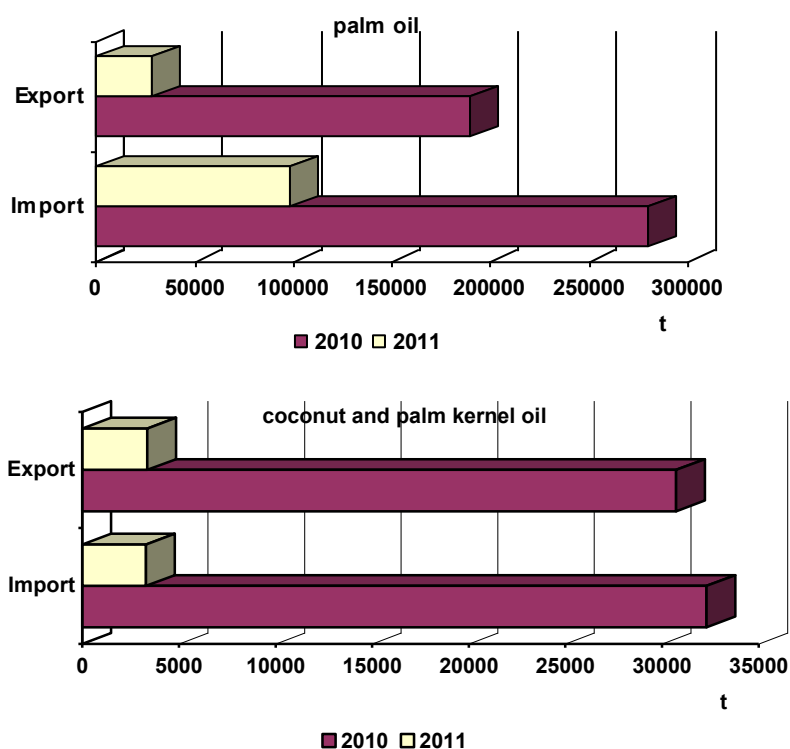


Fig. 1. Import and export by Ukraine of tropical oils in 2010 and 2011

Increasing areas under growing of oil-bearing cultures and extending the production of the vegetable refined oil, in 2010-2011 marketing year Ukraine gained the leading position in the world rating of producer of sunflower-seed oil (fig. 2) as evaluated by Ministry of agriculture of the USA.

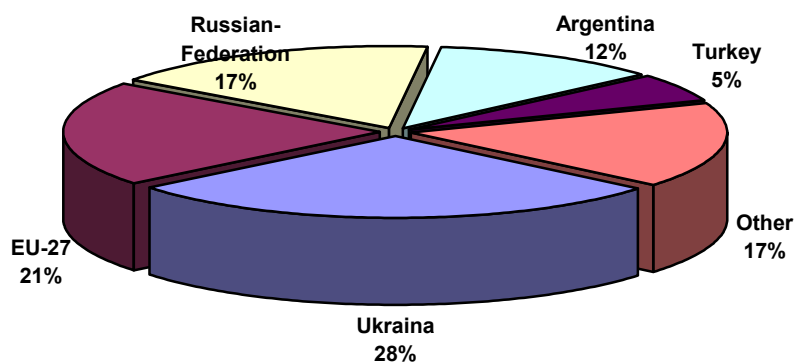


Fig. 2. There is the world rating of producers of sunflower-seed oil in 2011

Research is executed within the framework of scientifically research work of State |Organization "Institute for Economics and Forecasting, Ukrainian national academy of sciences" on the topic: "Structural transformations in the economy of Ukraine: dynamics, contradictions and influence on economic development".

Conclusions

Expansion and innovative development of powers on the production of fat-containing foods, introduction of new technologies of production and processing of vegetable oils allowed Ukraine to occupy leading positions in the world market of vegetable sunflower-seed oil. Expansion of oil production and reduction of fat go in the conditions of market. The production of foods of stock-raising must be supported at state level, entering grants from the state budget or giving to the producers of preferences and privilege at legislative level.

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PROMOTING MOLDAVIAN WINES ON INTERNATIONAL MARKETS AND DEVELOPMENT OF A NATIONAL BRAND

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Abstract: Moldovan wine industry has a long history, however it is currently in a very difficult position. In order to comply with the international standards several steps must be undertaken, starting from manufacturing process and ending with the marketing strategy.

In the article below we would outline some of the major problems of the wine sector, as well as offer solutions for properly branding moldovan wines in order to gain substantial shares of international markets.

Keywords: wine industry, marketing strategy, branding, international markets.

Historical overview

Winemaking in Moldova is a century old, interesting practice. It starts 4000 years ago, when the Dacs started to make wine from local grape varieties. In the second half of XIX century, European grape varieties have been brought into the country, thus determining the development of this industry. In XX century a series of events took place, which brought Moldova to the current situation

Starting 1991, once Moldova has gained its' independence, the wine industry started to flourish; most of the wineries were privatized. New owners started investing in modern equipment. Between 2000-2005, when a lot of companies decided to plant European clones, major capital was injected into the vineyards, and sales to Russia were a key factor for the continuous development of the sector. During this period, because the major market share was dedicated to Russia, not much attention was being paid to developing international markets. The local market also had a certain influence on most of the manufacturers, because making wine in home conditions is a vastly spread practice in Moldova, which determines the decrease of sales of bottled wine, but increase in wine consumption.

However the Russian Embargo on Moldovan wines and the 2006 agriculture, almost destroyed the industry, which currently is in a new rehabilitation phase. Being deprived by its major market, the industry significantly decreased, and the number of wineries has dropped considerably, as well as the vineyards surface. Today, the Moldovan Wine industry is slowly developing its' exports on international markets. However it is limited by a number of important factors such as high interest rates, global competition, lack of market experience, aroma profiles, the segmentation of the sector and little governmental support.

However the wine sector in Moldova, still continues to be a very important one for the national economy. During the last 5 years the number of wine companies reduced dramatically, from over 120 enterprises to approximately 50, and only around 30 of these are currently producing wines. In order for these wineries to be able to properly conduct and develop their businesses, a series of evaluations, permanent analysis, as well as programs of financial and governmental support are necessary.

Even if the sector is still fragmented, most companies have positive elements which can be used and developed to create a strong national brand.

Viticulture is an aspect that needs improvement, expertise and permanent diversification. The soil types in Moldova, determine a strong vigor of the vines, and in order to control this aspect it's necessary to work the "crown" of the vine. In the vineyards where the vine is directed and raised, the fruit is exposed and the vine shoots flex, a better wine quality has been observed.

The high acidity in the wine is also a problem for Moldova, but it can be reduced by viticultural techniques. Using adequate viticultural practices will decrease the sprinkling regime, which would later lead to improvements.

The soviet techniques of using too much pesticides, herbicides and fertilizers depleted the soil and considerably damaged the underground waters. Further degradation of the surface level of the soil due to the processing methods, predisposed land to erosion. In some cases the vineyards are abandoned or not properly taken care of, which represents a danger of contamination the nearby plantations. But there is also a positive combination of European and local grape varieties, several plantations have to be evaluated, in order to establish a balanced ration between the white and the red grapes.

Vinification is another aspect which needs a thorough approach. Although there was a certain transfer of international winemaking technologies, there still are companies which use obsolete techniques. The financing aspect is a major problem for most of the wineries, however many winemaking techniques can be applied by using the existing equipment at virtually no cost. The industry has to be as diversified as possible in order to increase the wine quality and minimize the harvest gaps form year to year. In many cases, processing high volumes, leads to losing some high quality wine lots. It is necessary for the grape processing to be done in lots, by individual vine parcels. As well, a lot of companies have to re-evaluate the logistical matters of the processing.

Marketing Moldovan wines on most of the export markets, still is in a incipient phase. Through gaining access to international markets, there is an extraordinary opportunity of growth and development.

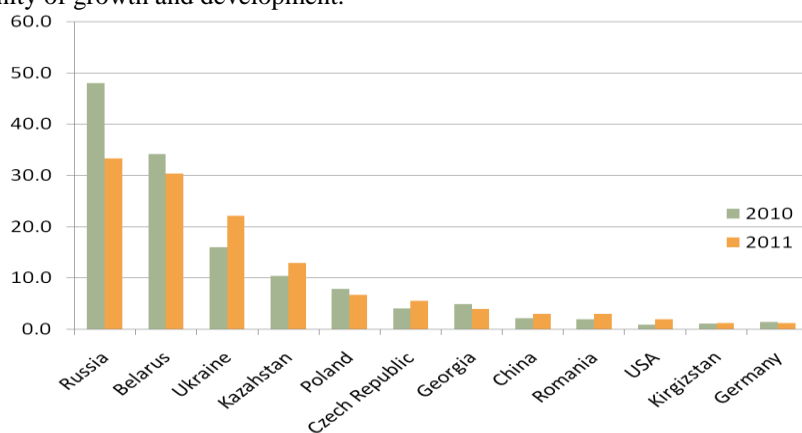


Fig. 1 Moldovan Wine Export on international market.

Source: Ministry of Agriculture and Food Industry

The Moldovan wines are basically unknown on international markets. The lack of recognition requires the adequate education of consumers regarding the quality and properties of Moldovan wine styles, because there is no preconception and the customers

crave more information. A lot of Moldovan wines are suitable for various international markets.

Bulk wines are an important part of Moldovan Wine Industry, especially on traditional Markets such as Belarussia, where the bottled wine export is complicated.

If adopted to the international markets, this strategy would only depreciate Moldova, as wine category, that is why bulk wine sales have to be restricted, otherwise local wine would be only a convenient low priced merchandise, compared to big wine producers such as Australia, Chile, South Africa.

It is absolutely necessary to create a national institution, which would be responsible for promoting Moldovan Wines, with both private and governmental support. This would be a permanent problem as long as the legal bureaucracy in the sector is restrictive and difficult. As mentioned above, financing is a major problem and the auto-financing capacity of the sector is low.

Expansion on international wine market

The main priorities should be as it follows:

- Establishing a Moldovan wine series that would promote the individuality and quality aspects of Moldovan wine products.
- Finding the right distributors for local wines.
- Educating and informing the business environment, the media and the costumers about Moldovan Wines
- Identifying an interesting market segment for wine industry development.
- Increasing the sales volumes of bottled wines.

The focal point and the most important aspect in developing a new export market for Republic of Moldova is creating a high added value for the brand. Because the brand's success is determined by customers' interpretation of the brand the advantages of a high value are the following:

- The wine can be sold at a higher price, compared to the competition, because the brand will be perceived as an indicator of higher quality.
- Capitalize on the relationships with the distributors and retail commercial networks, because the clients will demand access.
- Offers protection against price dropping
- Can easily be expanded, because the brand has credibility
- In case of joint marketing campaigns, the costs are considerably reduced.

Developing a National Brand

Developing a brand is a very difficult process, given the complexity of the markets, the consumers' mindset etc.

It is a well-known fact that the countries that are successful on target markets, have a sector institution in charge of the wine sector. In France, for example, one of the biggest wine exporters, this institution is Sopexa, while in Australia - Australian Wine and Brandy Corporation, which manage programs such as Wine Australia.

Moldova has to create an institution which will be able to act on behalf of the entire sector, and will have impact and authority. One of the goals of this institution would be deciding upon a wine series that will:

- Promote unicity and quality aspects of Moldovan Wine Industry.
- Find adequate distributors for local wineries.

- To educate and inform business environment, customers and media about the Moldovan wines.
- To capture an interesting market sector for wine industry development
- To increase the bottled wine sales by min 10% yearly.

In order to achieve these goals, this institution has to:

- Establish a generally accepted standard for the wines which are to be exported, which would comply with international quality standards, to create recognition of the wine quality, to respect the demands regarding labeling and other legal norms.
- To monitor the manufacturing process of the wines destined for export, in order to insure the compliance with quantifiable parameters which would reflect the wine quality.
- To monitor and publish statistical data on an yearly basis, related to winemaking and viticulture
- To collect a certain budget for the export activities, through volume based taxes and to attract foreign funds from projects such as USAID/CEED II
- To insure presence on target markets, as well as locally.
- To recommend a standard accepted by the sector for all the wine types., which would allow marketing under the “Approval Seal”. This would create recognition of the country and the Wine Moldova brand.

The full traceability of wines and implementing an integrity program of the brand are imperative for the sector, hence it is necessary and essential in order to insure the compliance with the quality standards.

The educational message which has to be conveyed by “Wine Moldova” to international markets, has to be controlled by the sector for the sector, in order to achieve the best possible result for the industry. It is necessary to set clear goals, that would later be revised, in order to insure a proper basis for the next step in gaining more market shares.

These goals have to be evaluated from traditional perspectives, such as:

- Increasing sales and export volumes
- Creating brand loyalty and increasing sales under the respective brand.
- Creating brand awareness a

Following these steps, as well as implementing the above mentioned strategies we can create a success story of Moldovan wines.

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ANALYSIS OF PUBLIC TRANSPORT IN CHISINAU

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Abstract: At the present stage of urban development, one of the main tasks is to create a safe system of public transport, one that is affordable, economical, reliable and environmentally friendly. The important role of passengers in the city transport economy and in the realisation of the social services dictates the need for harmonious, balanced and effective measures in this domain.

Analyzing the public transport in Chisinau and its organization, we will notice the progress and stagnation, so we will be knowing how to develop investment strategies in the public transport and measures of reducing the exploitation expenses for means of transport, and particularly the optimization of public transport, in result increasing the quality of the services for the passengers.

Keywords: public transport, urban passenger flow, transport agents, average driven distance, public transport fleet, routes, road infrastructure, quality index.

The urban transport is an ample, dynamic and live mechanism. Therefore all the problems and initiations for perfecting and reorganization can be examined only in terms of a systemic approach that is applied only per total in practice.

The main problems of urban transport in Chisinau are directly or indirectly related to the efficient use of energy resources.

Radical transformation processes of the economic and social spheres, the formation of product markets and services and the early integration of the country in the global economic system is unthinkable without creating a reliable system of operational, economic, safe and environmentally friendly transport, oriented towards satisfying the interests of the citizen, entrepreneurs, markets and the entire society.

It should be recognized that in the present moment the transport system of Republic of Moldova does not fulfill these requirements. In this area it still needs changes and capital investments. Tariff liberalization, the price growth of all types of energy resources, the increasement of private and independent transport agents, that are operating as small companies and also other factors have caused the formation and development of the current transport market .

World experience shows that such urban issues as:

- Ensuring the human rights of mobility and supporting the vulnerable groups of society
- Releasing the town center of private transport;
- Ecological and others

Prioritary can be solved through the municipal urban transport .

In recent years, the general mobility of the population increased due to:

- a) the growth of the municipality's territory;
- b) the increasing of the average driven distance;
- c) the city population growth;
- d) the increasing number of private cars;

The system of urban transport management in Chisinau has the following organizational structure as shown in Figure 2.

In Chisinau the passenger transportation services are done by the city companies "Regia transport electric" and "Parcul urban de autobuze" and other 19 agents transporters - administrators of buses and minibuses

Chisinau's passenger stream is served by 22 trolleybus routes, 27 bus routes (including 4 private routes) and 67 microbus routes. The average daily output on the routes of the municipality are: 258 trolleybus, 118 high capacity buses (including 25 private), 1840 minibuses. (Fig. 1)

In the base of the study of passenger's flow was established a program of basic routes for trolleybuses: no. 1, 4, 5, 7, 8, 10, 13, 17, 20, 21, 22, 24, 25, 28 and 29 acting up till 22.30 - 23.00 and secondary routes no. 2, 3, 9, 14, 16, 23 and 26 trolleybuses circulation is set up till 20.00 because after each hour the passenger flow is reduced essentially and the capacity of the trolleybus is used ineffective. The activity of buses and minibuses route is between the hours 05.30 - 24.00 depending on the flow of passengers on each route separately. Note that schedules of circulation of buses and minibuses are coordinated with the City Hall.

Fig.2. Structure of urban transport in Chisinau

1. Municipal Chisinau Council
 2. The City Hall of Chisinau
 3. General department of public transport and communication
 4. Administrators of the minibus routes
 - 4.2. Transport of persons or merchandise in the taxi regime.
 5. Transportators agents, transport operators, independent taxi drivers.
- Number of transported people.

From 1 June 2011 to 1 June 2012 on the regular routes of trolleybuses were transported 93.3 million passengers, including paid - 73.3 million (79%) and the free ones - 20.0 million (21%); by buses were transported 14.8 million passengers, including paid - 11.5 million (78%), free - 3.3 million (22%); with minibuses in this period were transported approximately 190.0 million passengers. (Fig3.)

Thus, during the nominated period were transported by trolley, buses and minibuses a total of 298.5 million passenger or calculated per day about 820.000 passengers.

It is known that up to 500m trips are usually made without transport, but those that are longer than 2 km have usually the transport priority. The average urban driven distance recommended is about 1-5 km.

Results show that an average trip is about 4.8 km. Same numbers we have for a trip with the minibus.

For sustaining the environmental quality parallel to the social, economical and cultural activities, it is necessary to impose public transport in the city life by improving the quality of the services offered by it.

The percentage of the transport agents in the urban passenger stream with the compliance of all quality requirements does not exceed 25%

Unfortunately, the goals and traditional criteria of appreciation of the effectiveness of the public transport only reveals it self by achieving some final quantitative results.

For example, in the municipal passengers transport contract for buses is often provided as the main index the transported volume. At the optimization of the bus routes system due to the reduction of the transportations number, this index will decrease. So it shows that a large transportation volume is not needed. Serving the population with public transport, the placement of buildings for living, commerce, culture and other centres of attraction for the

people should be organized in such a way that the maximum driven distances should be as short as possible and the necessity of transportation minimal.

The Chisinau road infrastructure consists of 900 streets with a length of 880 km and with the roadway surface about 10 million m². The Length of the sidewalks in a line constitutes 451.0 kilometers (1.8 million M²)

In the period 01.06.2011 - 01.06.2012 at the exploitation of communication paths have been made street repairs, of a total worth of 25249,6 thousand lei (78,700 m² of smooth asphalt), including:

- Works financed from the republican budget - 3682, 5 thousand (smooth asphalt-9300 m²)
- Work performed and financed from the municipal budget 328418 thousand lei (smooth asphalt - 114300 m²)

Electric transport

The report of activity of municipal company "Electric Transport" in the period 01.06.2011-01.06.2012, presents the following indices of activity:

Number of trolleybuses on 30.05.2012 - 320 units

Trolley routes - 23 routes

The average trolley activity on the route - 258 units

Utilization coefficient of the rolling stock - 0.81

The average driven distance - 45.0 thousand km

Average daily number of passengers -300.000 persons

Income from passenger transportation -126.6 million lei

Also it was fulfilled the repairment and prophylaxis of the cable and contact networks - 1200 km, repairs were made on 55 trolleybuses.

With the receipt of new trolleybuses in number of 102 units from the supplier of trolleybuses the consortium "BelkommunMash & Carpat BELAZ Service", since June 2011, it has stabilized the revenue collection, which was declining.

The bus transport

In the account of the municipal enterprise „Parcul Urban de autobuze" as from the accounting evidence, there were 136 buses designed for passengers transportation, of which 125 are involved in the transportation of passengers in the Chisinau municipality and 11 buses operating with an average of 20 -23 years are in conservative state of beeing, needing capital repairment, or disposal due to its malfunctions.

Today the company provides daily 93 buses on the line, serving 24 urban and suburban routes of the city, including 5 routes (or 21.0 percent) in urban traffic routes and 19 (or 79.0 percent) in suburban traffic. The total length of the route is 403 km.

The Average daily output of buses on the a period of time is 86 units, beeing in decline comparing to the same previous period. The decrease was caused by the old age rolling stock, the average age of a bus was 11.8 years compared to the norm of 8 years, situation which also makes additional expenses for maintenance and exploitation.

The volume of passengers for the period 1 June 2011 - June 1, 2012 constituted 14.8 million, of which 11.5 million. passengers (or 77.7 %) with full payment for travel, and 3.3 million with travel facilities.

During the management period was accumulated an income in the amount of 39.2 million lei, including: income from transporting passengers - 36.5 million lei, and incomes from other types of activity - 2.7 million lei.

The decrease of the paying passengers and accumulated income contributed to the reduction of running buses from 88 units per day to 86 units and the extension of minibus routes along with the bus routes.

Analyzing the total volume of passengers over the last years we can observe that the municipal company "RTEC" and "PUA" are losing their positions on the urban transport market.(Fig.4)

Fig.4 Dynamic volumes of passengers transported by "RTEC" and "PUA"
total, paid, free

Transport by minibuses

According to the provisions set out in the passengers and luggage auto transport Regulation, approved by the Government Decision no. 854 from 28.07.2006 to 31.12.2007 there were excluded from activity about 500 minibuses remade from trucks and in time were replaced by buses with smaller modifications and with a bigger number of seats (17-22 seats) designated to transport passengers.

Currently the Chisinau minibus fleet consists of 1840 units, of which:

- ☐ Up to 5 years - 10%
- ☐ 5-10 years - 43%
- ☐ 10-15 years - 41%
- ☐ 15-20 years - 6%

Urban minibuses are priority Mercedes-Benz type "Sprinter" which is characterized by reliability and economy and low emissions of gases. These minibuses according to the European classification are in the M2 category , A Class – Easy class buses for the transport of passengers in the quantity corresponding to the number of seats in the bus. This condition is not respected by transporters due to an increased number of passengers at stations during the rush hours.

The activity of the private minibus transporters is obviously more efficient than the urban bus fleet. But the unjustified increase of the general minibuses, the unfair competition with AUP and RTEC are leading to the apparition of problems, primarily for their own activity. In the case when they are driving "parallel" units of transport that belong to different transport companies , none of them can provide the actual exploitation of the rolling stock. Following this , appears the need of investments in the means of transport and requirements for price increase

According to our estimations at this stage, the general number of minibuses correspond to the requirements of the population (about 1800 units) in transportation. But the increase of their number will lead to a deepening in the existing problems created by them.

Conclusions

For public transport to be profitable, you need to follow certain quality parameters of passenger services as: accessibility, reliability and convenience.

In the DGTPCC, the control of the quality services provided by the passenger transporters is assured by the public transport management. In order to improve the public service of the passenger stream in Chisinau , were made several measures, including:

- There were introduced some modifications in the public transport network: a new bus route was opened no. 148 „Street Meleștiu (City Hospital no. 1)- village New Goiania " bus route no. 141 „ Cricova - str.Bulgara " the trolleybus routes had been prolonged no. 22 to bd. Dacia and no. 28 to bd. Iu. Gagarin, the minibuses were extended: no. 103, no. 119 till Street Arheolog Ion Casian-Suruceanu, no. 116 - up to Gradina Botanica street , nr. 161 – till the Andrei Doga street, modified the minibuses routes no. 180 to establish the circulation on Armeneasca Street, no. 169 - Street Bucuresti, and so on;
 - 102 contemporary trolleybuses of AKCM-321 model were purchased (factory "Belcomunmaş" Minsk city) with low floor (adopted for the transportation of disabled people), according to the public-private partnership project implemented jointly with the European Bank for Reconstruction and Development (EBRD) and European Investment Bank (EIB). The trolleybuses were distributed and are currently working on the municipal routes.
 - Municipal company "Electric Transport" made repairs on 55 trolleybuses and 3 trolleybuses were renovated , at 4 trolleys the doors were changed, and at 35 were put soft chairs. 16 pillars were changed and it was made a prophylactic repairs of 1200 km of network of contacts, replaced the contact wire on a distance of 5 km;
 - M.C. "Parcul Urban de Autobuze" had equipped 40 buses with special automatic announcement of the stations name (when the driver presses the button) and other useful information for travelers (route no. 3, 5, 23 and "A "). A MAN model bus was equipped with modern electronic audio-visual broadcast with full information and using GPS (without drivers involvement)
 - For the improvement of the road safety and increasement of the transport capacity , the Chisinau City Hall had decided through the disposition no. 411-d of 24.05.2011, that on the street M.Kogălniceanu the public transport was diverted on a part of the roadway and there was established the routes for trolleybuses number 3 and 4 Street on Bucuresti Street, and itinerary routes no. 104, 124, 127 and 129 on Street A. Şciusev
- In the period under analyzation in Chisinau there were recorded 439 road accidents involving passenger transportation : (trolley - 101, bus - 54, minibuses - 153, taxi - 138), of which 311 traffic accidents are produced by the transport drivers fault (trolley - 64, bus - 31, minibuses - 112, taxi - 114), after which 342 people were injured (trolley - 95, bus - 6, minibuses - 138, taxi - 108), 6 people died in accidents involving mini-bus on route.

RISK ASSESSMENT OF DUST CONTAMINATION OF AIR

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Abstract: Dust contamination is a risk factor for the population of the town of Ruse. It is very strongly expressed during the heating period. The paper presents the results from long-lasting investigations of dust content in the free air by residential districts, at different weather conditions and other factors. Results are compared with sociologic research among the townspeople. An attempt is made to assess the influence of Thermo-electric Power Plant and other industrial sources. Criticality is assessed according to the differential risk for dangerous action. It is compared with data about sick rate among population.

Keywords: dust, contamination, research, criticality

Introduction

Dust contamination of free air is a problem, which is unsolved and not studied enough. That fact is confirmed by a number of researches [2,3]. It can be classified as a phenomena, which causes critical situations and events, in differential and integral aspect, especially during the cold season, when cyclic trends of continuous and significant contamination are established [5].

At present criticality of dust contamination is assessed only by comparison of currently measured concentrations and allowable limits. Methods of measuring, used in Bulgaria, are based on discrete measurements over the course of time. Arithmetic mean values are determined and then they are compared to the standard values. Based on that comparison conclusions are made about degree of contamination. The time interval between measurements is adopted to be 4 hour. This is mainly because of organizational considerations.

Actually the process of dust contamination is very dynamic with significant fluctuations. Discrete measurements are not able to follow such dynamics and therefore assessment of contamination is not objective. This is confirmed by our research [3], as well as by the research of other authors [2] about the conditions in the town of Rousse.

To classify them as critical, situations of contamination of the components of environment are subject to several conditions, which determine their dangerous action [3,4,5]. The first condition is the spatial combination of object of exposure and risk factors. Therefore the object toward which criticality is assessed shall be defined precisely. The second condition is the above mentioned, namely the level of imissions of dangerous factors shall exceed the allowable limit. The third condition is combination over the course of time. This condition is expressed with the period of action of contaminating factors, during which the level of imissions exceeds the allowable limits. The fourth condition is that the period of action shall be larger than the allowable time of stay and work under the measured imission. This condition refers to factors, which exposition is standardized. The fifth condition is the coincidence of the time of action with the time of exceeding of allowable values.

The criticality of dangerous events resulting from contamination of environment according to the risk models, backed up with arguments by us [5,6], should be determined by the type of damage, its localization, heaviness and restorability. It is appropriate to compare them with the reactions of the objects of exposure.

The purpose of this study is to establish criticality of dust contamination of free air in the town of Rousse during the cold season.

The basic tasks that are solved are:

1. To provide grounds for a method of research and assessment of dust contamination, following its dynamics over the course of time;
2. To determine indicators of critical situations caused by dust contamination;
3. To establish critical events caused by contamination among population;
4. To establish dependence between criteria about criticality of contamination and criteria about criticality of health status of the population.

Material and Methods

Research was made in four districts in the town of Rousse. We used data from the automatic measuring stations and the fixed analytical sampling points of the Regional Inspectorate of Environment and Waters, of the Hygienic and Epidemiological Inspectorate and own measurements. When solving the first task we applied experimentally the probabilistic-statistical method of analysis and assessment. It is based on the theory of accidental processes [1]. Dust contamination is examined as a continuous accidental process with normal distribution, which is verified in [3]. Its basic statistical characteristics are function and density of distribution. We draw up the probabilistic characteristics of dust imissions for the time period t -mathematical expectation $m_x(t)$, equal to the average value $E[X]$; dispersion $\sigma_x^2(t)$; correlation function $R(\tau)$; spectral density $S(\omega)$ [1]. A check is made about stationariness and ergodicity of the process of dust contamination. Stationariness is examined in its wide sense, characterized with the equations: $m_x(t) = m_x = const.$, $\sigma_x^2(t) = \sigma_x^2 = const.$, $R_x(t_1, t_2) = R_x(t_2 - t_1) = R_x(t)$. Ergodicity is based on the coincidence of statistical characteristics, calculated by the multitude of realizations and the characteristics, calculated for continuous enough and averaged over the course of time realization of imissions. Thus determination of probabilistic characteristics is made according to the dependencies:

$$m[x] = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T x(t) dt; \quad \sigma_x^2[t] = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T [x(t)]^2 dt;$$

$$R_x(t) = \lim_{T \rightarrow \infty} \frac{1}{T} \int_0^T x(t) x(t+\tau) dt, \quad \text{where } \overset{o}{x}(t) = x(t) - m(x) \text{ is the centered realization of accidental processes.}$$

Correlation function and spectral density are applied to find the internal structure of the processes of dust imission in free air. They are connected with the transformations

$$R_x(\tau) = \int_0^\infty S_x(\omega) \cos \omega \tau d\omega; \quad S_x(\omega) = \int_0^\infty R_x(\tau) \cos \omega \tau d\tau, \quad \text{where } \omega \text{ is frequency. Through}$$

the correlation function $R_x(\tau)$ are established the process relations and the character of the process of contamination as a function of time, and through the spectral density $S_x(\omega)$ - its frequency composition. The standardized correlation functions $\rho(\tau)$ and the standardized

spectral densities $\sigma(\omega)$ [5] for the whole period of monitoring and their characteristics are determined, namely: - time τ_o of fall of correlation; - the average half-period τ_p ; - damping frequency of the correlation function; - shear frequency ω_c ; - frequency ω_o of the maximum value of spectral density; - spectrum width $\Delta\omega$. Modeling of the process of dust contamination is done by means of a typical correlation function [1]

$R(\tau) = \sigma_x^2 e^{-\mu|\tau|} (\cos \beta\tau + \frac{\mu}{\beta} \sin \beta|\tau|)$, where μ, β are the function factors. The number of exceedings n_x for a unit of time is:

$$n_x = \frac{\sqrt{\mu^2 + \beta^2}}{2\pi} e^{-\frac{(x_o - m_x)^2}{2\sigma_x^2}},$$

and their average duration τ_x is

$$\tau_x = \frac{\pi}{\sqrt{\mu^2 + \beta^2}} e^{-\frac{(x_o - m_x)^2}{2\sigma_x^2}} \left[1 - \Phi\left(\frac{x_o - m_x}{\sigma_x}\right) \right],$$

where $\Phi(x)$ is the Laplas function [1];

x_o - allowable limit of dust contamination.

Results & Discussion

To determine risk of occurrence of dangerous actions, according to the integrated model, adopted by us [4,5], we assume that:

- Dust distribution is only in free air, which supposes respiratory effect on people. Distribution of dust in other media is not followed;
- Duration of action is determined by the period of work of the central hot-water heating from the moment of turning on to the moment of turning off;
- Area of distribution covers four districts K1, K2, K3, K4;
- Probability of exceeding is the relative frequency of occurrence of dust volumetric concentration higher than the allowable value;
- Time of exceeding is equal to the total duration of all exceedings during the period of research.

The duration of action of imissions are related with the operation of the central hot-water heating, respectively its power source – Thermo-electric Power Plant “Iztok”, because our research [4,5] established correlation between its technical and operational indicators and the volumetric concentration of dust in the air of the town.

The duration of heating operation of Thermo-electric Power Plant “Iztok” in the period 2003-2007 was studied. It was found out that it obeys the normal law of distribution with the following values of basic characteristics: average value $E[X]=116,5$ days; dispersion $\sigma=12,4$ days; coefficient of variation $V=10,14$ days, probability of occurrence of the duration of imissions in the interval $E[X] \pm \sigma - P_{UM}=0,3451$.

Results from the research of dust concentrations as an accidental process in four districts in the town of Rousse are given as:

- Mathematical expectation m_x in mg/m^3 is close to, but lower than the maximum single concentration. The dispersion is large, which means that the allowable concentration is exceeded;

- The number of exceedings a day n_x for district K1 varies from 1,5 to 3,7. Analogous range is measured for district K4. For the two other districts the number of exceedings is higher and in wider interval - from 2,4 to 12,6. Average duration of exceedings in district K1 is from 1,03 to 1,79 h, in district K2- from 1,46 to 2,12h, in district K3 – from 1,32 to 2,45h, in district K4 from 1,14 to 1,67h. These characteristics allow to determine approximately the total interval during which the allowable value of dust contamination is exceeded;

- Our thesis that dust contamination in the town of Rousse is very dynamic process is confirmed. This is proven by the characteristics of the correlation function and the spectral density. To make an objective assessment it is appropriate to apply the proposed method. When comparing only the average value from the measurements of dust contamination with the allowable value, the duration and the number of exceedings may not be established. The reason is that the interval between measurements is not correctly chosen;

- It is established that there is big correlation between the discrete values of contamination, which is for a period of 1,03-2,93h for different districts. Maximum frequency varies in very wide limits – from 0,23 to $2,24\text{h}^{-1}$.

Except the typical characteristics for accidental processes, the probability of occurrence of one exceeding is established too. For its determination was made a check of the hypothesis of the law of distribution. Because the number of exceedings is a discrete accidental value, the check was made according to Poason's law and the binominal distribution. The hypothesis for the Poason's law was not rejected. Based on the values of its characteristics, the probability for occurrence of one exceeding a day was calculated. It varies in very wide limits by districts and by years - from 0,0914 to 0,4573.

For the purpose of passing from natural to accidental values and therefore to determine the risk of dangerous action of dust contamination, a check was made of the law of distribution of the duration of exceedings. The check established that this duration obeys the normal law of distribution, which characteristics for different districts vary in the following ranges: average value $E[X]=0,87-2,38\text{h}$; dispersion $\sigma=0,66-1,02\text{ h}$; quadratic mean deviation $\sigma^2=0,31-1,04\text{h}$; coefficient of variation $V=30,67-88,13\%$.

We calculated the probabilities for duration of exceedings within the range $E[X] \pm \sigma$ for every district. We obtained the following values: for district K1-0,8255, for district K2-0,6827; for district K3-0,6617 and for district K4-0,7629.

By taking into account the obtained values of the probabilistic components, we calculated the differential risk of dangerous action of dust contamination. We should mention the limiting conditions, namely: For a cold season with the above specified characteristics of the normal law of distribution of its duration, For occurrence of one exceeding of allowable limit a day (distribution of the number of exceedings according to Poason's law), Duration of the exceeding in the range from $E[X]-\sigma$ to $E[X]+\sigma$, again under normal law of distribution.

Under these conditions of the task, the following values of differential risk of dangerous action by districts were obtained: K1-0,0286; K2-0,0345; K3-0,0357; K4-0,0263. The analysis shows that risk is highest in district K3 and K2, which are located closest to

the basic producer of heat for the central heating of the town of Rousse – Thermo-electric Power Plant “Iztok”. Lowest is the risk in the districts, which are far from the thermo-electric power station, where local heating is used, mainly with solid fuel. The differential risk of occurrence of dangerous effects caused by dust contamination require establishing the type and the localization of damage, heaviness, restorability of the objects of risk research.

To establish the type of damages among the population of the studied districts we studied the pulmonary diseases from data received from the District Pulmonary Dispensary and the district polyclinics. We focused on diseases, connected with allergic reactions. For the period of the research were found 536 cases of such diseases with hospitalization. The check of the hypothesis for the law of distribution shows that the day number of diseases during the cold season obeys the Poason's law. This is due to the discrete character of the studied accidental value. The following results were obtained about the characteristics of the law for the four districts: Average value $E[X]=0,89-1,39$; dispersion $\sigma=0,94-1,39$; coefficient of variation $V=84,81-105,99\%$; day probability of occurrence of one pulmonary diseases from 0,3559 to 0,3678.

The check of the hypothesis for the law of distribution of the duration of hospitalization, as a criterion of the heaviness of damage, shows that the hypothesis for normal distribution is not rejected. The values of its characteristics for the four districts in the period 2003-2007 vary as follows average value $E[X]=12,7-16,34$ days; dispersion $\sigma=2,12-4,57$; quadratic mean deviation $\sigma^2=4,49-20,88$ days; Coefficient of variation $V=16,18-27,96\%$. The probability of occurrence of a disease with duration from $E[X]-\sigma$ to $E[X]+\sigma$ varies from 0,6327 to 0,6895.

The differential risk of occurrence of dangerous effects from the studied type – pulmonary diseases due to allergic reactions by districts is as follows: district K1-0,2342; district K2-0,2729; district K3-0,3139; district K4-0,2349. These results comply with the obtained values for the characteristics of dust contamination by districts, described above. In addition 883 cases of respiratory reactions with symptoms of allergies were registered. For these cases it is not possible to assess heaviness because there is no accurate information about the duration of treatment as it takes place at home. Thus the same values as for hospitalization were adopted. Distribution of respiratory reactions is again according to Poason's law with the following characteristic values: average value $E[X]=1,37-1,94$; dispersion $\sigma=1,15-1,39$; coefficient of variation $V=71,79-86,71\%$; the day probability of occurrence of one respiratory reaction with allergic symptoms of pulmonary disease is from 0,2788 to 0,3518. The differential risk of occurrence of dangerous effects of respiratory reactions with symptoms of allergies is: district K1-0,2151; district K2-0,2040; district K3-0,1894; district K4-0,2261.

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PROTECTION'S TECHNOLOGY OF CONTAMINATION OF RIVER'S WATERS WITH ANIMAL BLOOD

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Abstract: This paper presents a technology for utilization of animal blood, which is waste product in slaughter industry. By this technology blood-and-milk fodder concentrate with high protein amino acid content is obtained. The paper describes the basic technological processes and operations, their sequence, interrelations, some characteristics and modes. Comparative analysis of this technology and its previous versions is made in order to prove its higher efficiency. This technology has significant ecological effect since it prevents disposal of blood and pollution of wastewaters and respectively water reservoirs.

Keywords: environmental protection, waste utilization technology, slaughter blood

Introduction

Blood is the main pollutant of sewage waters in slaughter industry. In the process of transition from centralized economy to market economy the small-size and medium-size production business has intensified. The number of small slaughterhouses, meat processors and sausage-makers increased. Few of them have appropriate wastewaters purification systems, which results in uncontrolled and increased pollution of water reservoirs.

An efficient approach to environmental protection in such enterprises is the implementation of waste less blood utilization technology.

Materials and Method

Blood is suitable raw material for manufacture of feedstuffs. Such products are blood powder, blood meal, fodder mash, blood-and-milk protein concentrate, etc. [1,4,5].

Blood utilization prevents unspecified disposal of blood in wastewaters.

Considering the above, two technologies for manufacture of blood-and-milk protein concentrate were developed (BMPC). This product has high nutritive properties and healing effect. The first variant of the technology is intended for laboratory conditions (variant I)[1]. Based on the results from its implementation and the additional experimental research an improved variant was developed (variant II) [4,5].

The purpose of this work is to develop an advance technology – variant III for blood utilization in slaughterhouses.

To achieve that purpose the following tasks were solved:

- The basic technological processes, their sequence and interrelations were described;
- Optimal values of factors and parameters if technological operations were established;
- A product with higher protein, amino acids and vitamins content was obtained;
- Maximal production efficiency of the technological cycle was achieved;
- Waste minimization.

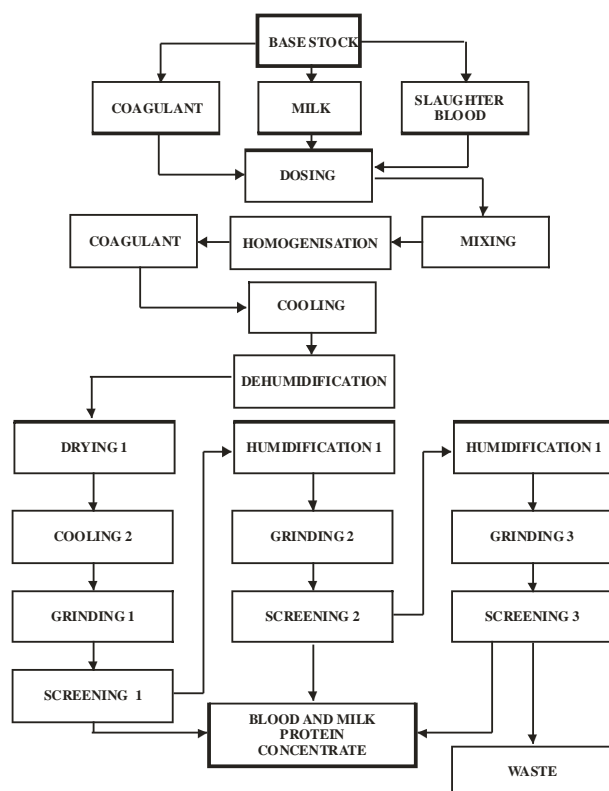


Fig. 1. Basic processes and operations of the advance slaughter blood utilization technology - variant III

studied and optimized [3,4].

The coagulated material has 65 - 68 % relative moisture content. After cooling to 20 - 25 °C the material is subject to filter press dehydration to reduce its relative moisture content to 40 - 42 %.

The lower moisture content of the coagulated material compared to the technology, described in [4] allows improvement of the temperature mode of drying 1. Power consumption and drying time is reduced.

Temperature/time drying mode 1 is shown on Figure 2. This mode was selected in order to reduce the relative moisture content of the material to 22, 5 - 23 %, while keeping the amino acids and the protein content in the stock.

The material is cooled to 25-30 °C, and then it is ground in ball mill for 22 min (grinding 1 – Figure 1) and undergoes vibration screening. In this way the fine fraction with dimensions 0, 8-1, 0 mm is extracted. This fraction composes 91, 6 % of the total dried substance. Other controlled factors of grinding process are optimized and published in [4].

Results and Discussion

Applied technological processes and their sequence are shown on Figure 1.

Animal blood and cow's milk, conforming to Bulgarian State Standards, are used as basic materials. These are mixed in proportions 1, 35. Obtained mixture is stirred for homogenization at rate 20 min⁻¹ for 0, 24 h. Then the mixture is heated at a specified temperature/ time mode.

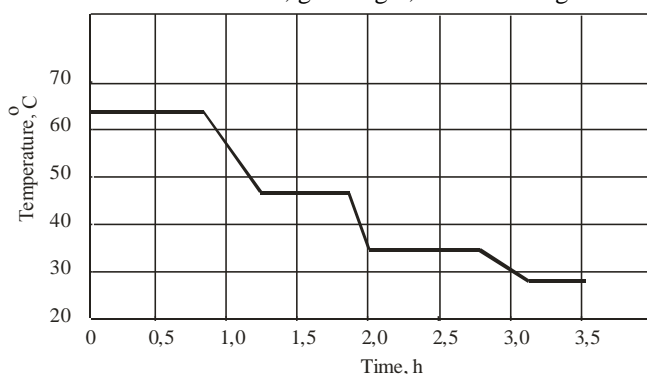
Calcium dichloride is used to coagulate the mixture. The coagulant in the specified proportion is added and is homogenized with the mixture. During this process a temperature of 40 °C is reached gradually and is maintained. Then the temperature is increased to 80 °C and the processing continues for 12 min. The process of coagulation is

Table 1. Comparative characteristics of slaughter blood utilization technology

Specifications	Unit of measure	Technology		
		Variant I [1]	Variant II [4]	Variant III
Technological cycle duration	h	18,4 - 18,6	7,9 - 11,8	4,8-5,7
Productivity	kg/cycle	6,9 - 7,4	11,8 - 13,7	16,2-17,5
Waste	%	0,8 - 1,8	0,2 - 0,6	0,12-0,23
Protein	%	70,7 - 72,4	76,5 - 78,2	78,1-79,3
Milk sugar	%	10,2 - 11,2	10,8 - 11,5	10,6-10,9
Mineral substances	%	2,2 - 2,5	1,7 - 2,1	2,4-2,6
Fats	%	1,1 - 1,3	1,2 - 1,3	1,1-1,2
Aspartic acid Asp	mg/100mg	2,432 - 2,845	3,445 - 3,78	3,612-3,714
Tyrosine Thr	mg/100mg	0,885 - 1,008	1,072 - 1,211	1,147-1,637
Serine Ser	mg/100mg	1,082 - 1,108	0,978 - 1,191	1,132-1,141
Glutamic acid Glu	mg/100mg	2,872 - 2,999	2,344 - 2,789	2,433-2,591
Proline Pro	mg/100mg	1,322 - 1,338	1,379 - 2,055	1,357-1,988
Glycine Gly	mg/100mg	1,172 - 1,213	1,612 - 1,544	1,513-1,631
Alanine Ala	mg/100mg	1,986 - 2,168	1,844 - 2,335	1,988-2,351
Valine Val	mg/100mg	2,562 - 2,713	2,612 - 2,712	2,633-2,788
Methyonine Met	mg/100mg	0,373 - 0,412	0,277 - 0,516	0,243-0,612
Leucine Leu	mg/100mg	3,448 - 3,569	3,331 - 3,781	3,129-3,562
Phenylalain Phe	mg/100mg	1,724 - 2,34	1,755 - 3,001	1,944-2,779
Histidine His	mg/100mg	1,843 - 2,56	1,912 - 2,665	2,314-2,655
Lysine Lys	mg/100mg	2,111 - 2,719	2,056 - 2,783	2,271-2,812

The fraction 0,8 - 1,0 mm is subject to drying 2 under the temperature/ time mode, shown on Figure 2. Drying results in reduction of moisture content to 5 - 7 %. Carried out experimental research has proved that such moisture content guarantees one-year durability of BMPC.

Coarse fraction (with dimension above 1 mm) has 28-31 % moisture content. It undergoes three operations - humidification 1, grinding 2 and screening 2 (Figure 1). Humidification is done by means of fine water spray until 20 - 22 % relative moisture is obtained. Grinding in ball mill for 8 min and then screening is performed. Fine fraction is separated. Dry at room temperature. The residue is subject to the same technological operations – humidification 1, grinding 3, and screening 3.

**Fig. 2.** Temperature/ time drying mode

The waste is coarse ungrindable fraction with hardness similar to that of polymer material.

BMPC is a product of full value, easy to assimilate and ecologically clean protein, used to enrich the fodder of poultry, swine and bees. It contains milk sugar, minerals, fats, vitamins

A, D, E, C, B₁, B₂, B₁₂, PP, K, as well as the basic amino acids, determined by means of acid hydrolysis (Table 1).

The comparative analysis of the characteristics of the presented advance variant (variant III) of the blood utilization technology and its initial variants (variant I and II) shows:

1. Duration of the technological cycle is significantly reduced (4,8-5,7h), and productivity is increased with approximately 62,3 %;
2. Waste is reduced to the minimum of 0,12-0,23%;
3. Protein content is increased to 78,1-79,3%;
4. Mineral substances content is reduced and the fats and milk sugar content is preserved;

5. Better amino acids balance is obtained. The relative share of the basic amino acids is increased; only for leucine the value is approximately the same as in the previous variants of the technology.

6. The presented advance variant of the blood utilization technology allows obtaining of BMPC – essential component for the fodder of poultry, swine and bees.

After consumption the mortality rate among poultry is significantly reduced and stops. Vitality, general condition, growth and muscular mass accumulation and later the laying capacity are improved. Similar effect is noticed for the physiology and general condition of pigs. The protein content is increased for bees and this product is used with great effect mainly in the process of brood cells formation.

Presented technology has significant ecological effect since the animal blood from slaughter industry is not disposed of in sewage waters and therefore it doesn't pollute water reservoirs. The implementation of this technology has proven that.

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