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THE ROLE OF BASIL, THYME AND TARRAGON IN REDUCING THE CONTENT OF NITRITE IN MEAT PRODUCTS

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Abstract. Reducing the content of nitrites and nitrates in food, including meat and meat products is a current issue. More and more studies are being done to reduce these synthetic food additives by using vegetative additives. This paper examines the issue of reducing nitrite content in meat products. The role of the basil, thyme and tarragon on nitrites and nitrates content in meat products is presented. The study showed the possibility of reducing the content of nitrites and nitrates in Lacta sausages, by using basil extract (BE 0,1%) by 0.4-0.94%, (BE 0.2%) by 0.80-1.88%. (BE 0.3%) by 1.20-2.82%; by thyme extract (ThE 0.1%) by 0.56-071%, (ThE 0.2%) by 1.12-1.42%, (ThE 0.3%) by 1.68-2.13%; by tarragon extract (TE 0.1%) by 0,08-0.66%, (TE 0.2%) by 1.16-1.32. (TE 0.3%) by 0.24-1.98%, maintaining the quality and safety of meat products.

Keywords: *nitrite, nitrate, basil, thyme, tarragon, meat products.*

Rezumat. Reducerea conținutului de nitriți și nitrați din alimente, inclusiv din carne și produse din carne este o problemă actuală. Numărul studiilor consacrate procedeelor tehnologice pentru reducerea acestor aditivi alimentari sintetici prin utilizarea aditivilor de origine vegetală este în creștere continuă. Această lucrare analizează problema diminuării conținutului de nitriți în produse din carne. Este prezentat rolul adaosurilor de busuioc, cimbru și tarhon asupra conținutului de nitriți și nitrați din produsele din carne. Studiul de caz a demonstrat posibilitatea reducerii conținutului de nitriți și nitrați din cârnații Lacta, prin utilizarea extractului de busuioc (BE 0,1%) cu 0,4-0,94%, (BE 0,2%) cu 0,80-1,88% (BE 0,3%) cu 1,20-2,82%; prin extract de cimbru (ThE 0,1%) cu 0,56-071%, (ThE 0,2%) cu 1,12-1,42%, (ThE 0,3%) cu 1,68-2,13%; prin extract de tarhon (TE 0,1%) cu 0,08-0,66%, (TE 0,2%) cu 1,16-1,32. (TE 0,3%) cu 0,24-1,98%, menținând calitatea și siguranța produselor din carne.

Cuvinte cheie: *nitrit, nitrat, busuioc, cimbru, tarhon, produse din carne.*

1. Introduction

Different spices and herbs, in different concentrations, are used to produced meat products [1]. Basil, thyme and tarragon are currently widely used in various food industries, including the meat industry. Their antioxidant and antimicrobial properties are increasingly being the subject of international research [2-5].

The microbial properties of these plants have been reported in numerous scientific papers: the authors of the studies [6-8] report antimicrobial activity of basil, authors [4, 9, 10] report antimicrobial activity of thyme, authors [11-13] report antimicrobial activity of tarragon. The chemical composition and antioxidant properties are also reflected in many scientific papers, for example: basil [14], thyme [15, 16], tarragon [17].

Nowadays, cured meat products are widely produced using nitrate and nitrite salts [18]. Nitrites and nitrates are used in the meat industry due to their beneficial effect on the quality and meat products microbiological safety [19-21]. Nitrates (sodium nitrate - E251, potassium nitrate - E252) and nitrites (sodium nitrite - E249, potassium nitrite - E250) are food additives listed as official preservatives according to Commission regulation (EC) No. 1129/2011 [22]. Nitrates and nitrites legal limits are reported in Regulation (EC) No. 601/2014 [23]. Sodium nitrite permissible dose according to GD No. 229/2013 [24]. The content of nitrates and nitrites in meat and meat products in the Republic of Moldova is regulated in [25]. But lately it is increasingly mentioned that these substances in meat products form carcinogenic N-nitrosogenic compounds, which pose a major risk to consumers [26-28]. Nitrite plays a distinct role in human physiology. In acidic environments or under oxidative stress conditions it may be converted to a number of reactive nitrogen species [29], such as nitric oxide (NO), nitrogen dioxide (NO_2) and peroxynitrite ($ONOO^-$) [30].

In living systems, they are involved in a variety of biological functions and their uncontrolled intracellular presence produces significant toxicity, as they can target a variety of biomolecules including proteins, DNA, lipids and carbohydrates [31]. Reducing the nitrite / nitrate content of meat is a global problem [32]. There are many studies that determine the content of nitrite in meat and meat products [33].

The benefits of aromatic plants (basil, thyme, tarragon) on the food stability, including meat products [8, 11, 16], and on the consumer's health [33-35] have been demonstrated and argued in various scientific papers. Nitrites are used in the production of various meat products, with the role of printing and stabilizing their color and flavor, as well as maintaining the products microbiological stability [36]. Currently, more and more international research is aimed at the use of various plant sources in order to reduce the content of nitrites / nitrates in meat products [35-37]. The stability of the meat and meat products color, quality and safety can also be achieved by using natural antioxidants that are able to regenerate Met-Mb to MbO_2 [18, 38], and have microbiostatic and microbicidal properties [11, 39].

In this work we have tried to reflect another property of basil, thyme and tarragon that of providing the opportunity to reduce the content of nitrites / nitrates in meat products.

2. Materials and methods

2.1. Sampling

The sausage samples were prepared in laboratory conditions (semi-industrial) according to classic technology of manufacturing “Lacta” sausages, included in the group of boiled sausages. In the control sample a mixture of salts was introduced, according to the classic recipe (NaCl salt and sodium nitrite $NaNO_2$ are introduced separately).

The research samples were prepared with extracts of aromatic plants (basil – SBE, basil, thyme SThE, tarragon STE) 0.1%, 0.2%, 0,3% concentration. Table 1 shows the recipes of the researched samples.

Table 1

Sausage recipes “Lacta“

Raw materials and ingredients	Unit	"Lacta" sausages		
		The classic recipe	Control	With an addition of 0.1% aromatic plants (SBE/ SThE/ STE)
Consumption rate for 100 kg of unsalted raw material				
Beef, high quality	kg	35	35	35
Pork, semi-fat	kg	60	60	60
Powdered milk	kg	2	2	2
Eggs mixture	kg		3	3
Ice	%	25	25	25
Consumption rate of spices and materials, g (per 100 kg of unsalted raw material)				
Food salt	g	2090	1870	1870
Sodium nitrite	g	7.1		
SBE/SThE/STE*	g	-	-	100
Granulated sugar	g	120	120	120
Ground black pepper	g	120	120	120
Nutmeg	g	40	40	40

*The sausages were prepared with extracts of aromatic plants (basil – SBE, basil, thyme - SThE, tarragon - STE).

2.2. Methods

2.2.1. Sensory Analysis of Sausages was determinate exterior appearance, color in section, taste, odor, and consistency using the 5-point system by an expert panel of eleven trained food technologists. The 5-point assessment system includes the following scores characteristics: 5 - very good; 4 - good, 3 - satisfactory, 2 - poor, 1 - bad, having as reference the sensory characteristics of the classic product according to GD 624 of 19-09-2020 on the approval of the Quality Requirements for meat preparations and products [26].

2.2.2. Moisture content was determined according to gravimetric method, based on the weight loss of the analyzed sample to constant mass, due to water evaporation by heating in an oven at a temperature of up to 130 °C [40].

2.2.3. Determination of the meat pH made with the pH meter Titroline 5000 at 20˚C.

2.2.4. Determination of water activity was performed by the express method using the LabSwift-a_w device (Novasina AG, Lachen, Switzerland).

2.2.5. Determination of nitrites and nitrates content (predictive method) based on bibliographic sources, regarding their content in aromatic plants.

2.3. Statistical analysis

All calculations were performed using Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA). Data obtained in this study are presented as mean values ± the standard error of the mean, calculated from three parallel experiments. The comparison of average values was based on the one-way analysis of variance (ANOVA), according to Tukey’s test, at a significance level of $p \leq 0.05$, using the Staturphics program, Centurion XVI 16.1.17 (Statgraphics Technologies, Inc., The Plains, VA, USA).

3. Results and Discussion

Reducing food additives, especially nitrates used in the manufacture of meat products, is one of the most important problems facing the meat industry. Consumers prefer natural additives instead of chemicals used in meat products because of the health risks of nitrous compounds. Therefore, studies on the use of natural compounds as nitrites / nitrate alternatives have increased in recent years.

Processed meat producers have begun to use "natural" sources of nitrates, such as celery juice, beet extract or spinach, where the nitrates present are reduced to bacterial nitrites and as a result contribute to the formation of nitrosamine [41]. Nitrate reduction in nitrites is mainly caused by bacteria possessing nitrate reductase activity (*staphylococcus* and *micrococcus*), naturally contained in the raw material or in the case of addition to manufacture [42].

Most scientific papers mention the antimicrobial and antioxidant properties of herbs (basil, thyme and tarragon) and their use in various foods. There are insufficient studies on the possibility of using these plants in order to reduce the content of nitrates / nitrites in the food processing. In this research we conducted a reliability study in this direction.

The reduction in the salt amount introduced in the samples did not diminish the products quality and stability, Table 2.

Table 2

Physicochemical indicators and sensory characteristics of "Lacta" sausages

Tested samples	Quality indicators						
	Physicochemical			Sensory			
	Moisture content, %	Active acidity, pH	Water activity a_w	Color in section	Odor	Taste	Consistency
Control sample	64.37± 0.09 [a]	6.38± 0.06 [c,d]	0.875± 0.001 [e,f]	5.00± 0.00 [e]	5.00± 0.00 [g]	5.00± 0.00 [h]	5.00± 0.00 [d]
„Lacta" sausages with basil extract (SBE)							
SBE 0.1%	67.38± 0.06 [b]	6.15± 0.02 [b]	0.873± 0.001 [c,d]	5.00± 0.00 [e]	5.00± 0.00 [g]	4.75± 0.11 [f,g]	4.99± 0.01 [d]
SBE 0.2%	69.07± 0.06 [c]	6.13± 0.06 [b]	0.873± 0.001 [c,d]	4.75± 0.02 [d]	4.87± 0.04 [f]	3.83± 0.05 [c]	4.98± 0.02 [d]
SBE 0.3%	69.16± 0.05 [c]	6.12± 0.06 [b]	0.873± 0.001 [c,d]	4.45± 0.01 [b]	3.83± 0.01 [b]	3.46± 0.04 [b]	4.98± 0.01 [d]
„Lacta" sausages with thyme extract (SThE)							
SThE0.1 %	69.33± 0.09 [c]	6.16± 0.08 [b,c]	0.875± 0.001 [e,f]	5.00± 0.00 [e]	5.00± 0.00 [g]	4.95± 0.02 [h]	4.99± 0.01 [d]
SThE0.2 %	69.44± 0.06 [c]	6.12± 0.03 [b]	0.875± 0.001 [e,f]	4.75± 0.02 [d]	4.37± 0.02 [d]	4.27± 0.08 [d,e]	4.95± 0.02 [c,d]
SThE0.3 %	71.48± 0.12 [e]	6.10± 0.05 [b]	0.873± 0.001 [c,d]	4.60± 0.04 [c]	4.00± 0.01 [c]	3.35± 0.01 [b]	4.87± 0.07 [b,c]
„Lacta" sausages with tarragon extract (STE)							
STE 0.1%	69.48± 0.11 [c]	6.29± 0.04 [c]	0.874± 0.001 [d,e]	5.00± 0.00 [e]	4.75± 0.02 [e]	4.70± 0.10 [f,g]	4.99± 0.01 [d]
STE 0.2%	69.56± 0.08 [c,d]	6.14± 0.03 [b]	0.870± 0.001 [b]	4.50± 0.02 [b,c]	3.83± 0.01 [b]	4.33± 0.05 [d,e]	4.98± 0.02 [d]
STE 0.3%	69.64± 0.06 [c,d]	5.12± 0.03 [a]	0.867± 0.001 [a]	3.75± 0.00 [a]	3.63± 0.02 [a]	3.25± 0.04 [a]	4.45± 0.03 [a]

Values in the table represent the means of three replicated trials ± standard deviation. Different letters ([a–h]) designate statistically different results ($p \leq 0.05$).

Probably this effect was achieved by introducing aromatic herbs, with remarkable antioxidant and microbiological properties, which were mentioned in another study [23]. Other authors also report that herbs control microbiological risk and do not affect color, and flavor of products [38, 43].

Levels of nitrite and nitrate in vegetable are dependent on several factors [44 - 46]: type of soil, temperature, humidity, light intensity, plant maturity, genetic, harvesting time, storage time and source of nitrogen [47 - 49]; method of plant cultivation and the early stage of their vegetative development [50]. Jolanta Molas says that according to the susceptibility degree to nitrate accumulation in plants, they are arranged as follows: lettuce> spinach> basil> mint> oregano> thyme [50].

Currently, great attention is being paid to the determination of nitrate / nitrate content in food, including meat products [53, 54], in the context that no substance has yet been found which would completely replace the functions of nitrites or nitrates. Positive effect of nitrites / nitrates on meat products are related to the improvement of color, the development of the typical meat flavor, the antimicrobial role and the antioxidant effect [55]. The reaction of nitric oxide with myoglobin, contributes to the formation of the nitrosylmioglobin complex, which imprints the meat color's characteristics. Following the interaction of nitrates / nitrites with meat lipids and proteins [56], several compounds are formed that influence the aroma of the meat product. Also the antimicrobial effect of nitrites which is related to the inhibition of bacteria metabolic enzymes, limiting oxygen absorption and breaking the proton gradient [30]. However, some plant origin raw materials can replace nitrates / nitrites due to their increased nutritional and biological value as well as their microbiological stability.

The estimated percentage reduction in nitrite content in the analyzed samples was based on the nitrite content reported in the bibliographic sources, Table 3.

Table 3

Estimation of the probability to reduce the nitrite / nitrate content in the examined sausages samples

Concentration of extract / Evaluated parameters	Classic recipe	Control	SBE	SThE	STE	Levels of nitrite, nitrate, mg/100g / Bibliographic sources*
The amount of salts, nitrites	2097.1	1870				
percentage reduction, Δ%		6.09				
0.1%						
percentage reduction NO_2^-, Δ%			0.22 - 0.54	0.52 - 0.64	0.07 - 0.64	154 - 381 (B) 369 - 453 (Th) 50.4 - 450 (T) [47, 51, 52]
percentage reduction NO_3^-, Δ%			0.18 - 0.40	0.04 - 0.07	0.01 - 0.02	1872 - 3972 (B) 369 - 702 (Th) 83 - 139 (T) [47, 51, 52]
NO_2^- + NO_3^-, Δ%			**0.40 - 0.94**	**0.56 - 071**	**0,08 - 0.66**	
0.2%						

Continuation Table 3

percentage reduction NO_2^-, Δ%	0.44 - 1.08	1.04 - 1.28	0.14 - 1,28	[47, 51, 52]
percentage reduction NO_3^-, Δ%	0.36 - 0.80	0.08 - 0.14	0.02 - 0.04	
NO_2^- + NO_3^-, Δ%	**0.80 - 1.88**	**1.12 - 1.42**	**1.16 - 1.32**	
		0.3%		
percentage reduction NO_2^-, Δ%	0.66 - 1.62	1.56 - 1.92	0.21 - 1.92	[47, 51, 52]
percentage reduction NO_3^-, Δ%	0.54 - 1.20	0.12 - 0.21	0.03 - 0.06	
NO_2^- + NO_3^-, Δ%	**1.20 - 2.82**	**1.68 - 2.13**	**0.24 - 1.98**	

* The nitrate / nitrite content taken into account is the average value reported in the target bibliographic sources. B- basil, Th- thyme, T- tarragon.

In the 0.1% SBE samples, the NO_2 - reduction percentage are of 0.22% to 0.54%. If we take into account the level of nitrates reported in the bibliographic sources, this percentage would be higher (0.40 - 0.94%).

The quality and microbiological stability of the examined samples were achieved due to their antioxidant, antimicrobial properties and the nitrite / nitrate content of the aromatic plants, although the amount of salt and nitrites in the samples was reduced by 6.09% compared to the classic recipe.

4. Conclusions

Herbs (basil, thyme and tarragon) with remarkable antioxidant and antimicrobial properties are recommended ingredients for use in the meat products manufacture. In the context of the bibliographic and experimental study, it was found that these plants could reduce the consumption of nitrites and salts that we use in the meat products manufacture.

So it is possible to replace sodium nitrite with basil, thyme or tarragon (plant extracts, powders or pears) which result in the nitrite content elimination / reduction of the end product. Herbal extracts appear to be a suitable alternative to synthetic nitrite, may prevent meat products discoloration and, in appropriate quantities, shall not impair the flavor, texture and consistency of the product.

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