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Abstract

Introduction. Studies of the physico-chemical composition of the carob beans and the pulp of the pods originating from four countries are present.

Materials and methods. The physicochemical properties of carob (*Ceratonia siliqua* L.) cultivated in different countries, Moldova, Algeria, Italia and Spain, were characterized in terms of mineral (Ca, Mg, and Fe), carotenoids (β-carotene, lycopene, and zeaxanthin) and chlorophyll (a chlorophyll and b chlorophyll) content. The antioxidant activity of biologically active compounds was determined using simulated gastrointestinal digestion.

Results and discussion. The samples of Moldovan carob compared to those grown in Algeria, Spain, and Italy contain higher amounts of biologically active compounds, some positions far exceeding those of carob from the mentioned regions. Thus, the mineral content in terms of Ca, Mg and Fe in Moldovan carob samples was 1.1–1.7 times higher. The same trends were recorded for the content of carotenoids in Moldovan carob beans: β-carotene, 13.610 mg/100 g of dry matter (DM); lycopene, 19.882 mg/100 g DM, and zeaxanthin, 20.709 mg/100 g DM, which were much higher in comparison with samples from Algeria, Spain, and Italy. The differences concerning the amounts of biologically active compounds between Moldovan and other regions of carob beans were significant. Samples from Italy were distinguished by the highest content of chlorophyll and it was up to 1.1 mg/100g DM. The evolution of the antioxidant activity of biologically active compounds, which was done via gastrointestinal digestion, confirmed the functional profile of carob pods and beans. Thus, the DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant activity of bioactive compounds in carobs from different regions of the world, during gastric digestion simulation, increased from 38–48% to 60–74%.

Conclusions. The studied four carob bean and pod samples originating from different world regions were similar by their bioactive potential. Nevertheless, it was found that Moldovan carob is the best in terms of the content of minerals, β–carotene, lycopene, zeaxanthin, and antioxidant activity.

Introduction

Carob (*Ceratonia siliqua* L.) is a tree that can grow in varied latitudes, from those with temperate climates to tropical ones (FAOSTAT, 2022) (Figure 1).

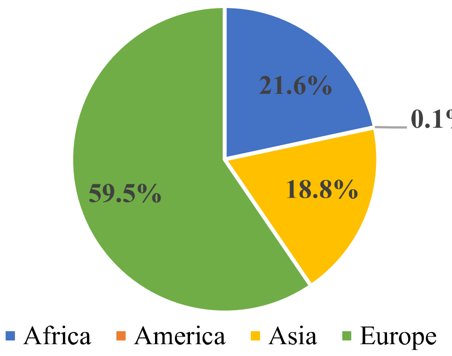

Figure 1. Production share of carob beans by region, sum 1994 – 2021 (FAOSTAT, 2022)

According to the 2022 data of Food and Agriculture Organization, Portugal takes the first place for carob tree cultivation. The second largest producer in the world is Italy, although Morocco, Turkey, and Greece also produce carob on a large scale (FAOSTAT, 2022). Among European countries with a Mediterranean climate, Romania, the Republic of Moldova, and Ukraine are geographically advantageous in terms of favorable climatic conditions for carob cultivation (Capcanari et al., 2022). Unfortunately, according to the same report carob production is decreasing around the world (Figure 2).

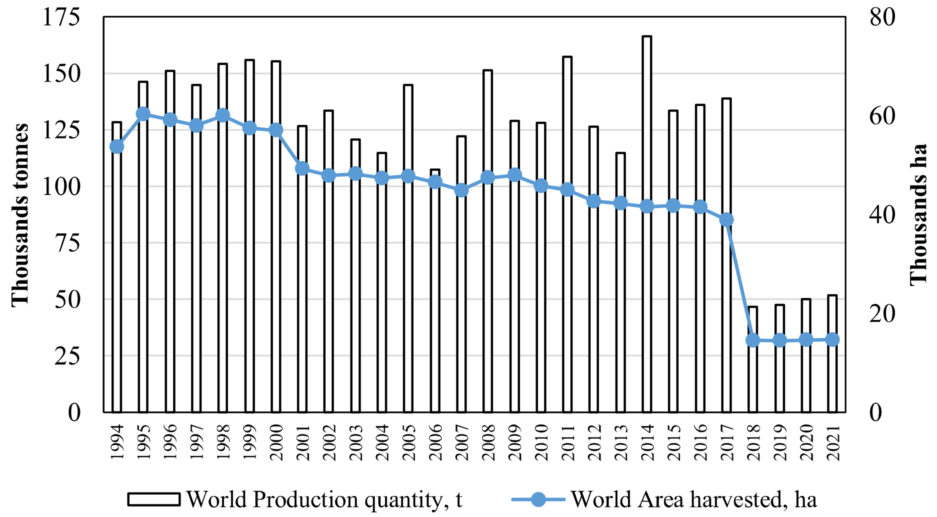

Figure 2. Area harvested/ Production quantity of carob beans in the World, 1994 – 2021 (FAOSTAT, 2022)

This fact is probably caused by several factors, among which those ones can be mentioned: (1) ignorance of nutritional and biological potential of fruits; (2) reduced individual consumption; (3) low fruit prices (FAOSTAT, 2022; Şahin et al., 2016).

It was reported that carob fruits (pod pulp and beans) contain biological active substances, such as polyphenols, vitamins, and minerals (Bouzdoudi et al., 2012; Santonocito et al., 2020; Vekiari et al., 2012). Carob beans could be considered as a valuable source of such minerals as K (850.8–1169.3 mg/100 g dry matter (DM), Ca (237.1–350.6 mg/100 g DM), and Mg (45.30–137.70 mg/100 g DM) (Fidan et al., 2015). According to Fidan and Sapundzhieva (2015), the content of mineral elements varies from one grade to another, respecting the following series: K > Ca> Mg> P> Fe> Zn> Mn. Large variations between results can be caused by environmental factors including climate, soil, and growing region.

Due to its chemical composition, the carob has many nutraceutical uses. The antioxidant activity of carob is primarily attributed to polyphenolic constituents, including ellagitannins present mainly in carob pod pulp (Chait et al., 2020). However, most of the existing research is devoted to the chemical composition, technological properties, transformation processes and culinary processing of carob beans, while there is much less studies on the pulp of the pods. Currently, the beans from the carob pods are transformed into flour that closely resembles cocoa powder, naturally sweet, aromatic and ideal in sweet dishes, being one of the natural and healthy additives (unfortunately too little used), in bakery products, ice cream, salad dressings and other food products (Loullis et al., 2018; Stabnikova and Paredes-Lopez, 2023).

It was shown that carob powder has certain advantages over cocoa because it contains a lower amount of fat and significantly higher amounts of dietary fiber. The lower fat content adds fewer calories, while the high dietary fiber content with its unique composition along with the polyphenolic compounds offers numerous health benefits. The key to the potential of carob in substituting cocoa is the cocoa-like aroma and flavor. Overall, the nutritional and economic advantages that carob presents make it a great candidate for the substitution of cocoa (Gunel et al., 2020). There is experience in the incorporation of carbon flour in the recipes of nutritious snacks for children (Aydın et al., 2017), milk and dark compound chocolate (Akdeniz et al., 2021), pastry sauces (Capcanari et al., 2022), and muffins (Pawłowska et al., 2018).

The aim of the present paper was to study the bioactive profile of carob cultivated in European and North Africa agrifood sectors in terms of mineral (Ca, Mg, Fe), carotenoids (β-carotene, lycopene, and zeaxanthin) and chlorophyll (a chlorophyll and b chlorophyll) content. The antioxidant activity of biologically active compounds was determined using simulated gastrointestinal digestion.

Materials and methods

Materials

Carob samples from different world regions (Republic of Moldova, Italy, Algeria, and Spain) have been used in the study. The unroasted carob bean samples from Spain, Italy and Algeria were bought from supermarkets in Italy and Romania. The pod pulp is not commercially available for the above-mentioned samples, as it is considered a by-product. The Moldovan samples (pods pulp and beans) were collected from different country geographical regions (Center, South and East).

a. Industrial powder of carob beans originated from Spain

b. Industrial powder of carob beans originated from Algeria

c. Industrial powder of carob beans originated from Italia

d. Powder of carob pods pulp originated from Moldova

e. Carob pods and beans originated from Moldova

f. Powder of carob beans originated from Moldova

Figure 3. Carob samples used in the present study

In order to avoid major experimental errors in the comparative analysis, Spanish, Algerian, and Italian unroasted carob samples were selected with the same particles size (≤90 µm).

Preparation of carob powder extracts

In order to assess the bioactive profile of carob, hydroalcoholic extracts were obtained according to the technological scheme shown in Figure 4. It should be noted, that after Moldovan carob beans and pod pulp grinding, particles with the same particles size (≤90 µm) as the bought ones were selected for determination. The extracts were further used as raw material for laboratory determinations.

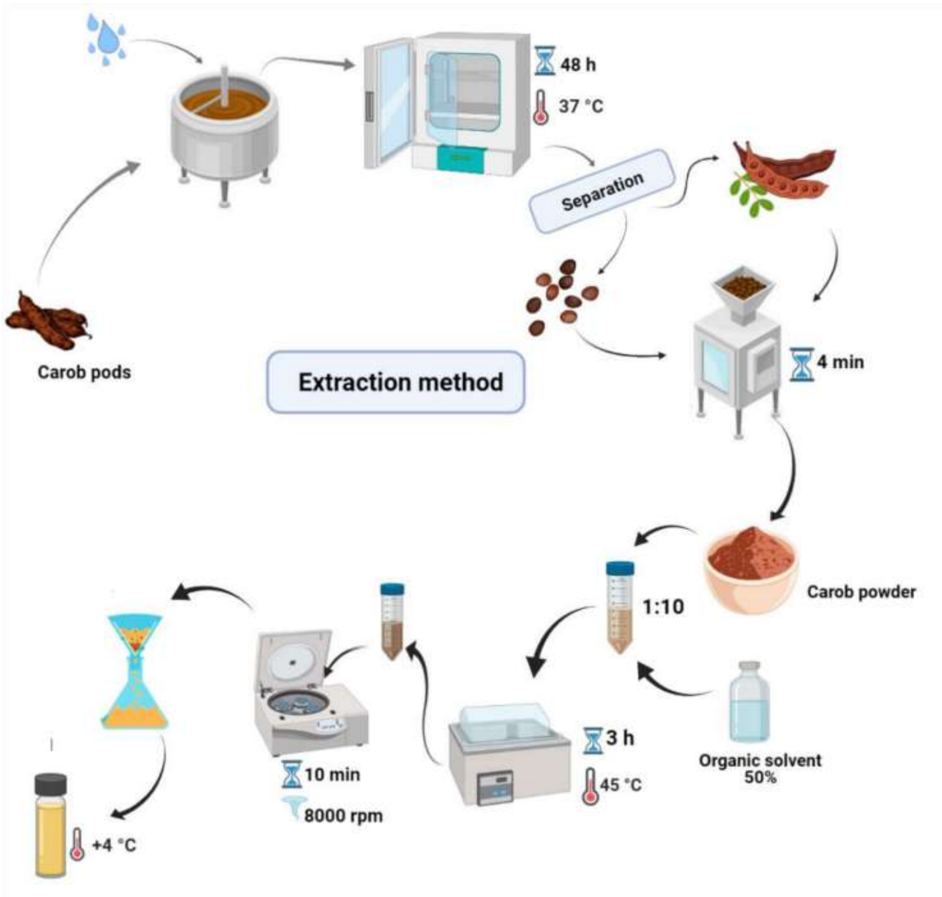

Figure 4. The technological flow of carob extract preparation

Mineral content

The content of minerals, Ca, Mg and Fe, was determined from the resulting solution using AOAC (Association of Official Analytical Chemists) methods as described by (James, 1995).

Chlorophyll and carotenoid content

Chlorophylls a and b were extracted with refined sunflower oil from carob powder and the absorbance of the extract solutions was measured in a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 661.6, 644.8 and 470 nm. Pigment contents were estimated as described by (Lichtenthaler, 1987). Carotenoids lipid extractions were analysed in the range of 448 and 480 nm wavelength for determining β-carotene, lycopene and zeaxanthin content (Meléndez-Martínez et al., 2007).

Antioxidant activity expressed via gastrointestinal *in vitro* digestion

Product samples were dissolved in HCl (pH 1.5–2.0), pepsin being added. Similar product samples were dissolved in $NaHCO_3$ (pH 8.2), and trypsin was added. The samples were incubated at 37ºC by continuous stirring at 95 rpm for 60 minutes. Aliquots were extracted during different incubation periods (0 h, 1 h, 2 h, and 3 h). Each aliquot was centrifuged for 10 minutes at 6000 rpm then the upper phase was isolated and further used for the determination of antioxidant activity using DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical. DPPH (60 µM) was added to the sample filtrate (0.1 mL). Methanol was used as a reference solution. The samples with DPPH were incubated for 30 min in a dark place. The absorbance was recorded on the spectrophotometer LLG uniSPEC-2 at the wavelength λ = 515 nm (Pavan et al., 2014).

Statistical analysis

All experiments were carried out in triplicate. The results were given as mean standard deviation (SD). Statistical analysis was performed using XLstat (2020 version) software.

Results and discussion

Mineral content

For the quantitative analysis of mineral compounds there were selected 10 lots of Moldovan carob products and 5 lots of carob beans samples from different regions of Spain, Italy and Algeria (Table 1).

Table 1

Mineral content in carob cultivated in European and North Africa agrifood sectors, mg/kg dry matter (DM)

Sample	Ca		Mg		Fe	
	Min-Max	Mean	Min-Max	Mean	Min-Max	Mean
Spanish carob beans (n=5)	2519.3–2526.4	2522.5[b]	1232.4–1239.4	1235.5[ab]	39.48–46.12	42.57[ab]
Italian carob beans (n=5)	2698.7–2705.4	2702.2[b]	1042.5–1052.2	1047.1[a]	42.39–48.71	45.42[b]
Algerian carob beans (n=5)	2174.3–2181.6	2178.5[a]	1344.7–1352.4	1348.1[b]	34.89–43.75	39.65[a]
Carob pods pulp from Moldova (n=10)	4501.2–4511.5	4506.7[d]	1860.7–1869.2	1864.4[c]	75.17–82.94	78.19[d]
Carob beans from Moldova (n=10)	3813.7–3819.6	3816.4[c]	1483.9–1491.4	1487.7[bc]	58.24–64.62	61.28[c]

[a–d] The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n is number of samples from each region.

Data concerning the mineral content of carob pods is practically lacking in literature, a part of Ayaz et al. (2007), who stated that the mineral amount of Ca, Mg and Fe in Anatolian carob pods was 3000, 600 and 18.8 mg/kg, respectively. Later, the same author reported an amount of 3040, 554 and 15.1 mg/kg of Ca, Mg and Fe, respectively, in home-prepared carob pods flour (Ayaz et al., 2009). It is worth mentioning that carob pods pulp had a greater amount of each determined element, emphasizing once again the importance of the valorization of these by-products.

The amount of Ca, Mg and Fe in Moldovan carob pods is almost twice higher (4506.7, 1864.4 and 78.19 mg/kg DM, respectively) than Spanish, Italian or Algerian beans (Table 1).

According to studies (Fidan et al., 2020; Musa Ozcan et al., 2007; Pazir et al., 2018), the amount of Ca, Mg and Fe in carob beans consists 2510–4207 mg/kg, 630–894 mg/kg and 12.5–42.57 mg/kg, respectively. The studies have mainly focused on the mineral content of Turkish carob, while data for European carob are lacking. Thus, the obtained data are valuable for the European agrifood sector. Analyzing and comparing the content of the selected elements in the studied samples the following chains can be formed:

– $Ca_{MD} > Ca_{IT} > Ca_{ES} > Ca_{DZ}$;
– $Mg_{MD} > Mg_{DZ} > Mg_{ES} > Mg_{IT}$;
– $Fe_{MD} > Fe_{IT} > Fe_{ES} > Fe_{DZ}$.

(MD, Republic of Moldova, IT, Italy, ES, Spain, DZ, Algeria)

Carotenoid content

Table 2 provides data on the carotenoid content of carob cultivated in European and North Africa agrifood sectors.

Table 2

Carotenoid content in carob cultivated in European and North Africa agrifood sectors, mg/100 g DM

Sample	β–carotene		lycopene		zeaxanthin	
	Min–Max	Mean	Min–Max	Mean	Min–Max	Mean
Spanish carob beans (n=5)	0.534–0.612	0.542[b]	0.639–0.659	0.647[ab]	0.712–0.718	0.715[ab]
Italian carob beans (n=5)	1.118–1.161	1.126[ab]	1.549–1.569	1.558[b]	1.451–1.457	1.453[b]
Algerian carob beans (n=5)	0.258–0.279	0.267[a]	0.458–0.483	0.467[a]	0.421–0.433	0.427[a]
Carob pods pulp from Moldova (n=10)	2.648–2.841	2.749[c]	3.874–3.892	3.879[c]	3.787–3.817	3.804[c]
Carob beans from Moldova (n=10)	13.541–13.724	13.610[d]	19.869–19.897	19.882[d]	20.681–20.735	20.709[d]

[a–d] The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n– number of samples from each region.

Carotenoids are the most common pigments in nature, also known as the source of dietary vitamin A (Delgado-Vargas et al., 2000). However, data concerning the carotenoid content in carob are practically lacking. According to Khatib and Vaya (2010), carob pods from the Mediterranean region contained 0.2 mg total carotenoids/100 g DM, with α- and β-carotene (0.08 mg/100 g DM), lycopene (0.03 mg/100 g DM), and lutein (0.02 mg/100 g DM).

In the present study, major carotenoids present in carob included β-carotene, zeaxanthin and lycopene. Lycopene was the most abundant carotenoid in Italian carob beans (1.558 mg/ 100 g DM), while in Spanish and Algerian samples this indicator had a mean value of 0.647 and 0.467 mg/100 g DM, respectively. Italian carob beans were most abundant in carotenoids (β-carotene 1.126 mg/100 g DM, lycopene 1.558 mg/100 g DM, zeaxanthin 1.453 mg/100 g DM), while in Spanish and Algerian samples these indicators had mean values of 0.267 mg/100 g DM β-carotene for Algerian samples, 0.542 mg/ 100 g DM β-carotene for Spanish; 0.467 mg/100 g DM lycopene for Algerian samples, 0.647 mg/100 g lycopene for Spanish; 0.427 mg/100 g DM zeaxanthin for Algerian samples, 0.715 mg/100 g DM zeaxanthin for Spanish.

The data presented in Table 2 confirm that the difference between Moldovan and rest of the samples was significant ($P \leq 0.05$). The carotenoid content in samples of Moldovan carob beans was more than 10 times higher than the biological potential of industrial bought samples, especially in the case of zeaxanthin, which concentration was up to 20.71±0.42 mg/100 g DM.

This can be explained by different conditions of pre-treatment of raw materials. Nevertheless, it is necessary to mention the biological potential of carob pod pulp originated from Moldova. Although this product is considered as industrial waste and does not find use in the food industry, it contains high amounts of β-carotene, lycopene and zeaxanthin.

Chlorophyll content

Chlorophyll has antioxidant properties and on a par with vitamins A, C and E could neutralize the damaging effect of free radicals (Inanc, 2011; Lanfer-Marquez et al., 2005; Queiroz Zepka et al., 2019). There are two main types of chlorophyll: chlorophyll a and chlorophyll b, the contents of which in carob samples are shown in Table 3.

Taking into account that chlorophyll is not an essential parameter in carob pods or beans, its presence in these products has not been studied before. The data presented in Table 3 show that in all beans samples, except for Algerian carob beans, chlorophyll b is more abundant than chlorophyll a, and its content values ranging within the limits from 0.122 to 0.605 mg/100 g DM.

The content of chlorophyll in Moldovan beans is comparable to the data of other carob bean samples. However, the content of these compounds is higher in carob pod pulp and is up to 0.749±0.04 for chlorophyll a and 0.482±0.02 mg/100 g DM for chlorophyll b. This indicates the antioxidant potential of Moldovan carob, including carob pod pulp. It is worth mention that in Moldovan carob pods the content of chlorophyll a prevails over chlorophyll b. The content of a chlorophyll from pods (0.749 mg/100 g DM) was higher than the one from all beans.

Evaluation of antioxidant activity by simulated *in vitro* gastrointestinal digestion

In order to evaluate the antioxidant activity of carob samples, a simulation of gastrointestinal digestion in time was performed. The evaluation of the antioxidant activity following induced gastric digestion (in acid medium) (Figure 5) shows lower values for the

Spanish samples (40.00–64.25)±0.1%, Italy (38.15–60.25)±0.2% and Algeria (39.28–62.61)±0.6% compared to the Moldovan carob samples, which values vary between (47.00–70.07)±0.05% for carob pods pulp and (48.00–74.24)±0.03% for carob beans.

Table 3

Chlorophyll content in carob cultivated in European and North Africa agrifood sectors, mg/100 g DM

Sample	Chlorophyll a		Chlorophyll b	
	Min–Max	Mean	Min–Max	Mean
Spanish carob beans (n=5)	0.149–0.157	0.152[a]	0.231–0.237	0.234[a]
Italian carob beans (n=5)	0.497–0.507	0.501[b]	0.602–0.608	0.605[b]
Algerian carob beans (n=5)	0.139–0.156	0.146[a]	0.116–0.129	0.122[c]
Carob pods pulp from Moldova (n=10)	0.742–0.758	0.749[c]	0.478–0.487	0.482[d]
Carob beans from Moldova (n=10)	0.275–0.283	0.278[d]	0.347–0.359	0.352[ad]

[a–d] The means in columns followed by the same letter are not statistically different ($P \leq 0.05$); n– number of samples from each region.

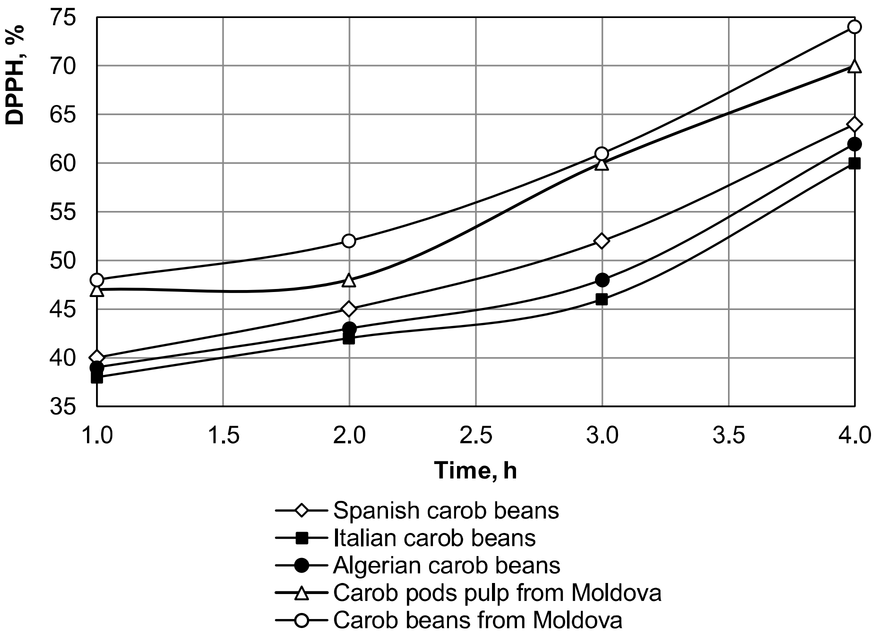

Figure 5. Antioxidant activity expressed via gastric digestion of carob powders

The gradual increase of antioxidant activity in 3 hours is explained by the gradual release of biologically active compounds in the process of gastric digestion. Another important factor was the influence of the pH of the solution and the enzymatic interactions in the studied samples. It was shown that changes in the content of biologically active compounds with antioxidant capacity, including polyphenols, flavonoids, tannins and other compounds such as carotenoids and chlorophyll groups can increase the antioxidant capacity of the analyzed samples. The data showed that gastric digestion did not essentially change the qualitative and quantitative composition of biologically active compounds with the antioxidant capacity of the studied carob samples. These compounds demonstrated high stability under low pH (acidic medium) conditions. The acidic environment along with the digestive enzymes favored the release of the biologically active compounds, which also affects the increase in the antioxidant capacity of the studied carob samples.

Following the gastrointestinal digestion process, the simulation of the intestinal digestion phase induced by incubating the samples in an alkaline environment (pH=8.2) (Figure 6) and determining the antioxidant activity in time for a period of 3 hours was performed. The data showed that the antioxidant activity of both types of Moldovan carob samples (from pod pulp and beans) was higher compared to the carob beans samples from Spain, Algeria and Italy. The values obtained varied between (40.05–19.89)±0.06% for Moldovan carob pod pulp; (35.25–18.69)±0.12% for Moldovan carob beans; (31.22–14.65)±0.08% for Italian carob beans; (30.04–17.47)±0.34% for Algerian carob beans and respectively (32.56–16.78)±0.58%, for Spanish carob beans.

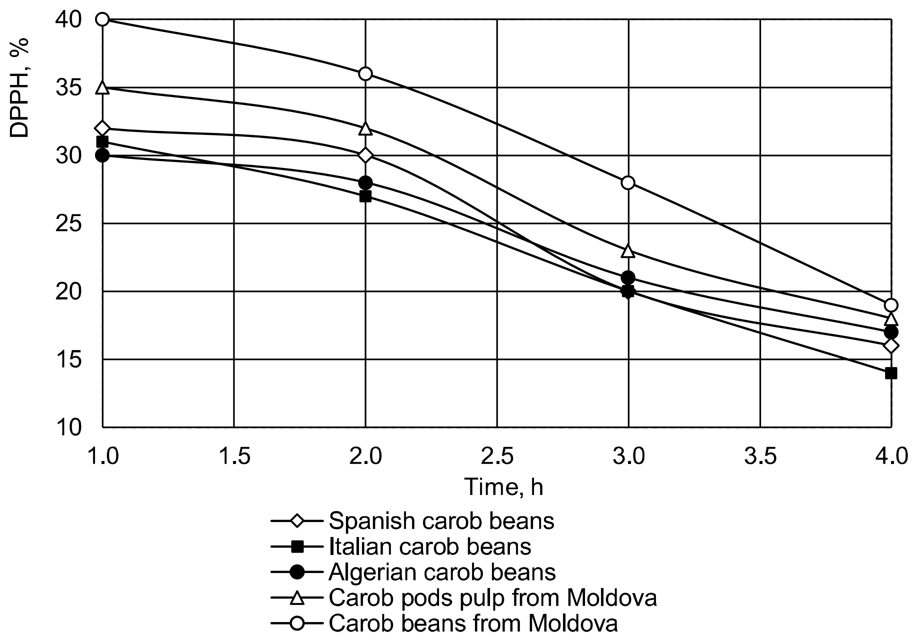

Figure 6. Antioxidant activity expressed via intestinal digestion of carob powders

Following intestinal digestion, there was a gradual decrease in antioxidant activity within 3 hours for both Moldovan carob samples and samples from Spain, Italy, and Algeria. This can be explained by the low stability of biologically active compounds in alkaline conditions (pH=8.2) and the formation of metabolites that inhibit their antioxidant activity in the analyzed samples. This is a normal physiological process. Absorption of biologically active substances occurs within 2–3 hours during the period of their maximum antioxidant activity.

Conclusions

The obtained results demonstrated the biological profile and antioxidant potential of carob pods and beans, originating from different world regions. The content of minerals Ca, Mg, and Fe in Moldovan carob pods and beans was found to be 1.1–1.7 times higher than in samples from Algeria, Spain, and Italy. The content of biologically active compounds β–carotene, lycopene and zeaxanthin in Moldovan carob pods, which are usually considered as food residue, is higher than in carob beans originating from Italy, Spain and Algeria. In the same time, the content of β-carotene, lycopene, and zeaxanthin (13.610, 19.882 and 20.709 mg/100 g DM respectively) in Moldovan carob beans exceeds by over 10 times the content of these substances in Italian (1.126, 1.558 and 1.453 m/100g DM), Spanish (0.542, 0.647 and 0.715 m/100 g DM), and Algerian (0.267, 0.467 and 0.427) carob beans. The biologically active compounds from carob pods and beans are stable at low pH values, exhibiting an increase in the antioxidant capacity from 38 - 48% to 60 - 74 % during the simulated gastric digestion.

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