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Nutritional and antioxidant potential of carob (*Ceratonia siliqua)* flour and evaluation of functional properties of its polysaccharide fraction

Nadezhda Petkova[1*], Ivanka Petrova[2], Ivan Ivanov[1], Rumen Mihov[2], Raina Hadjikinova[3], Manol Ognyanov[4], Vania Nikolova[2]

[1]Department of Organic Chemistry and Inorganic Chemistry, [2]Department of Catering and Tourism, [3]Department of Technology of Sugar, Confectionery, Starch and Starch Hydrolysates, University of Food Technologies, 26 Maritsa Blvd., Plovdiv, 4002, Bulgaria

[4]Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria

Abstract

The aim of the current study was to evaluate nutritional and antioxidant potential of commercial carob flour. The evaluation of functional properties of carob polysaccharide presented additional interest for pharmaceutical and food application of this flour. Ash, moisture, protein, carbohydrate, lipid, total phenolic and total flavonoids contents and antioxidant potential were studied. The polysaccharide from carob flour was isolated and their monosaccharide composition and functional properties as solubility, swelling, water and oil-holding capacities were also studied. Moisture content did not exceeded 7.6 %. The carob flour contained 2 % ash, 5.9 % protein, carbohydrates (85.5%), low lipids content (0.5 %), total phenols and flavonoids 8.1 mg GAE/g dw and 8.1 mg QE/g dw, respectively. Antioxidant potential of this flour were 70.4 mM TE/g dw (DPPH assay) and 84.2 mM TE/g dw (FRAP assay). The isolated polysaccharide from carob flour was characterized as galactomannan (M/G ratio 3.5). Two polysaccharide fractions were isolated with weight molecular weights 1724 kDa and 665 kDa, respectively. The carob galactomannan showed promising water solubility (84 %), higher oil-holding capacity 4.0 g oil/g sample than water-holding capacity 1.4 g water/g sample. The carob flour was evaluated as high nutritional and antioxidant food with low fat content, but rich of dietary fibers (galactomannan).

Key words: carob flour, carbohydrates, antioxidant activity, nutrition, galactomannan

INTRODUCTION

Nutritional and functional properties of food significantly influence user's preferences and food choices. Nutritional value is linked to the quantity and quality of nutrients found in the food and their digestibility. Food industry faces the challenge of producing and designed foods that improve the human health. This also provokes scientific interest concerning food components with appropriate nutritional, technological and functional properties, which will improve the value of food.

Carob tree (*Ceratonia siliqua* L.) belongs to the *Leguminosae* family and it is mainly cultivated in Mediterranean and Aegean regions. The world annual production of carob fruit increased 315000 tons, which is distributed among Spain (42 %), Italy (16 %), Portugal (10 %), Morocco (8 %), Greece (6.5%), Cyprus (5.5 %) and Turkey (4.8 %) [1]. Carob flour is obtained from seeds and pods of carob fruit. Chemical composition of the carob pod depends on varieties, origin and harvesting time [2]. Seeds are used as a source to produce the carob gum, also known as locust bean gum. This gum containing high amounts of galactomannans and can be added to a range of various foodstuffs aimed for the general population as a natural thickener and stabilizer [3-5]. Locust been gum included in the composition of baby foods should be used under medical supervision [6].

Carob flour might be considered as a natural sweetener, this is due to the high content of sugars [7]. The chemical composition of carob flour also shows a high content of insoluble fiber [8-10], this may find application for enrichment of food with dietary fiber [11].

Carob pods (pulp and seeds) have multiple uses, both in food and industrial purposes. Carob flour can be used as a cocoa powder substitute due to the similar flavor and appearance [12]. Nowadays, carob found enormous application, especially the pulp, as an animal feed [13], in pharmaceutical products [14] and ethanol production [15]. Carob syrup and medicines such as laxatives and diuretics. Seeds are mainly used to produce the natural food additive, "locust bean" gum (additive E 410), valued for its galactomannan content, as a stabilizer of emulsions and dispersions, thickening agents in food industry [17, 18]. The locust bean gum is also applied in pharmaceutical industry as drug delivery [19]. Carob pods is used for manufacturing of citric acid [20] and antiemetic products, and in pastry baking [14]. Hariri et al. [21] reported that the pod fiber content play a role in hypocholesterolemic and hypoglycemic regulation, whereas phenolic compounds can be used as antioxidant additive.

The aim of the current study was to evaluate the nutritional and antioxidant potential of the bio carob flour available on the local market in Bulgaria and to characterized the isolated polysaccharides concerning its future application in food technology and pharmacy.

MATERIAL AND METHODS

Materials

The carob flour (Biosviat, Avgeri Sofia, Bulgaria, Lot number: 143260912) was purchased from the local market in Plovdiv, Bulgaria. It was labelled as Bio product (Bio HELLAS Inspection institute for organic production) and it is a product from biological agriculture with origin Cyprus. All other reagents were analytical grade.

Chemical analysis

Moisture, ash, fat of the carob flour were determined according to the AOAC methods (2007) [22]. The crude protein content of the samples was estimated by the micro-Kjeldahl method [23]. The nitrogen as ammonia content in the digested sample was determined by acetylacetone-formaldehyde colorimetric method using ammonium sulfate as a standard [24]. For the calculation of crude protein, a value of the nitrogen to protein conversion factor 6.25 was used.

Extraction procedure

For the extraction of phytochemical compounds (1 g) carob flour was extracted with d. H_2O in solid to liquid ratio 1:10 ($w.v^{-1}$). The extraction procedure was performed in an ultrasonic bath (SIEL, Gabrovo, Bulgaria, 35 kHz and 300 W) for 20 min, at 75 °C. The obtained extract was filtered and the residue was extracted again under the above mentioned conditions. The combined extracts were used for further analysis.

Total carbohydrates

The total soluble carbohydrate content were estimated by phenol-sulphuric acid method [25]. The absorbance was measured at 490 nm against blank with d. H_2O. The amount of presented carbohydrates was determined from the calibration curve with glucose. The results were calculated as percent (%) of dry weight (dw).

Reducing sugars content

The reducing sugars were estimated by PAHBAH method [26]. The absorbance was measured at 410 nm against the blank, prepared with d. H_2O. The assay was set up by preparing glucose standard in the concentration range 5–100 μg/ml.

HPLC analysis of carbohydrates

Chromatographic separations and determination of presented sugars were performed on a HPLC instrument Elite Chrome Hitachi, coupled with refractive index detector (RID) Chromaster 5450. The separation was done on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with Pb^{2+} and a guard column Shodex SP - G (5 μm , 6 × 50 mm) operating at 85°C, mobile phase d. H_2O with flow rate 1.0 ml/min and the injection volume 20 μl [27].

Total phenolic contents

Total phenolic content was measured using a Folin-Ciocalteu reagent. Briefly, 1 ml Folin-Ciocalteu reagent diluted five times was mixed with 0.2 ml sample and 0.8 ml 7.5% Na_2CO_3. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as mg equivalent of gallic acid (GAE) per g dried weight (dw), according to calibration curve [28].

The total flavonoids content

The total flavonoids content was analyzed by $Al(NO_3)_3$ reagents [29]. The absorbance was measured at 415 nm against blank. The results were presented as mg equivalents quercetin (QE) per g dry weight (dw) according to the calibration curve, linear in range of 10-100 μg/mL quercetin as a standard.

The DPPH radical-scavenging ability

To conduct the assay, 0.15 ml from carob water extract was mixed with 2.85 ml freshly prepared 0.1mM solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance was measured at 517 nm in comparison to the blank containing methanol and % inhibition were calculated [28].

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain [30] with slight modification. The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in d. H_2O. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with methanol. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g dry weight (dw) [28].

Isolation of polysaccharides from carob

The polysaccharide extraction of commercial carob flour was performed with ethanol and d. H_2O. Carob flour (38 g) was suspended in 800 ml 96 % ethanol (purity 99.8%) at 70 °C. The sample was stirred on magnetic stirrer for 15 min to inactivate the enzymes and eliminate low-molecular-weight compounds [31]. The ethanol was decanted and hot distilled water was added in a proportion of 1:5 (endosperm:water). The suspension was stirred for approximately 24 h. Then water, in a proportion of 1:10, (suspension:water) was added and mixed in a blender for 5 min. Suspension was filtered through nylon cloth. The precipitation of the galactomannan was achieved by adding cold ethanol at a ratio of 1:2. The resulting precipitate was washed with acetone, lyophilized and kept in a dry place until further use [32].

Determination of monosaccharide composition and mannose/galactose ratio

The hydrolysis of isolated polysaccharide from carob flour was performed as follows: polysaccharide sample (100 mg) was hydrolyzed with 3 ml 1 M H_2SO_4 at 100 ºC for 2 h [32]. The solution was neutralized by addition of 10% Na_2CO_3. The sample were centrifuged at 1300 g for 10 min. The supernatant was filtered through a 0.45 μm nylon membrane. High-performance liquid chromatography analyses of monosaccharides composition were performed on a Shodex® Sugar SP0810 (300 mm × 8.0 mm i.d.) with $Pb^{2+}$and a guard column Shodex SP - G (5 μm, 6 × 50 mm) at 85°C, mobile phase d. H_2O with flow rate 0.5 ml/min.

Homogeneity and molecular weight

Number average molecular weight (Mn) and weight average molecular weight (Mw) of carob

polysaccharide were determined by high performance size-exclusion chromatography (HPLC-SEC). The separation was conducted using HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) equipped with a column Shodex OH-pack 806 M (ID 8mm and length 300 mm), (Shodex Co., Tokyo, Japan) and a RI detector (VWR Hitachi Chromaster, 5450, Japan) with mobile phase aqueous 0.1M $NaNO_3$ solution at 30 °C, with a flow rate of 0.8 mL/min. The column was maintained at 30.0 ± 0.1°C. The samples (3 mg/mL in 0.1 M $NaNO_3$) were passed through a 0.45µm syringe filter, PTFE 45/25mm (Isolab, Germany) before injection. The injection volume of samples was 20 µL. Pullulans with known molecular weight was use for calculation [33]. Polydispersity index of inulin was calculated as the ratio of the two molecular weights (Mw/Mn).

Functional properties

Swelling properties

Swelling properties of isolated galactomannan was evaluated as previously described by Robertson et al. [34]. Polysaccharide (100 mg dry weight) was hydrated in known volume of distilled water (10 mL) in a calibrated cylinder (1.5 cm diameter) at room temperature. After equilibration (18 h), the bed volume was recorded and expressed as volume/g original substrate dry weight [34].

Solubility

Alcohol insoluble residues (AIR) samples (100 mg) were suspended in distilled water (30 mL) and stirred for 3 h at room temperature. Each suspension was centrifuged (3000 rpm), the supernatant discarded and the pellet washed twice with distilled water. Pellets were dried at 105 ºC to constant weight and water insoluble AIR determined from loss in sample weight during extraction [34].

Water holding and oil holding capacities

Water holding and oil holding capacities of carob flour polysaccharide were determined in duplicate [35]. The samples (0.1g) were put into tared 50 ml polypropylene centrifuge tubes to which 10 ml deionized water or sunflower oil were then added. The tube was capped before the contents were vigorously mixed. They were then held for 24 h at 20 °C before centrifuging at 3500 rpm for 15 min, the excess water or oil decanted and the tubes inverted for 1 h at 20 °C. The tubes were then weighed and dried at 105 ºC to constant weight.

Statistical analysis

All determinations were performed in triplicate (n = 3) and the data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using MS Excel 2010. A difference was considered statistically significant, when $P < 0.05$.

RESULTS AND DISCUSSION

Nutritional characteristics of carob flour

Carob flour had been considered as a food supplement in various cultures and countries [36]. The nutritional value of commercial carob flour was evaluated and the results were summarized in Table 1. The moisture content was 7.56 % dw, that was higher than reports of Youssef et al. [36] and near to data of carob flour [37]. However, the ash content was 2.25 % dw, that was less than found by Youssef et al. [36]. The moisture and ash content was in a good agreement with Turkish commercial carob flour (7 % and 2.89 %, respectively) [38]. The carbohydrate was the most abundant phytochemical compounds in carob flour – 87. 5 % dw. Our results were higher than previous reports for Turkish, Sicillian, Marocanian and Tunasian carob flour [7, 32, 39-46] but were in good agreement with nine variety of Lebanese carob flour [42].

The average proximate composition of raw carob pods is 8–10% moisture, 90–91% carbohydrate (total sugars of sucrose (34–46%), glucose (2–5%) and fructose (2–5%)), 30–36 % dietary fibre, 3–4% protein, 3% polyphenols with gallic acid being the most abundant phenolic acid, 0.5–0.9% fat and 2–3% ash rich in Ca, P and K [36].

The total soluble carbohydrate and reducing sugar contents in commercial carob flour was 55.44 and 8.60 % dw, respectively. Our data for total soluble carbohydrate were higher than our previous result for carob pods from Bulgarian and Turkish origin [10]. Our results were in accordance with earlier reported data for carob species from Marconian origin (31.5 -53 g/100 g dw) [7, 41], for wild varieties from the territory of Turkey [46] and for Libyan carob (500 g/kg) [42]. The reducing sugars content coincided with results reported for carob from Saudi Arabia after roasting [44].

The quantity of reducing sugars was lower than some results (19–25 g/100 g dw) [10, 45]. The individual sugar profiles were obtained after HPLC-RID analysis. The presence of 1-kestose, sucrose, glucose and fructose was detected (Table 1). From all detected sugars, sucrose dominated in carob flour 34.13% dw collected from the territory of Bulgaria and Turkey with 34.2±0.7 and 16.5±0.4 g/100 g dw, respectively. Similar to our results were reported for Sicilian and Turkish carobs pods [10,39, 40]. The content of glucose (3.25 % dw) and fructose (4.16% dw) in commercial carob flour from Cyprus was near to results reported by Avallone et al. [39] and Fidan et al. [10] for Sicilian and Turkish carob pods. Avallone et al. [39] determined ~34-3.6%sucrose, ~4-1% glucose and ~6-2% fructose in eight different carob pods from *C. siliqua* grown in Sicily (Italy). The content of prebiotic 1-kestose (0.97 % dw) was higher than our previous report for Turkish carob pods (0.5 g/100 g dw) [10].

The carob flour contained low values of total lipids - 0.53±0.05 % dw (Table 1). The lipid content in our study was approximately three times lower than reported by Youssef et al. [36] values. The reported by us lipid content was in good agreement and supported the statement of Haddarah et al. [42] and Gubbuk et al. [46] for low lipid. Total fat content of carob flour coincided with Turkish genotypes - 'Sisam' (0.53%) [46].

Protein content of carob flour was found to be 5.9 %. Our results were near to the protein content of carob pods collected from southern and western Anatolia (4.45±0.40 g protein/100 g dry weight) [40] and similar to Sicilia [39]. In addition Youssef et al. [36] reported up to 6 % protein content in carob flour and evaluated this product

as the best vegetables and animal protein source. Gaisford et al. [37] also reported protein content in range 10 to 6.9 % for carob flour. The nutritional value of this commercial carob is near to reports of Youssef et al. [36] - 370 kcal/100 g.

Table 1. Nutritional characteristics of carob flour

Characteristics	Value
Moisture, %	7.56±0.16
Ash %	2.25±0.02
Carbohydrates, %	85.50±0.02
Total soluble carbohydrates, %	57.44±3.30
Reducing sugars, %	8.60±1.72
Glucose	3.25±0.42
Fructose	4.16±0.21
Sucrose	34.13±1.45
1-Kestose	0.97±0.01
Total lipids, %	0.53±0.05
Protein, %	5.90±0.10
Energy, kcal/100 g	370.37

Polyphenols and antioxidant activity of carob flour

In addition, the carob pulp has considerable amounts of dietary fiber and polyphenols (hydrolyzable tannins, derived from gallic acid and condensed tannins, derived from flavan-3-ol, anthocyanidines, and flavan-3,4-diol) [47, 48]. Moreover, the carob flour was evaluated as an ingredient with a marked nutritional value due to its high levels of phenol compounds. In investigated flour total phenols reached 8.11 ±1.15 mg GAE/g dw. The level of total flavonoids was 8.13±0.34 mg QE/g dw. The polyphenols possessed antioxidant activity evaluated by DPPH and FRAP assay (Table 2), which is important for the prevention or delay the oxidative damage. Consequently, polyphenols are involved in protection against several diseases (cardiovascular and neuronal, among others). Our values for total polyphenol coincided with the reported data for some Lebanese carob populations [42]. In addition, our results for total phenols and flavonoids were near to the results reported for carob flour with seeds [49]. However an apparent variation in phenolic contents has been found between Sicily and Anatolian carob pods. The total phenolic compounds for Anatolian carob pod were higher 13.51 mg GAE/g dry weight [36] but coincided with results for the roasted carob flour at 135 ºC for 30 min [43]. Therefore, our data were in the range of previous report reported that total phenols were 1.3–20 g/100 g [48]. However, the radical scavenging activity of carob flour evaluated by DPPH method was higher than reported results [49].

The flavonoids content were in accordance with [4] who found that carob contained between 0.41 and 0,48 mg/g DM of flavonoids.

The characteristic of carob flour for further uses is important for application in food and pharmaceutical formula. It is known that the climatic and geographic origin of carob seeds and the cultivation mode influence its chemical and rheological properties [32].

Polysaccharide from carob flour

The yield is one of the most economically important aspects of polysaccharide extraction and purification. The isolated polysaccharides from commercial carob flour yielded 7 %. Our yield was lower than the results for purified galactomannansobtain from carob 27% to 33%. The mean value of purification yield is 30% [32]. Lower yield of polysaccharides could be explained with carob variety, climate and postharvest conditions, as well as the parts form carob pods used in flour production.

Monosaccharide composition of carob polysaccharides after hydrolysis

It is known that Man:Gal ratio depends on both the plant growing conditions and the process it is subjected to [13]. The mannose/galactose ratios were not the same for carob samples [32].

The purified samples exhibited higher M/G ratios than did the crude gum. The contents of galactomannan for the 7 samples were deduced from a calibration curve obtained from five synthetic mixtures of β-D-galactose and β-D-mannose in known proportions. The M/G ratios for the sample of was 3.36 the mean value 3.23 for the crude LBG; this value increased to 4.06 the mean value of 3.91 for the purified LBG. As it is seen in Table 2, the purification process improved the yield of galactomannan and gave good resolution in chromatograms.

The results of polysaccharide analyses confirmed that mannose (Man) and galactose (Gal) are the major monosaccharides present in the polysaccharide material isolated from commercial carob flour (Table 3). The extracted galactomannans contain minor amounts of other monosaccharides such as xylose (Xyl) and glucose (Glc). Arabinose was not detected in our samples. The M/G ratio in our study was 3.5 for isolated polysaccharide from carob flour after purification with acetone (Table 3). Our results were similar to those reported by other researches [32, 37, 50, 51]. Therefore, the isolated polysaccharide from carob flour is typical galactomannan.

Average degree of substitution of galactose (DS_{Gal}) is important characteristics of the molecular structure of the galactomannan polysaccharide family. In our case (Table 3) DS_{Gal} was estimated to 0.28. As previously reported carob galactomannan (assumed as DSGal - 0.2–0.4) is generally regarded as partially soluble, and is reported to form weak gels after freeze–thaw treatment, in the presence of high concentrations of sucrose, or in ‘‘single-component’’ solutions when held near the freezing point of water [34].

Table 2.Total phenolic compounds and antioxidant activity of carob flour

Sample	Total phenols, mg GAE[1]/g dw	Total flavonoids mg QE[2]/g dw	DPPH	FRAP
			mMTE[3]/g dw	
carob flour	8.11±1.15	8.13±0.34	70.45±3.29	84.23±5.08

[1]GAE-gallic acid equivalent, [2]QE–quercetin equivalent, [3]TE – Trolox equivalents

Table 3. Comparison between monosaccharide composition for polysaccharide of commercial available carob flour and galactomannans

Sample	Monosaccharide composition (mol %)					
	Ara	Glc	Gal	Man	Xyl	M/G
Carob flour in this study	-	2±0.4	20.1±0.5	70.5±0.5	10.5±0.5	3.50±0.05
Carob gum or Locus bean gum (LBG) [51]	1.3-1.9	1.7-4.1	14.6-18.0	51.9-67.2	0.4-0.6	3.5-3.7 3.05-4.32 [32]
Galactomannan from *D.gardneriana* [52]	-	1.1	34.7	64.2	-	1.84
Guar gum [53, 54]	1.8-4.4	3.1	34.2-35.2	58.3-58.5	0-0.9	1.70-1.66

Table 4. Homogeneity and molecular weight of isolated galactomannans from carob flour

	Polysaccharide Fraction	
	Fraction 1	Fraction 2
Mw, kDa	1724	665
Mn, kDa	1100	595
Polydispersity index	1.15	1.12

Table 5. Functional properties of isolated galactomannans form carob flour

Sample	SP, ml water/g sample	Solubility, %	WHC, g water/g sample	OHC g oil/g sample
Carob flour polysaccharide	11.4	84.1	1.4±0.2	4.0±0.1

SP – swelling properties, WHC – water-holding capacity, OHC – oil holding capacity

Molecular weight of isolated galactomannans from carob flour

In the current study, HPLC SEC-RI analysis was used for the determination of the weight-average molecular weights (Mw), number-average molecular weights (Mn) and the polydispersity of isolated polysaccharide from carob flour (Table 4). Two fractions were observed from SEC chromatograms as the first fraction has a high weight-average molecular weight of about 1724 kDa and number molecular weight 1100 kDa. Our results were in a good agreement with molecular weight characteristics of isolated galactomannans from carob flour by 96 % ethanol precipitation [55]. The chain length polydispersity of carob polysaccharides fractions, as estimated by these SEC measurements, is in the range of 1.12-1.15 (Table 4). Our data coincided in the previous reports for narrow range 1.1-1.3 [55].

The relation between galactomannan chemical structure and this fractionations behaviors have been characterized extensively [55].

Functional properties

Functional properties of isolated galactomannans from carob flour were summarized in table 5. According to these results, galactomannans from carob flour is highly soluble in cold-water (~ 84% at 25°C). However, in our study water solubility of isolated polysaccharide were not in good agreement with the findings of Dakia et al. [51] and Farahnaki et al., [56] for locust bean gum, who reported the solubility of carob gum of about 50% at 25°C and 70-85% at 80°C. The major reason for this observation is that the isolated by us galactomaman was not only from seeds, but also from other carob husk parts. In addition climate and harvest conditions could caused significant influence on some function properties of carob flour. Commercial products do not always consist of pure endosperm, but may contain residual hull and germ parts [57].

Moreover, WHC of isolated polysaccharide from carob flour is four times less than this of locust bean gum 565 g/100 g [58]. In our case isolated polysacchride from commercial carob flour absorbed 1 g of water per gram samples. This could be due to the extraction process and particle size, which increased the contact surface between hydrocolloid and water. However, the isolated polysaccharide showed better OHC (4 g per g sample). This is important properties for application of these flours in foods and pharmaceuticals with high lipid content.

Composition and structure of proteins and their interactions with others substances control the functional properties of the carob flour. WHC is very important for the sensory evaluation of the food formulated products. This functional property represents the ability to absorb and retain bound hydrodynamic, capillary, and physically entrapped water against gravity. WHC affects the texture, juiciness, and taste, particularly the shelf life of bakery products.

OHC represents the ability of the product to absorb fats. The oil absorption is attributed to the physical entrapment of oil, the obtained results show that oil holding capacity OHC is higher than water holding capacity (Table 5). These results can be used to investigate the emulsifying and stabilizing properties of the carob flour, which would allow carob flour to be used in the manufacture of dressings and other food emulsions [59].

The soluble fibres in particular are thought to exert a preventative role against heart disease, as they appear to have the ability to lower serum cholesterol. Besides the polyphenols have antioxidant activity, which mainly enhances the prevention or delay the oxidative damage. Consequently, polyphenols are involved in protection against several diseases (cardiovascular and neuronal, among others). As a result, carob powder should be increasing interest as an ingredient in the food industry

such as functional and healthy foods formulations as biscuits, bread, creams, fillings and cakes.

CONCLUSION

The analyzed carob flour was characterized with high sugar content, relatively moderate protein content and low fat content. Additionally, it is well established that carob is rich source of polyphenols with well-pronounced antioxidant activity. The polysaccharides isolated from carob flour demonstrated promising physico-chemical properties for future application in emulsions or other dispersed system. According the obtained results carob flour was evaluated as a natural healthy food that could be used in many energy foods.

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CONFLICTS OF INTERESTS

All authors declare no conflicts of interests.

REFERENCES

1. Santos M., A. Rodrigues, A. J. Teixeira .2005. Production of dextran and fructose from carob pod extract and cheese whey by leuconostoc mesenteroides NRRL B512(f). *Biochemical Engineering Journal*. 25: 1–6.
2. Rtibi K., S. Selmi, D. Grami, M. Amri, B. Eto, J. El-benna, H. Sebai, L. Marzouki. 2017. Chemical constituents and pharmacological actions of carob pods and leaves (*Ceratonia siliqua L.*) on the gastrointestinal tract: A review. *Biomedicine & Pharmacotherapy*. 93: 522–528.
3. Dakia P. A., B. Wathelet, M. Paquot. 2007. Isolation and chemical evaluation of carob (*Ceratonia siliqua* L.) seed germ. *Food Chemistry*. 102 (4): 1368–1374.
4. Sęczyk Ł., M.Swieca, U. Gawlik-Dziki. 2016. Effect of carob (*Ceratonia siliqua* L.) flour on the antioxidant potential, nutritional quality, and sensory characteristics of fortified durum wheat pasta. *Food Chemistry*. 194: 637–642.
5. Moreira T. C., A. T. da Silva, C. Fagundes, S. M. Rodrigues Ferreira, L. M. Bileski Cândido, M. Passos, C. C. HeckeKrüger. 2017. Elaboration of yogurt with reduced level of lactose added of carob (*Ceratonia siliqua* L.). *LWT - Food Science and Technology*. 76: 326-329.
6. Meunier L. J.A. Garthoff, A. Schaafsma, L. Krul, J.S chrijver, J.B. van Goudoever, G. Speijers, Y. Vandenplas. 2014. Locust bean gum safety in neonates and young infants: An integrated review of the toxicological database and clinical evidence. *Regulatory Toxicology and Pharmacology*, 70: 155–169.
7. Batal H. E., A. Hasib, A. Ouatmane, F. Dehbi, A. Jaouad, A. Boulli. 2016. Sugar composition and yield of syrup production from the pulp of Moroccan carob pods (*Ceratonia siliqua* L.). *Arabian Journal of Chemistry*. 9: S955–S959.
8. Özcan M. M., D. Arslan, H. Gökçalik. 2007. Some compositional properties and mineral contents of carob (*Ceratonia siliqua*) fruit, flour and syrup. *International Journal of Food Sciences and Nutrition*. 58 (8): 652–658.
9. Durazzo A., V. Turfani, V. Narducci, E. Azzini, G. Maiani, M. Carcea. 2014. Nutritional characterization and bioactive components of commercial carobs flours. *Food Chemistry*. 153: 109–113.
10. Fidan H., N. Petkova, T. Sapoundzhieva , En. Isik Abanoz, 2016. Carbohydrate content in Bulgarian and Turkish carob pods and their products, *CBU international conference proceedings innovations in science and education*. 4: 795-801
11. Biernacka B., D. Dziki, U .Gawlik-Dziki, R. Rózyło, M. Siastała. 2017. Physical, sensorial, and antioxidant properties of common wheat pasta enriched with carob fiber. *LWT - Food Science and Technology*. 77: 186-192.
12. Hoda H., M. Fadel, A. Magda, A. Mageed, A. Kader, M. E. A. Samad, Sh. N. Lotfy. 2006. Cocoa substitute: Evaluation of sensory qualities and flavor stability. *European Food Research Technology*. 223: 125–131.
13. Manzi A.E., A.S. Cerezo, J.N. Shoolery. 1986. Higher solution 13C NMRspectroscopy of legume-seed galactomannans. *Carbohydrate Research*, 148 (2): 189–197
14. Calixto F.S., J. Canellas. 1982. Components of nutritional interest incarob pods *Ceratonia siliqua*. *Journal of the Science of Food and Agriculture*. 33: 1319–1323.
15. Turhan I., K.L. Bialka, A. Demirci, M. Karhan, 2010. Ethanol production from carob extract by using *Saccharomyces cerevisiae*. *Bioresource Technology*. 101: 5290–5296.
16. Yousif A. K., H. M. Alghzawi. 2000. Processing and characterization of carob powder. *Food Chemistry*. 69: 283–287.
17. Batlle I., J. Tous. 1997. Promoting the conservation and use of underutilized and neglected crops (carob tree, *Ceratonia siliqua* L.). International Plant Genetic Resources Institute (IPGRI), Rome, Italy.
18. Neukom H. 1989. Galactomannans: Properties and applications. *Lebensmittel Wiss. Technol.* 22: 41–45.
19. Sandolo C., T. Coviello, P. Matricardi, F. Alhaique. 2007. Characterization of polysaccharide hydrogels for modified drug delivery. *European Biophysics Journal*. 36 (7): 693–700.
20. Roukas T. 1998. Citric acid production from carob pod extract by cell recycle of Aspergillus niger. *Food Biotechnology*. 12: 91–104.
21. Hariri A., Ouis, N., Sahnouni, F., Bouhadi, D., 2009. Mise en oeuvre de la fermentation de certains ferments lactiques dans des milieux a base des extraits de caroube. Rev. microbiol. ind. san et environn., 37-55
22. AOAC (2007). International, Official methods of analysis, 18th edn. 2005; 2007 (On-line). AOAC International, Gaithersburg, MD.
23. Bradstreet, R. B. The Kjeldahl method for organic nitrogen. 1965, Academic Press: New York.
24. National Food Safety Standard of the People's Republic of China. 2010. Determination of protein in foods. GB 5009.5—2010.
25. Dubois M., K. Gilles, J. Hamilton, P. Rebers, F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 28(3): 350-356.
26. Lever M, 1972, A new reaction for colorimetric determination of carbohydrates. *Analytical Biochemistry*, *47*: 273-279.
27. Petkova N., R. Vrancheva, P. Denev, I. Ivanov, A. Pavlov, 2014. HPLC-RID method for determination of inulin and fructooligosacharides. *Acta Scientifica Naturalis*. 1: 99—107.
28. Ivanov I., R. Vrancheva, A. Marchev, N. Petkova, I. Aneva, P. Denev, V. Georgiev, Pavlov A., 2014. Antioxidant activities and phenolic compounds in Bulgarian Fumaria species. *International Journal of Current Microbiology and Applied Sciences*. 3(2), 296-306.
29. Kivrak I., M. Duru, M. Öztürk, N. Mercan, M. Harmandar, G. Topçu, 2009. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. *Food Chemistry*. 116 (2): 470-479.
30. Stintzing C., M. Nerbach, M. Mosshammer, R. Carle, W. Yi, S. Sellappan, C. Acoh, R. Bunch, P. Felker. 2005. Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. *Journal of Agricultural and Food Chemistry*. 53: 442-451.
31. Egorov A. V., N. M. Mestechkina, V. D. Shcherbukhin, 2003. Determination of the primary and fine structures of a galactomannan from the seed of *Gleditsia triacanthos* f. *inermis* L. *Applied Biochemistry and Microbiology*. 39 (4): 398–402.
32. Bouzouita N., A. Khadi, S. Zgoulli, L. Chebil, R. Chekki, M. M. Chaabouni, P. Thonart. 2007. The analysis of crude and purified locust bean gum: a comparison of samples from differentcarob tree populations in Tunisia. *Food Chemistry*. 101: 1508-1515.
33. Murdzheva D., N. Petkova, M. Todorova, I. Vasileva, I. Ivanov, P. Denev. 2016. Microwave-assisted synthesis of methyl esters of alginic acids as potential drug carrier. *International Journal of Pharmaceutical and Clinical Research*. 8(10): 1361-1368.
34. Robertson Fr., D.de Monredon, P. Dysseler, F. Guillon, R. Amado, J.-Fr. Thibault. 2000. Hydration properties of dietary fibre and resistant starch: a European collaborative study. *LWT - Food Science and Technology*. 33(2): 72-79.

35. Holloway W.D., R. I. Greig. 1984. Water holding capacity of hemicelluloses from fruits, vegetables and wheat bran. *Journal of Food Science*. 49: 1632-1633.
36. Youssef M. K., M. Moshera. El-Manfaloty, H.M. Ali. 2013. Assessment of proximate chemical composition, nutritional status, fatty acid composition and phenolic compounds of carob (*Ceratonia siliqua* L.). *Food and Public Health*. 3(6): 304-308.
37. Gaisford S. E, S.E. Harding, J.R. Mitchell, T.D. Bradley. 1986. A comparison between the hot and cold water soluble fractions of two locust bean gum samples. *Carbohydrate Polymers*. 6 (6): 423-442.
38. Herken E., N. Aydin. 2015. Use of carob flour in the production of tarhana. *Polish Journal of Food Nutrition Science*. 65 (3): 167–174
39. Avallone R., M. Plessi, M. Barldi, A. Monzani. 1997. Determination of chemical composition of carob (*Ceratonia siliqua*): protein, fat, carbohydrates, and tannins. *Journal of Food Composition and Analysis*. 10:166-172.
40. Ayaz F. A., H. Torun, R. Glew, Z.D. Bak, L. T. Chuang, J. M. Presley, R. Andrews. 2009. Nutrient content of carob pod (*Ceratonia siliqua* L.) flour prepared commercially and domestically. *Plant Foods for Human Nutrition*. 64: 286–292.
41. M. Khlifa, A. Bahloul, S. Kitane. 2013. Determination of chemical composition of carob pod (*Ceratonia siliqua* L.) and its morphological study. *Journal of Materials and Environmental Science*. 4(3): 348-353
42. Haddarah A., A. Ismail, A. Bassal, T. Hamieh, I. Ioannou, M. Ghoul. 2013. Morphological and chemical variability of Lebanese carob varieties. *European Scientific Journal*. 9:18
43. Şahin H., A. Topuz, M. Pischetsrieder, F. Özdemir, 2009. Effect of roasting process on phenolic, antioxidant and browning properties of carob powder. *European Food Research Technology*. 230:155–161.
44. Jambi H. A., 2015. Effect of roasting at 180˚C for 30 minutes on the sugars content of carob powder. *Life Science Journal*. 12(11): 21-24.
45. Kahkah El. R., R. Zouhair, M. Diouri, M. Ait Chitt, R. Errakhi. 2015. Morphological and biochemical characterization of Morocco carob tree (*Ceratonia siliqua* L.). *International Journal of Biological and Medical Research*. 6(2):4946-4952.
46. Gubbuk H., E. Kafkas, D. Guven, E. Gunes. 2010. Physical and phytochemical profile of wild and domesticated carob (*Ceratonia siliqua* L.) genotypes. *Spanish Journal of Agricultural Research*. 8(4): 1129-1136.
47. Owen R.W., R. Haubner, W.E. Hull, G. Erben, B. Spiegelhalder, H. Bartsch, B. Haber. 2003. Isolation and structure elucidation of the major individual polyphenols in carob fibre. *Food and Chemical Toxicology*. 41: 1727–1738.
48. Kumazawa S, M. Taniguchi, Y. Suzuki, M. Shimura, M-S. Kwon, T. Nakayama. T 2002. Antioxidant activity of polyphenols in carob pods. *Journal of Agricaltural and Food Chemistry*. 50:373–377
49. Benković M., A. Belščak-Cvitanović, I. Bauman, Dr. Komes, S. Srečec. 2017. Flow properties and chemical composition of carob (*Ceratonia siliqua* L.) flours as related to particle size and seed presence. *Food Research International*. doi: 10.1016/j.foodres.2017.08.048
50. Lopes da Silva, J. A., M. P. Goncalves. 1990. Studies on a purification method for locust bean gum by precipitation with isopropanol. *Food Hydrocolloids*. 4: 277–287.
51. Dakia, P. A., C. Blecker, C. Roberta, B. Watheleta, M. Paquota, 2008. Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocolloids*, 22:807–818.
52. Cunha P. L.R., I. G.P. Vieira, A. M.C. Arriaga, R.C.M. de Paula, J. P.A. Feitosa, 2009. Isolation and characterization of galactomannan from *Dimorphandra gardneriana* Tul. seeds as a potential guar gum substitute. *Food Hydrocolloids*. 23: 880–885.
53. Cunha, P. L. R., R. R.Castro, F. A. C.Rocha, R. C. M de Paula, J. P. A. Feitosa. 2005. Low viscosity hydrogel of guar gum: preparation and physicochemical characterization. *International Journal of Biological Macromolecules*. 37: 99–104.
54. Debon S. J. J., R. F. Tester. 2001. *In vitro* binding of calcium, iron and zinc by nonstarch polysaccharides. *Food Chemistry*, 73: 401–410.
55. Pollard M. A., R. Kelly, C. Wahl, P. Fischer, E. Windhab, B. Eder, R. Amadó, 2007. Investigation of equilibrium solubility of a carob galactomannan. *Food Hydrocolloids*. 21: 683-692.
56. Farahnaky A., N. Darabzadeh, M. Majzoob, Gh. Mesbahi. 2014. Physicochemical properties of crude and purified locustbean gums extracted from Iranian carob seeds. *Journal of Agricultural Science and Technology*. 16: 125-136.
57. Wielinga, W. C. and Maehall, A. G. 2000. Galactomannans. In: "Handbook of Hydrocolloids", (Eds.): Phillips, G. O. And Williams, P. A.. Woodhead Publishing Limited, North and South American, PP.472
58. Boulos N. N., H. Greenfield, R. B. H. Wills. 2000. Water holding capacity of selected soluble and insoluble dietary fibre. *International Journal of Food Properties*. 3(2): 217-231.
59. Petravić-Tominac V., V. Zechner-Krpan, K. Berković, P. Galović, Z. Herceg, S. Srečec, Ig.Špoljarić. 2011. Rheological Properties, Water-Holding and Oil-Binding Capacities of Particulate b-Glucans Isolated from Spent Brewer's Yeast by Three Different Procedures. *Food Technology and Biotechnology*. 49 (1): 56–64.