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ORIGINAL ARTICLE

Evaluation of chemical composition, antioxidant potential and functional properties of carob (*Ceratonia siliqua* L.) seeds

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Abstract The interest in carob pod as an ingredient of functional foods is constantly increasing due to its beneficial health effect and functional properties. The aim of this study was to evaluate the chemical composition and antioxidant potential of carob seeds, as well as the functional properties of its isolated galactomannan fraction. The lipid, protein, carbohydrate and phenolic composition were analysed. The obtained results demonstrated that the main detected fatty acids were oleic (45.0%), linoleic (32.4%), and palmitic (16.6%) acid. The primary tocopherol in the tested tocopherol fraction was γ-tocopherol (53.1%). It was established that β-sitosterol (74.2%) and stigmasterol (12.8%) predominated in the sterol fraction. Carob seed was characterized by high protein (25.7%) content, while mannose and galactose were the dominating monosaccharides. Moreover, the isolated galactomannan from carob seed demonstrated good swelling properties—30.1 ml per g sample and oil-holding capacity (27.9 g/g sample). The total polyphenolic and flavonoids content of carob seeds was 1.76 mg Gallic acid equivalent/g dry weight and 0.30 mg quercetin equivalents/g dry weight, respectively. The carob seeds showed the highest antioxidant potential by cupper reduction assay—15.71 mM Trolox® equivalent/g dry weight. The mineral composition was also defined as the macroelements Ca and Mg were the predominant minerals in the seed. The obtained results showed that carob seeds were a valuable source not only of phenolic compounds and antioxidants, but also of proteins, lipids, galactomannan with functional properties that could improve the nutritional value of foods in which are incorporated.

Keywords *Ceratonia siliqua* L. · Seeds · Chemical composition · Antioxidant activity

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Introduction

Nowadays, there is a growing interest in supplements derived from natural, traditional and non-traditional foods as possible sources of biologically active substances with proven health properties for inclusion in the human diet (Baumel et al. 2018).

The carob tree (*Ceratonia siliqua* L.) holds potentially significant importance for the food industry due to its chemical constituents, flavoring properties, and nutrition benefits. This tree thrives in the semi-arid growing conditions of the Mediterranean region and has an annual worldwide production of over 315,000 tons of carob products (Baumel et al. 2018). The carob bean consists of pulp (90%) and seeds (10%) with the seeds being obtained after the carob pods are broken. Carob seeds are characterized by a brown colour, significant hardness, a length of about 10 mm, and a weigh of about 0.2 g per seed. The manufacture of primary carob products such as flour, powder, and syrup requires pre-separation of seeds from the pod, and the carob seeds remain as a by-product or food waste (Mekhoukhe et al. 2018).

Carob seeds consist of three layers—an outer shell, the endosperm, and the embryo with approximate proportions of 30–35% shell, 40–50% endosperm, and 20–25% embryo. Carob was characterized by a high diversity in the yield of pulp and seeds (El Batal et al. 2016). The carob germ (consisting of fine fragments of husk and endosperm) is 8.3% moisture, 6.5% ash, 6.6% lipids (neutral and polar) containing approximately 21% polar lipids, 54.7% crude proteins, and has an energy value of 17.5 kJ/g (Dakia et al. 2007, 2008; Matthaus and Özcan 2011; Mahtout et al. 2018). The carob fruit has been the subject of research with regard to its application in various industrial sectors (Papaefstathiou et al. 2018). Due to differences in the composition and functional properties, carob pulp and seeds are used separately in the production of many products. For example, seed separation is warranted because the difference in carbohydrate composition would alter the solubility and sweet taste of the carob flour and syrup. Another reason for seed exclusion is the difference in the composition and the amount of fats contained in the pulp and seeds that determine the rate of oxidative changes occurring in the end product (Mekhoukhe et al. 2018).

The composition of the carob seed includes approximately 9% moisture, 1% ash, 1% protein, 1.1% fat, 0.4% sucrose, 0.1% D-glucose, fructose, 0.1% starch, and a total phenols content of 0.661 mg/g. In the endosperm, the main polysaccharide is a galactomannan (Dakia et al. 2007). Carob seeds are primarily used for production of locust gum (galactomannan) which is widely used as a stabilizer in various commercial applications in the food, pharmaceutical, cosmetic, and biotechnology industries. Commercial production and processing of carob pod was presented (Fig. 1). Locust gum is obtained by grinding the endosperm layer that is situated between the shell and embryo of the seeds. It is a globally approved food additive, proved with its properties to improve the nutritional properties as used in different food matrixes. This gum is the most important constituent of many products such as yogurt, pudding, cheese, water-based jellies, candies, fish products, ketchup, mayonnaise, bakery products, and frozen foods. Carob byproducts are supplemented in diets as an inexpensive feed source (Calislar and Kaplan 2017).

The fatty acid composition of the seed germ of *C. siliqua* includes palmitic (16.2%), stearic (3.4%), oleic (34.4%), and linoleic (44.5%) acids (Dakia et al. 2007). Fatty acids are essential for human health; consequently, the quantitative and qualitative composition of the unsaturated fatty acids of seeds determines the possibility of their application in different food products with pronounced functional properties and proven health benefits.

The attention in carob pulp as a component of various food products is continuously increasing due to its beneficial health effects. However, most of the researches were devoted to the characterization of whole carob seeds or some phytochemical components. Therefore, the complex approach to detailed analysis of carob seeds was necessary to be implemented. The objective of this study was to evaluate the chemical composition and antioxidant potential of carob seeds, including their functional properties (especially swelling, oil and water-holding capacity).

Materials and methods

Plant material

Ceratonia siliqua pods were purchased from local markets in Mersin, Turkey during early September, 2018, and kept in refrigerated storage (4–6 °C) until being analyzed. The pods were separated; the carob seeds were removed and placed in paper sacks in order to allow adequate air circulation, and then stored in a cool, dark place. The seeds were finely ground in a laboratory mill (Clatronic KSW 3307 Grinder) and then sieved through a laboratory sieve to the final particle size of 0.5 mm.

The moisture content of the seeds (10.8 ± 0.09%) was determined by drying the seeds up to a constant weight at 105 °C (Nielsen 2010). The biologically active substances in the samples were analyzed and the values were presented on the basis of absolute dry weight.

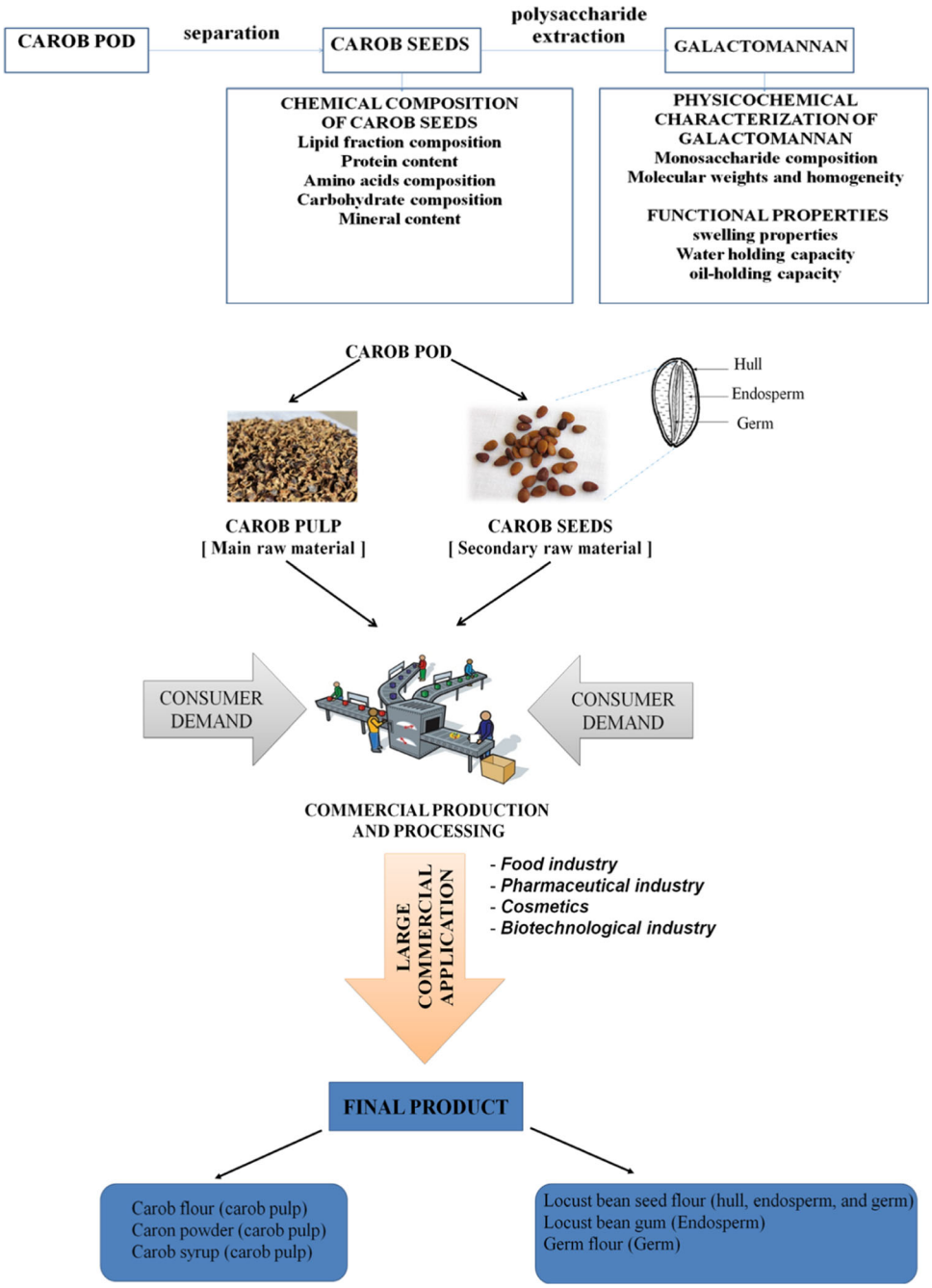

Fig. 1 Commercial production and processing of carob pod

Lipid composition

The lipid fraction was extracted with *n*-hexane in a Soxhlet apparatus for 8 h. (ISO 659:2014).

Fatty acids

The total fatty acid composition of the lipid fraction was determined (ISO 12966-2:2011). The identification was performed by comparing the retention times with those of a standard mixture of FAME injected into GC under identical experiment conditions (ISO 12966-1:2014).

Tocopherols

Tocopherols were determined directly in the lipids by HPLC analysis (ISO 9936:2016). The instrument Merck-Hitachi (Merck, Darmstadt, Germany) was equipped with 250 mm × 4 mm Nucleosil Si 50-5 column and fluorescent detector Merck-Hitachi F 1000. The operating conditions were mobile phase of *n*-hexane: dioxane, 96:4 (v/v)

and flow rate 1 ml/min, excitation 295 nm, emission 330 nm. 20 µl 2% solution of crude oil in *n*-hexane were injected. Tocopherols were identified by comparing the retention times with those of authentic individual ones. The tocopherol content was calculated on the base of tocopherol peak areas in the sample vs. tocopherol peak area of standard tocopherol solution (ISO 9936:2016).

Sterols

The nonsaponifiable lipid fraction was determined by weighing after saponification of the lipids with 2 N KOH in ethanol, extraction with *n*-hexane (ISO 18609:2000), and taking into consideration ISO 12228-1:2014. Sterol composition was determined on HP 5890 gas chromatograph (Hewlett Packard GmbH) equipped with 25 m × 0.25 mm DB—5 capillary column and flame ionization detector. The temperature gradient was from 90 °C (hold 2 min) up to 290 °C at a rate of change 15 °C/min and then up to 310 °C a rate of 4 °C/min (hold 10 min); the detector temperature was 320 °C; the injector temperature was 300 °C and carrier gas was hydrogen. The identification was confirmed by comparison of retention times with those of a standard mixture of sterols (ISO 12228-1:2014).

Protein content

The total protein content was analyzed according to the method of AOAC 976.06 (2016). The sample (1.0000 g) was mineralized with concentrated H_2SO_4 (15 ml) and catalysts: anhydrous K_2SO_4 and $CuSO_4$. The process was run at 420 °C for 60 min. With this method, 40% NaOH was used to produce an alkaline distillation medium and 4% H_3BO_3 in order to collect the distilled ammonia. The titrations were carried out with a standard HCl (0.1 N) solution.

Amino acid composition

The protein was hydrolyzed to free amino acids as 30 mg of dried seeds were placed in a glass ampule with a 3 ml 6 N HCl solution. The ampule was thoroughly sealed and left in a drying chamber at 105 °C for 24 h. The ampule content was then transferred to a crystallizer and dried in a vacuum chamber at 40–50 °C. After the evaporation of the water, the residue was fully diluted in 2 ml 20 mM HCl. The solution was filtered through a paper filter and 20 µl of the collected filtrate was derivatized with an AccQ-Fluor kit (WATO52880, Waters Corporation, USA). Initially, 60 µl of AccQ-Fluor borate buffer was added to the filtrate and homogenized. Then, 20 µl of AccQ-Fluor reagent was added and the sample was homogenized again for 30 s. Before injection, the solution was heated in a water bath at 55 °C. The resulting AccQ-Fluor amino acid derivatives were separated by an ELITE LaChrome high-performance liquid chromatography (HPLC) (Hitachi) equipped with a diode array detector (DAD) and a reverse phase column C18 AccQ-Tag (3.9 mm × 150 mm) operating at 37 °C. The volume of the injected sample was 20 µl and the elution was made with a gradient system of two mobile phases: A—buffer (WATO52890, Waters) and B—60% acetonitrile. Amino acids were detected at 254 nm. Subsequently, the chemical score was calculated.

Carbohydrate composition of carob seeds

Sugar composition

The determination of sugars present in the water extract of carob seeds was performed on an HPLC Elite Chrome Hitachi instrument with a refractive index detector (RID) Chromaster 5450 operating at 35 °C with a Shodex® Sugar SP0810 column (300 mm × 8.0 mm i.d.) with Pb^{2+} and a Shodex SP—G guard column (5 µm, 6 × 50 mm) maintained at 85 °C. The separation was performed with mobile phase distilled water at a flow rate of 1.0 ml/min, and the sample injection volume was 20 µl (Petkova et al. 2017).

Isolation of polysaccharides from carob seeds flour

The polysaccharide extraction of carob seeds was performed with ethanol and distilled water as previously described (Bouzouita et al. 2007).

Determination of monosaccharide composition and mannose/galactose ratio into carob galactomannan

The isolated polysaccharide from carob seeds (100 mg) was hydrolyzed with 3 ml 1 M H_2SO_4 at 100 °C for 2 h (Bouzouita et al. 2007). 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 Shodex SP—G guard column (5 µm, 6 × 50 mm) at 85 °C with mobile phase distilled water at a flow rate of 0.5 ml/min (Petkova et al. 2017).

Homogeneity and molecular weight

The number average molecular weight (M_n) and weight average molecular weight (M_w) of carob seed polysaccharides were determined by high-performance size exclusion chromatography (HPLC-SEC). The separation was performed using an HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) equipped with a Shodex OH-pack 806 M column (ID 8 mm and length 300 mm;

Shodex Co., Tokyo, Japan) and an RI detector (VWR Hitachi Chromaster, 5450, Japan), both operating at 30 °C with 0.1 M $NaNO_3$ in mobile phase with a flow rate of 0.8 ml/min. The injection volume of samples (3 mg/ml in 0.1 M $NaNO_3$) was 20 µl. Pullulans with a known molecular weight were used for the calculation and the polydispersity index was calculated accordingly (Murdzheva et al. 2016).

Functional properties

Swelling properties

Galactomannan (100 mg dry weight) was hydrated with 10 ml distilled water in a calibrated cylinder (1.5 cm diameter) at room temperature (25 ± 1 °C). After equilibration (18 h), the bed volume was recorded and expressed as volume/g polysaccharide dry weight (Robertson et al. 2000). The analysis was performed in duplicate.

Water holding and oil holding capacities

The water holding capacity (WHC) and oil holding capacity (OHC) of carob seed polysaccharides were determined in duplicate (Holloway and Greig 1984).

Determination of total polyphenol (TPC), flavonoids content, and antioxidant activity of carob seeds

Determination of total polyphenolic content (TPC)

The total polyphenol content was measured at 765 nm according to the Folin–Ciocalteu method (Kujala et al. 2000). The TPC was expressed as mg Gallic acid equivalent (GAE) per g dry weight (dw).

The total flavonoids content

The total flavonoids content was analyzed by $Al(NO_3)_3$ reagents and measured at 415 nm against a blank (Kıvrak et al. 2009). The results were presented as mg quercetin equivalents (QE) per g dry weight.

Antioxidant activity

DPPH radical scavenging activity The DPPH radical scavenging activities of carob seeds were evaluated (Brand-Williams et al. 1995), and the absorbance was measured at 517 nm. The radical scavenging activity of carob seeds was expressed as mM Trolox® equivalent (TE) per g dry weight.

ABTS radical action decolourization assay The scavenging activity against radical cation 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) was estimated (Re et al. 1999), and the results were expressed as mM Trolox® equivalent per g dry weight.

Ferric reducing antioxidant power assay (FRAP) The FRAP method was performed as previously described (Benzie and Strain 1996) and the absorbance was recorded at 593 nm. The results were expressed as mM Trolox® equivalent per g dry weight.

Cupper reduction assay (CUPRAC) The CUPRAC assay was used (Ak and Gülçin 2008) and the absorbance was measured at 450 nm. Trolox® was used as a standard and the total antioxidant activity was expressed as mM Trolox® equivalent per g dry weight.

Mineral analysis

Macroelements (calcium, magnesium) and microelements (iron, copper, manganese, zinc, boron) in the carob seeds were determined according to a validated laboratory method (in Food Research and Development Institute Plovdiv). Samples were washed with tap water and deionized water. Carob seeds samples (1 g) were treated with 2 to 3 ml of 0.2% HNO_3 and 2 to 3 ml H_2O_2 in a hermetically sealed Teflon pressure vessel. The mineralized sample was filtered through a paper filter into a 10 ml volumetric flask which was then filled with 0.2% HNO_3. ICP-OES Spectroflame (Spectro Analytical Instruments) with a monochromator, linear range of 165 to 440 nm, Nebulizer type Minhard, coolant gas—42 bar, and auxiliary gas—26 bar, is based on the pulverizing of studying products by acid mineralization in an inductively coupled plasma. This process results in the excitation of the atoms of the chemical elements and subsequent measurement of their specific emission at specific wavelengths. The measured intensities were compared with the intensity of a series of standard solutions containing determined elements measured under the same conditions.

Statistical analysis

The measurements were performed in triplicate and the results were presented as the mean value of the individual measurements with the corresponding standard deviation (SD), using Microsoft Excel.

Results and discussion

Lipid fraction composition

The lipid fraction content of carob seeds was determined as 2.1 ± 0.01%. The extracted lipid fraction was observed to be a yellow liquid with a specific odor and was composed of nonsaponifiables (17.2 ± 0.13%), sterols (4.0 ± 0.04%), phospholipids (7.2 ± 0.07%), and tocopherols (2801.0 ± 40.16 mg/kg). Matthaus and Özcan (2011) determined the total tocopherol (208.45–223.14 mg/100 g) and sterols (16400.94–30191.55 mg/kg) content from the seeds of wild and cultivated carobs (*C. siliqua*) from Turkey that was lower than the sterol content in the investigated fractions in our study. Due to the biological function and promising potential as cholesterol lowering effect, anti-cancer, antiatherosclerosis, anti-inflammation, and antioxidant activities, the consumption of plant sterols from natural sources should be encouraged. Our results confirm that carob seeds are good natural source of plant sterols and could be used for enrichment of food products.

Fatty acids

The fatty acid composition of the lipid fraction is presented in Table 1. The data showed that 11 fatty acids were detected in the lipid fraction samples, constituting 100% of the total oil content. Three groups of fatty acids were identified—with high, average and low amounts of fatty acids. The primary fatty acids were oleic (45.0 ± 0.42%) and linoleic (32.4 ± 0.30%). Palmitic (16.6 ± 0.15%) and stearic (4.7 ± 0.15%) fatty acids were of average amounts. The saturated-to-unsaturated fatty acids ratio was 21.9:78.1. It was identified that 21.9% of the fatty acids were saturated fatty acid with palmitic and stearic acids, and all other saturated fatty acids combined contributed less than 1% of the total fatty acids. The monounsaturated-to-polyunsaturated fatty acids ratio was 45.4:32.7. Data obtained from other studies showed a high diversity in the fatty acid composition of carob seeds. For example, previous studies (Dakia et al. 2007) showed that the oil of *C. siliqua* seed germ contained oleic (34.4–38.5%) and linoleic (43.6–44.5%) acids at relatively high levels. The main fatty acids in cultivated and wild carob seed oils from Turkey were determined to be linoleic (49.1% and 51.0%), oleic (30.4% and 26.5%), palmitic (10.3% and 12.0%), and stearic (3.5% and 4.6%), respectively (Matthaus and Özcan 2011). Our results for oleic (45.0 ± 0.42%) fatty acids in the carob seed were higher than those reported in the literature. Bojilov et al. (2013) studied the chemical composition of *Gleditsia triacanthos* L. belonging to the family of Fabaceae and reported that the correlation between unsaturated and saturated fatty acids was 74.5: 25.5. Their results showed that 13 types of fatty acids were detected in the oil and the linoleic acid (54.5%) predominated in the oil followed by palmitic (17.1%), oleic (18.6%) and stearic (7.5%) acid. The results show that carob seed oil is rich in unsaturated fatty acids. Dietary lipids supply the essential fatty acids needed by the body to maintain proper health and functioning. Essential fatty acids are essential to human health but cannot be produced by the body; consequently, these fatty acids must be obtained from food. Essential fatty acids insulate the body against heat loss, prevent the skin from drying, and have an important role in cardiovascular and immune system functioning. Omega-6 fatty acids are represented by linoleic acid and omega-3 fatty acids by α-linolenic acid. The balance of omega-6/omega-3 fatty acids is an important determinant in decreasing the risk of a coronary heart disease, both in the primary and secondary prevention of the disease. Because of the increased amounts of omega-6 fatty acids in the modern diet, the eicosanoid metabolic products are formed in larger quantities than those formed from omega-3 fatty acids. Moreover, a high linoleic acid intake interferes with the desaturation and elongation of α-linoleic acid. Studies show that it is essential to increase the omega-3 and

Table 1 Fatty acid, tocopherol, and sterol composition of the lipid fraction

Fatty acids, % (w/w)		
$C_{12:0}$	Lauric	0.1 ± 0.00
$C_{14:0}$	Myristic	0.2 ± 0.00
$C_{15:0}$	Pentadecanoic	0.1 ± 0.00
$C_{16:0}$	Palmitic	16.6 ± 0.15
$C_{16:1}$	Palmitoleic	0.2 ± 0.00
$C_{17:0}$	Margaric	0.3 ± 0.00
$C_{17:1}$	Heptadec-10 enoic	0.1 ± 0.00
$C_{18:0}$	Stearic	4.7 ± 0.05
$C_{18:1}$	Oleic	45.0 ± 0.42
$C_{18:2}$	Linoleic	32.4 ± 0.30
$C_{18:3}$	Linolenic	0.2 ± 0.00
Tocopherols, % (w/w)	α-Tocopherol	41.1 ± 0.40
	β-Tocopherol	1.7 ± 0.20
	γ-Tocopherol	53.1 ± 0.50
	δ-Tocopherol	4.1 ± 0.10
Sterols, % (w/w)	Cholesterol	0.8 ± 0.00
	Campesterol	3.8 ± 0.04
	β-Sitosterol	74.2 ± 0.22
	Stigmasterol	12.8 ± 0.08
	Fucosterol	7.7 ± 0.08
	Δ^5-Avenasterol	0.7 ± 0.00

decrease the omega-6 fatty acid intake in order to have a balanced omega-6 and omega-3 intake in the diet. The obtained values determined that carob seed oil is rich in omega-6 but unfortunately deficient in omega-3 fatty acids.

Tocopherols

The tocopherol composition of the lipid fraction is presented in Table 1. The results show that γ-tocopherol (53.1 ± 0.50%) and α-tocopherol (41.1 ± 0.40%) are the major tocopherols in carob seed oil. Tocopherols have antioxidant properties that protect against oxidative damage that is associated with a number of chronic disease states, including cardiovascular diseases and cancer (Matthaus and Özcan 2011). The alpha form of tocopherol predominates in the blood and tissue, although the absorption of all tocopherol homologues in the diet is comparable. Matthaus and Özcan (2011) demonstrated that the major tocopherol in cultivated and wild carob seed oils from Turkey was γ-tocopherol. Our values are barely distinguishable from (Matthaus and Özcan 2011) who reported the tocopherol composition as follows: α-tocopherol, δ-tocopherol (8.70 mg/100 g and 10.66 mg/100 g) and β-tocopherol (2.30 mg/100 g and 1.85 mg/100 g). The composition of tocopherols obtained in this study was different from *Gleditschia triacanthos* oil (Fabaceae family) where the content of α-tocopherol predominated in tocopherols fraction (85.5%), followed by β- γ- and δ-tocopherols (Bojilov et al. 2013).

Sterols

The individual sterol composition of the lipid fraction is summarized in Table 1. The results show that *β*-sitosterol (74.2 ± 0.22%) was the predominant sterol that accounted for the total sterols followed by stigmasterol (12.8 ± 0.08%). It has been shown that *β*-sitosterol has biological activities such as inhibition of oxidative reactions and inhibition of carcinogenesis. Sterols have a similar structure to that of cholesterol, but have different side chain configurations that inhibit the absorption of LDL cholesterol (Azab 2017). This composition was different from *Gleditschia triacanthos* seed oil (from Fabaceae family) where the content of *β*-sitosterol (86.3%) and cholesterol was higher than the obtained data about carob seed oil (Bojilov et al. 2013).

In their lipid evaluation of cultivated and wild carob seeds from Turkey, Matthaus and Özcan (2011) showed that the total content of sterols of both oils were determined as 16400.94 mg/kg and 30191.55 mg/kg, with β-sitosterol as the predominant sterol (78.62–72.04%) that accounted for more than 70% of the total amount of other sterols.

Protein content

Carob seeds are characterized by high protein content (25.7 ± 0.18%). Moderate amounts of protein (18.6 ± 0.3%) were reported in carob seeds that grow naturally in the Tazmalt region of northern Algeria (Mahtout et al. 2018). The differences in chemical composition between the present investigation and the reported data may be due to the plants' environmental conditions. Carob seeds could be used as a food additive in order to enrich the protein content and enhance the biological activity of the food system. The expressed hydrophilic-lipophilic properties of seeds and their amino acid composition constitute an important physiological feature that emphasizes their widespread use of these seeds.

Amino acid composition

The amino acid composition of the protein fraction is summarized in Table 2. A significant amount of nonessential amino acids like arginine (27.8 ± 0.25 g/100 g), alanine (17.0 ± 0.16 g/100 g), and the essential amino acid lysine (15.0 ± 0.14 g/100 g) was detected. Moderate amounts of essential amino acids isoleucine (8.6 ± 0.08 g/100 g) and valine (7.3 ± 0.06 g/100 g) were presented in carob seeds. Our results contrast with the values obtained by Dakia et al. (2007), in which glycine (2.8 ± 0.1 g/100 g), arginine (7.3 ± 0.1 g/100 g), alanine (2.4 ± 0.02 g/100 g), proline (1.9 ± 0.02 g/100 g),

Table 2 Amino acid composition of the protein fraction

Amino acids	Content, g/100 g	Chemical score
Asparagine	0.9 ± 0.00	
Serine	1.0 ± 0.00	
Glutamic acid	0.9 ± 0.00	
Glycine	3.4 ± 0.02	
Histidine	1.3 ± 0.01	
Arginine	27.8 ± 0.25	
Thryptophan	7.3 ± 0.06	2.2
Alanine	17.0 ± 0.16	
Proline	9.4 ± 0.09	
Cysteine	0.4 ± 0.00	
Tyrosine[a]	7.8 ± 0.07	
Valine	7.3 ± 0.06	2.1
Threonine	1.1 ± 0.00	
Methionine	2.1 ± 0.02	
Lysine	15.0 ± 0.14	2.6
Isoleucine	8.6 ± 0.08	3.1
Leucine	1.6 ± 0.01	1.6
Phenylalanine[a]	0.8 ± 0.00	1.4

[a]Tyrosine + phenylalanine

tyrosine (1.8 ± 0.1 g/100 g), and lysine (3.2 ± 0.01 g/100 g) are significantly lower than our values. The results showed that essential amino acids were presented in high amounts. Carob seed protein is complete because it contains all of the essential amino acids needed to support the protein synthesis and the determination of the quality of protein.

Carbohydrate composition of carob seeds

Table 3 shows the physicochemical characterization of galactomannan isolated from carob seed flour. Only sucrose (8.1 ± 0.04%) and glucose (2.2 ± 0.01%) were found in the water extract of carob seeds. The quantity of sucrose was higher than glucose. Fructose was not detected. The monosaccharide composition after hydrolysis of polysaccharide isolated from carob seeds included mannose (54.0 ± 0.50%), galactose (15.5 ± 0.14%), glucose (2.2 ± 0.01%), arabinose (1.0 ± 0.00%), and xylose (0.4 ± 0.00%). Mahtout et al. (2018) reported amounts of sucrose (7.2 ± 1.8%) and glucose (0.4 ± 0.1%) in Algerian carob seeds which were comparable with our reported values for free sugars in carob seeds of Turkish origin. In comparison with carob flour prepared from pulp and seeds (Petkova et al. 2017), sugars values were significantly lower.

The results confirmed that mannose and galactose were the dominating monosaccharides (Table 3). The ratio mannose/glucose was 3.5 for isolated polysaccharide from carob seeds after purification with acetone. Carob seed galactomannans differ in their mannose/glucose ratio, depending on the origin, the variety and age of the plant (tree), the growing conditions (climate, soil), and the method used for extraction of the polysaccharide in terms of purification of crude gum (Dakia et al. 2008). Bouzouita et al. (2007) presented 3.55–4.32 mannose/galactose ratios that are in agreement with our results. The average degree of substitution of galactose is an important characteristic of the molecular structure of the galactomannan polysaccharide family. In our case, galactose was near to previously reported carob galactomannan (assumed as galactose 0.2–0.4) (Petkova et al. 2017).

Table 3 Physicochemical characterization of galactomannan isolated from carob seed flour

Monosaccharide composition, % dw	
Arabinose	1.0 ± 0.00
Glucose	2.2 ± 0.01
Galactose	15.5 ± 0.14
Sucrose	8.1 ± 0.04
Mannose	54.0 ± 0.50
Xylose	0.4 ± 0.00
Molecular weights and homogeneity	
Weight-average molecular weights (Mw), kDa	1801.9a (61.1)b
Number-average molecular weights (Mn), kDa	1468.0a (54.7)b
Polydispersity index	1.23a (1.12)b

a—Fraction 1, b—Fraction 2

Homogeneity and molecular weight

The weight-average molecular weight (M_w), the number-average molecular weight (M_n) and the polydispersity of isolated galactomannan from carob seeds were determined (Table 3). Two fractions were observed from HPLC-SEC chromatograms. The first one was characterized by the highest weight-average molecular weight of about 1801.9 kDa and the number molecular weight—1468.0 kDa, while the values for M_w and M_n of the second fraction were significantly lower—61.1 kDa and 54.7 kDa. These results were in agreement with the molecular weight characteristics of isolated galactomannans from carob flour (Pollard et al. 2007). The chain length polydispersity of carob polysaccharides fractions, as estimated by the HPLC-SEC analysis, is in the range of 1.23–1.12 for the first and second fraction. Our data coincided with some previous reports for narrow range 1.1–1.3 (Pollard et al. 2007; Petkova et al. 2017).

Functional properties

Figure 2 shows the functional properties of isolated galactomannans from carob seeds. The sample demonstrated very good swelling properties—30.1 ± 0.01 ml per g sample and oil-holding capacity (27.9 ± 0.05 g per g sample), followed by the water holding capacity (8.3 ± 0.02 g per g sample) respectively.

Water holding capacity of isolated polysaccharide from carob flour is higher than this of locust bean gum 565 g/

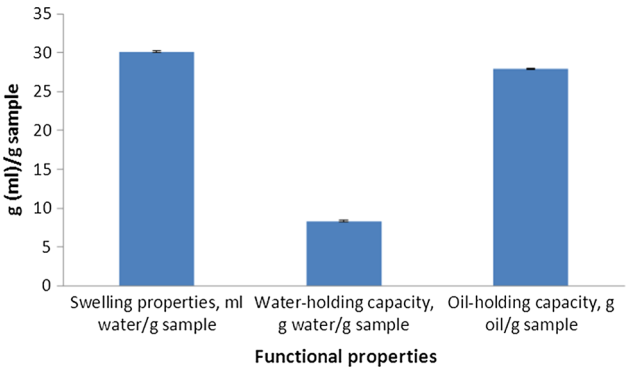

Fig. 2 Functional properties of isolated galactomannan from carob seeds

Table 4 Total phenolic content (mg GAE/g dw), flavonoid content (mg QE/g dw) and antioxidant activity (mM TE/g dw) of carob seed (mean ± SD)

Sample	TPC, mg GAE[a]/g dw[b]	TFC, mg QE[c]/g dw	Antioxidant activity, mM TE/g dw			
			DPPH	ABTS	FRAP	CUPRAC
Carob seed	1.76 ± 0.29	0.30 ± 0.02	3.34 ± 0.12	14.01 ± 0.67	6.19 ± 0.25	15.71 ± 2.81

[a]GAE—gallic acid equivalent, [b]dw—dry weight, [c]QE—quercetin equivalent

TPC, TFC: total phenolic content and total flavonoid content, respectively

100 g (Boulos et al. 2000) and carob flour 1.4 ± 0.2 g/100 g (Petkova et al. 2017). However, the isolated polysaccharide showed better oil-holding capacity (27.9 ± 0.05 g per g sample) than water-holding capacity. The soluble and insoluble dietary fiber content is a potential opportunity for the application of carob seeds as a functional component in a number of food systems. This is an important property for application of carob flour in foods and pharmaceuticals with high lipid content.

Determination of total phenolic, flavonoids content, and antioxidant activity of carob seeds

Total phenolic, flavonoids content, and antioxidant activity of carob seeds were evaluated (Table 4). The total polyphenolic and flavonoid content of carob seeds was 1.76 mg GAE/g dw and 0.30 mg QE/g dw respectively. The carob seeds showed low antioxidant potential that was evaluated by four methods as follows: DPPH, FRAP, ABTS, and CUPRAC respectively. Our results for carob seeds collected from Turkey were lower than reported values for different extracts for carob seeds from Morocco (El Kahkahi et al. 2015). In contrast, the authors (Fadel et al. 2011) reported that there was no significant difference between the total phenols of pulps and seeds. El Khamlichia et al. (2017) studied the phenolic profile, the flavonoid content, and the antioxidant activity of methanol and the ethyl acetate extracts of the seeds of *Calycotome villosa*, belonging the family of Fabaceae, and the results show that methanol extracts were with higher values than that reported for carob seeds extracts.

Mineral analysis

The values of two macro elements (Ca and Mg) and four microelements minerals (Fe, Cu, Mn, B, and Zn) in carob seed were determined. The data revealed that carob seeds could be considered as a source of Ca (8300.0 ± 30.0 mg/kg dw) and Mg (894.0 ± 3.0 mg/kg dw). The amounts of other minerals were considerably lower with concentrations of Mn (188.0 ± 1.00 mg/kg dw), Zn (124.0 ± 0.5 mg/kg dw), B (90.0 ± 0.4 mg/kg dw), Fe (71.0 ± 0.2 mg/kg dw), and Cu (49.0 ± 0.1 mg/kg dw). Our results are in agreement with those previously reported by El Bouzdoudi et al. (2017) for Fe (69.4 mg/kg dw) but different for Mn (12.7 mg/kg dw), Zn (29.5 mg/kg dw), and Cu (12.5 mg/kg dw). Some differences in the results could be due to the influence of environmental factors as the pods investigated in different studies were collected from different regions with specific climatic conditions at different times of the year.

Conclusion

Based on our results, carob seeds are a by-product with high nutritional value and promising functional properties, and should therefore be considered as an additive in food and pharmaceutical products. Carob seeds have balanced protein content, galactomannans with good swelling and oil-holding capacities, phenolic content, and important Ca macroelements and Mg microelements. This chemical composition of carob seeds reflects the nutritional, dietary, and antioxidant potential of this product. However, further research on carob seeds is warranted. For example, considering the high level of protein in carob seeds, it would be interesting to determine the protein digestibility and bioavailability of amino acids as basic parameters in determining the quality of protein source. The soluble and insoluble dietary fiber content of carob seeds could be determined due to its potential application as a functional component in a number of food systems. In addition, our results provide the basis for further study of the application of carob seeds in food in which a cause-effect relationship could be established between the functional properties of carob and the quality of the final products. Carob seeds are thus a prime potential additive or ingredient for application in the food industry as a value-added product in the design of new functional foods and could be used in optimizing the formulation of functional foods and nutritional supplements.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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