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ORIGINAL PAPER

Chemical composition and antioxidant activity of commercial flours from *Ceratonia siliqua* and *Prosopis* spp.

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Abstract

Ceratonia siliqua and some species of *Prosopis* (*Fabaceae* family) are commonly known as carob trees. The flours obtained from their pods are used in the food industry, as cocoa substitute in the confectionery and also used in beverages and mixed with products derived from cereals. The aim of this study was to compare and characterize the physical and chemical properties, specially the antioxidant activities, of the two commercial carob flours. Commercial *Prosopis* spp. (mainly from *P. alba*) flour exhibited high content of protein, starch and fat, while commercial flour from *C. siliqua* had a lower content of these compounds, but higher antioxidant activity. By nuclear magnetic resonance (NMR) the aqueous extracts of the two carob flours were analysed and concluded that they had similar content of sucrose, but *C. siliqua* had more monosaccharides and pinitol. This important cyclitol has beneficial physiological effects, improving the glycaemic level and, thus, having a great potential in the food industry. We conclude that the commercial flour of *C. siliqua* has a better nutritional potential than that of *Prosopis* spp., owing to dietary fiber, total phenols, pinitol and antioxidant activity. Our results corroborate the nutritional benefits of the commercial supplements already available for healthy food formulations.

Keywords Food quality · Phenolics · Chromatography analysis · Sugars · Antioxidants

Introduction

Ceratonia siliqua and some *Prosopis* species are trees of the *Fabaceae* family that are known as have high ecological value and useful agroforesty characteristics. They are extremely resistant to the adverse environment (heat, drought, soil, alkalinity and salinity) and contribute to soil stabilization and improvement and to sustainable agriculture [1].

Ceratonia siliqua is abundant and adapted to the Mediterranean area of poor soils, harsh summer conditions and mild winters, and is also grown in Mediterranean type climates of South Africa, North America and Australia [2]. The world production of Ceratonia carob is estimated for 2016 as about 158,609 t/year, in an area of approximately 66,874 ha with highly variable yield that depends on cultivar, region, climate and farming practices [3–5]. Europe produces about 68% of the total, mainly in Portugal, Italy, and Spain, Asia and Africa produce each about 16%, mainly in Turkey and Morocco [5].

Prosopis spp. grow in a large American area that extends from the south-western part of the United States to the Patagonia. In Argentina they are characteristic of the Monte desert, from Salta to Chubut provinces where *P. alba* and *P. nigra* are the main *Prosopis* species used for food [6].

Carob pods of both *Ceratonia* and *Prosopis* species are constituted by about 90% pulp and 10% seeds [7, 8].

Unripe *Ceratonia* pods are green, succulent and very astringent, turning sweet when ripe [9, 10]. Ripe pods (Fig. 1a) are dark brown with an elongated, straight or curved form, and attain 25 cm in length, 4 cm in width, 1.3 cm in thickness and 5–30 g in weight [11]. Each pod has 9–12 seeds that weight between 150 and 250 mg each.

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Fig. 1 Pods and seeds of *C. siliqua* (**a**) and *P. alba* (**b**). (Color figure online)

Ripe *Prosopis alba* pods (Fig. 1b) are straw-colored, very compressed, with a linear or arched form, 12–25 cm long, 1.2–1.8 cm wide, 0.5–0.6 cm thick and weighing on average 10 g. Each pod has 28–32 seeds that weight about 45 mg each [12, 13].

For a long time *C. siliqua* seeds have been the source of locust bean gum, one important food additive (E 410), used as thickening agent and stabilizer and also in the textile industry [7]. The flour from the seed germ of *C. siliqua*, has about 50% protein that has been used to produce protein isolates rich in arginine and glutamine (for instance Glutarcarob) with great potential for sportsman foods supplements [14] and also for baking [15].

Chemical composition of the carobs pulp depends on the species, geographical origin, climate conditions, harvesting and storage [16]. However, it has a high content of carbohydrates (mainly sucrose), minerals, protein, insoluble dietary fibres and tannic acid and is low in fat [7, 17, 18]. Recently, attention has been drawn to the health promoting effects of carob pulp, not only due to dietary fiber [15, 19], but also to polyphenols, as expressed by their antioxidant activity [3, 6, 7, 20].

Raw *C. siliqua* pods are traditionally consumed by the Mediterranean people, and the use of their flour in confectioneries is also well-established. The flour can be a cocoa substitute [4] in the confectionery and also used in beverages, bread [15] or pasta [11] with the advantage of having fewer calories and fat, and great fiber and calcium contents than cocoa. Moreover, it is free of caffeine and theobromine (stimulants) or oxalic acid, and cholesterol [21]. The higher D-pinitol richness of *C. siliqua* pods, compared to other species, makes this species an important source of this compound [22].

The studies focused on the nutraceutical compounds of *C. siliqua* pod indicate they are effective in reducing LDL-cholesterol in blood and postprandial blood glucose in patients with Type II diabetes mellitus as a consequence of the presence of D-pinitol [23].

Concerning *Prosopis*, research in Argentine has also focused on the pods flour as coffee and cocoa substitutes [13, 24] and for baking [25].

Although the milled pods of *C. siliqua* and *Prosopis* are indiscriminately designated as carob flour, there are few studies that compare their composition. The aim of the present study is the comparison of commercial carob flours obtained from the two species by the determination of color, chemical composition (moisture, ash, protein, fat, carbohydrates, starch, fiber and hydrosoluble metabolites), total phenolic content, antioxidant activity and the identification of other water soluble nutraceutics using nuclear magnetic resonance (NMR) and HPLC.

Materials and methods

Plant material

Commercial carob flour from *C. siliqua* was obtained from a local de-seeding factory (Industrial Farense, Faro, Portugal) and flour from *Prosopis* spp. (mainly *P. alba*) was purchased from a local market (Todo Dieta Srl., Buenos Aires, Argentina). Carobs flours were stored in a closed container at 4 °C.

Colour of the carob flours was determined on 10–12 g of sample in an opaque recipient by using a Minolta Minolta CR300 colourimeter [26]. The tristimulus colour parameters included: L*—lightness, a*—red/green index, b*—yellow/blue index. Moisture was determined by the International Standard Method [27].

Chemical analyses

International standard methods were used to determine: total crude fat, total dietery fiber, protein (N × 6.25) and total ash [28]. Starch was determined by a spectrophotometric method based on the starch hydrolysis to D-glucose by the enzyme amyloglucosidase at pH 4.6 using the Boehringer enzymatic kit of analysis [29].

Sucrose, glucose, fructose and pinitol were identified by NMR (nuclear magnetic resonance) and quantified by HPLC (High-Performance Liquid Chromatography).

Organic components identification by NMR

Enzymes of carob flours (1 g) were inactivated by boiling in water for 5 min. The slush was filtered through ten layers of gauze and the resultant liquid adjusted to pH 6.0 with NaOH, and then lyophilized. The lyophilized residue was solubilized in a solution containing 5.8 M D_2O, 2.5 mM Na_2EDTA and 2.5 mM NaN_3. All experiments were performed with

a Bruker Avance II$^+$ 400 spectrometer (Bruker Biospin, Rheinstetten, Germany) operating at ^{13}C frequency of 100.61 MHz, using a 5 mm diameter broadband probe head. ^{13}C-NMR spectra were acquired using a zgig pulse program with inverse gated decoupling of protons, with a total of 750 scans collected into 64 k points over a spectral width of 24 kHz, a recycle time of 3.36 s, and a pulse angle of 30°. The temperature of the probe head was kept at 25 °C. Data was processed with 4 Hz exponential line broadening. Chemical shifts are expressed in ppm relative to dioxan (66.7 ppm) that was used as an internal reference. Sucrose, glucose, fructose and pinitol were identified through NMR spectra of pure compounds solutions.

Organic components determination by HPLC

The sugar compounds and pinitol were analyzed by HPLC according to AACC 80-04.01 method [28].

One gram of dry mass per replicate was weighed and extracted with 10.0 mL of boiling water for 5 min. The mixture was centrifuged at 10,000 rpm for 5 min, and an aliquot of the supernatant (0.5 mL) was filtered through a 0.22 µm PET-20/25 syringe filter. The first two drops of the filtrate were discarded, and the remainder filtrate was collected and stored at − 20 °C until analysis. Sugars (fructose, glucose, and sucrose) and pinitol were separated and quantified by the HPLC system (LaChrom-Hitachi, USA) using a refractive index detector (IR) on a Purospher Star NH_2 (5 µm) silica with an amino-bonded phase column, 4 × 125 mm, 5 µm particle size, (Merck, Darmstadt, Germany), operated at 23 °C. 20 µL of aliquots were injected and eluted with isocratic solution [acetonitrile/ water (80:20 v/v)] during 30 min at a flow rate of 1.0 mL/min, to separate compounds. The linear range for the sugar standard curves were 1–9.5 mg mL^{-1} for pinitol, 0.7–7 mg mL^{-1} for fructose and 2–20 mg mL^{-1} for glucose and sucrose in acetonitrile. For calibration, regression coefficients achieved were as follows: pinitol (y = 175.2 x + 117.6; r^2 = 0.999), fructose (y = 159.4 x + 3.3; r^2 = 0.998), glucose (y = 48.3 x + 51.0; r^2 = 0.998), and sucrose (y = 131.0 x + 333.9; r^2 = 0.994). The sugars and pinitol peaks were measured and identified by comparison with the retention times of the standards. Two technical repeats were used for each flour sample. The sugars data were expressed as g 100 g^{-1} flour dry basis.

Total phenols and antioxidant activity

Extract preparation

Extraction of flours followed the procedure described by Rodriguez-Saona [30]. 1 g of carob flour was blended with 20 mL acetone and filtered on a Buchner funnel. The filter cake residue was re-extracted with aqueous acetone (70:30 v/v) until a clear solution was obtained. The solvent ratio was the most appropriate for extracting carob flour, according to Avallone [17]. Filtrates were combined, shaken in a separator funnel with chloroform (2:1 chloroform:acetone) and stored overnight at 5 °C. The aqueous portion (top portion) was collected and placed on a rotovapor (Buchi model R-114 assembly A, Flawil, Switzerland) at 40 °C until all residual acetone was evaporated. The extract was brought to a known volume with distilled water.

Total phenols

The total phenols content was determined spectrophotometrically using the Folin–Ciocalteu method. This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (FC reagent), based on the Slinkard and Singleton method, and modified by Asami [31]. The concentrations of total phenolics were determined as grams per liter of gallic acid equivalent (GAE). The linearity range for this assay was determined as 2.5×10^{-2}–88 g L^{-1} GAE (r^2 = 0.998, σ = 0.019), and an absorbance range of 0.08–1.23 AU. All determinations were performed four times.

Antioxidant activity

Antioxidant activity was determined by two complementary methods: determination of the radical scavenging activity by the DPPH (2,2-diphenil-1-picrylhydrazyl) radical scavenging method [32] with the modification of using ethanol instead of methanol and resting time of 60 min and the ferric ion reducing antioxidant power by the FRAP (ferric ion reducing antioxidant power) method [33, 34].

DPPH radical scavenging method

A DPPH solution was prepared dissolving 4 mg in 100 mL of 95% ethanol. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the antioxidant standard reference at concentrations of 25–800 µM. A sample of 100 µL of a standard solution or extract solution was added to 2 mL DPPH solution and the mixture was shaken and allowed to stand for 60 min at room temperature in the dark. Absorbance of samples was measured at 517 nm, using a UV–Vis spectrophotometer (double-beam; Hitachi U-2010, Cincinatti, Illinois, USA). The antioxidant activity was expressed as trolox equivalent (TE) on the basis of the standard curve y = − 0.0009 x + 0.9863 (r^2 = 0.996) in terms of micromole equivalents of trolox per g of carob flour (µmol TE g^{-1} carob flour). All determinations were performed in triplicate.

FRAP method

FRAP measures the formation of a blue-coloured Fe^{2+} tripyridyltriazine compound from the colourless oxidised Fe^{3+} by the action of electron donating antioxidants [33]. A standard curve was prepared using different concentrations of iron sulfate (linearity range 0.13–3.50 µmol L^{-1}; $r^2 = 0.995$, s = 0.021). FRAP values are presented as µmol Fe^{2+} g^{-1} of sample (ferric reducing power). All determinations were performed in triplicate.

Statistical analysis

Data are presented as mean ± standard deviation of triplicate samples. Variation between the two species flours where tested by analysis of variance (ANOVA) with level of significance defined at $p < 0.05$.

Results and discussion

Moisture, colour, ash, protein, fat, total dietary fiber, starch, sugars and pinitol contents of the carobs commercial flours are shown in Table 1.

The colour of both carob flours are brown, but *Prosopis* has a significant higher L-value (59.11 ± 0.13) than *Ceratonia* (44.39 ± 0.15), and also a higher b-value indicating that *Prosopis* is more yellow than *Ceratonia*, what corroborates with visual perception.

Table 1 Moisture, colour parameters (L, a, b) and organic composition of *Ceratonia* and *Prosopis* commercial flours (g 100 g^{-1} dry weight)

	Ceratonia	*Prosopis*
Moisture	7.20 ± 0.07	7.39 ± 0.08
Protein	4.90 ± 0.03	8.01 ± 0.14
Ash	3.18 ± 0.09	2.30 ± 0.05
Fat	0.75 ± 0.05	2.22 ± 0.06
Total dietary fiber	47.00 ± 0.19	9.20 ± 0.20
Starch	0.12 ± 0.02	6.11 ± 0.21
Fructose	2.02 ± 0.06	n.q.
Glucose	7.27 ± 0.16	n.q.
Sucrose	18.86 ± 0.57	23.89 ± 1.59
Pinitol	5.46 ± 0.21	n.q
Colour (tristimulus)		
L*	44.39 ± 0.15	59.11 ± 0.13
a*	8.25 ± 0.05	9.39 ± 0.05
b*	12.60 ± 0.01	18.24 ± 0.02

Values are expressed as mean ± standard deviation (n = 3)

n.q. not quantifiable

The amounts of ash in *Ceratonia* flour (3.18 g 100 g^{-1}) are in the range 2.1–3.3 g 100 g^{-1} previously reported [10, 35], while for *Prosopis* was 2.30 g 100 g^{-1}.

Prosopis flour contained more protein (8.01 g 100 g^{-1}) than *Ceratonia* flour (4.90 g 100 g^{-1}). These values are in accordance with the reported values of 2.7–4.2 g 100 g^{-1} for *Ceratonia* [10, 35] and 7.00 to 11.20 g 100 g^{-1} for *Prosopis* [7]. *Ceratonia* flour cannot be labelled as a source of protein due to this low value.

The fat content of *Ceratonia* flour is very low (0.75 g 100 g^{-1}) compared with *Prosopis* (2.22 g 100 g^{-1}), in agreement with the previously reported values of 2.0 g 100 g^{-1} for *Prosopis* and 0.6 g 100 g^{-1} for *Ceratonia* [18, 36]. A higher content (3.17 g 100 g^{-1}) was reported for *Prosopis* by Galán [37]. As is well known the consumption of high fat food is associated with increased risk of coronary and circulatory diseases, obesity and some types of cancers [38], carob may therefore be regarded as a healthy food source [39], due its low amount of fat content.

The total dietary fiber content of *Ceratonia* and *Prosopis* flours were 47.0 g 100 g^{-1} and 9.2 g 100 g^{-1}, respectively, although for *Ceratonia* the value of 29.9–36.1 g 100 g^{-1} has previously been reported [35]. The high content of carob dietary fiber together with high polyphenols content (Table 2) have valuable health-promoting attributes such as cholesterol-lowering activity, anti-oxidative properties and the reduced risk of gastro-intestinal cancer [40, 41]. In fact, the potential of carob dietary fiber concentration has been exploited and commercial ingredients already exist, like Caromax® (Nutrinova, GmbH, Germany) which is a preparation of more than 80% dietary fibre obtained by supercritical extraction [16]. It should be refered that the value of 9.2% of dietary fiber we found for *Prosopis* is significantly lower than the value of 26.5–35.3% reported for different *Prosopis* spp. [7], what is probably due to the pods processing.

Starch content was 0.12 g 100 g^{-1} and 6.11 g 100 g^{-1} for *Ceratonia* and *Prosopis*, respectively, although Avallone [17] reported 0.8% for *Ceratonia* and Becker [42] reported trace amounts for *Prosopis*. The higher *Prosopis* starch content obtained sugest that flour is not pure.

Table 2 Total phenols content and antioxidant activity by FRAP and DPPH methods of commercial carob flours

	Total phenols (mg GAE g^{-1})	FRAP (µmol Fe^{2+} g^{-1})	DPPH (µmol TE g^{-1})
Ceratonia	17.7 ± 0.9[a]	249.2 ± 28.8[a]	58.9 ± 0.2[a]
Prosopis	0.5 ± 0.01[b]	6.7 ± 0.3[b]	2.9 ± 1.0[b]

Values are expressed as mean ± standard deviation (n = 3)

Different letters in a column show statistically significant differences at $p < 0.05$

In order to identify the water soluble compounds of the carob flours, ^{13}C-nuclear magnetic resonance spectra were obtained (Fig. 2). The most abundant metabolites are sugars (sucrose, glucose and fructose) and pinitol, a cyclic polyol that belongs to the group of inositols. The presence of some minor unditified compounds in the range 18–47 ppm, which are probably organic acids and amino acids, is also visible. The two species presented similar content of sucrose, but *Prosopis* has less glucose, fructose and pinitol than *Ceratonia*.

Using HPLC chromatography, sucrose, glucose, fructose and pinitol have been quantified (Table 1). Sucrose is the predominant sugar in both flours, its mean concentration being 18.86 g 100 g^{-1} for *Ceratonia* and 23.85 g 100 g^{-1} for *Prosopis*. Mahtout et al. [43] also referred sucrose as the main sugar present in pulp of *Ceratonia*, though the amounts were higher. Glucose, fructose and pinitol are negligible in *Prosopis*, while in *Ceratonia* their content is respectively, 7.27 g 100 g^{-1}, 2.02 g 100 g^{-1} and 5.46 g 100 g^{-1}. Similar conclusions were reached by Felker et al. [7] and Batlle and Tous [44] for the two flours. The *Prosopis* reducing and nonreducing carbohydrates, mainly sucrose was previously determined with different methodology and higher amounts was obtained 23.89 g 100 g^{-1}, while in was 18.89 g 100 g^{-1} of sucrose content [25].

The presence of pinitol in *Ceratonia* has great dietetic significance. Several studies concerning the importance of this inositol in animal and human nutrition have been performed. It was found that pinitol can exert an insulin-like effect lowering the glycemic index [23, 45] and it is therefore claimed to be anti-diabetic. Although pinitol also occurs in other species of the *Fabaceae* family, *Ceratonia* carob is the best source of pinitol with maximum concentration of 84.6 g kg^{-1} obtained in wild type species [22]. D-Pinitol® isolated from carob pod flour is already comercialized by Euronutra (Málaga, Spain) and the company claims a synergetic effect between pinitol and insulin at sub-maximal concentrations. D-Chiroinositol, to which pinitol is metabolized, seems to be the key substance for the positive effects observed, as type-2 diabetic people have a higher excretion of this substance [23]. Other positive effects of D-pinitol are relevant, for sportsmen coingestion with creatine enhances creatine metabolic uptake, promoting the building of muscular mass in the body and in this way facilitating training adaptations to exercise [46]. Therefore pinitol can play a valuable role in sportsmen.

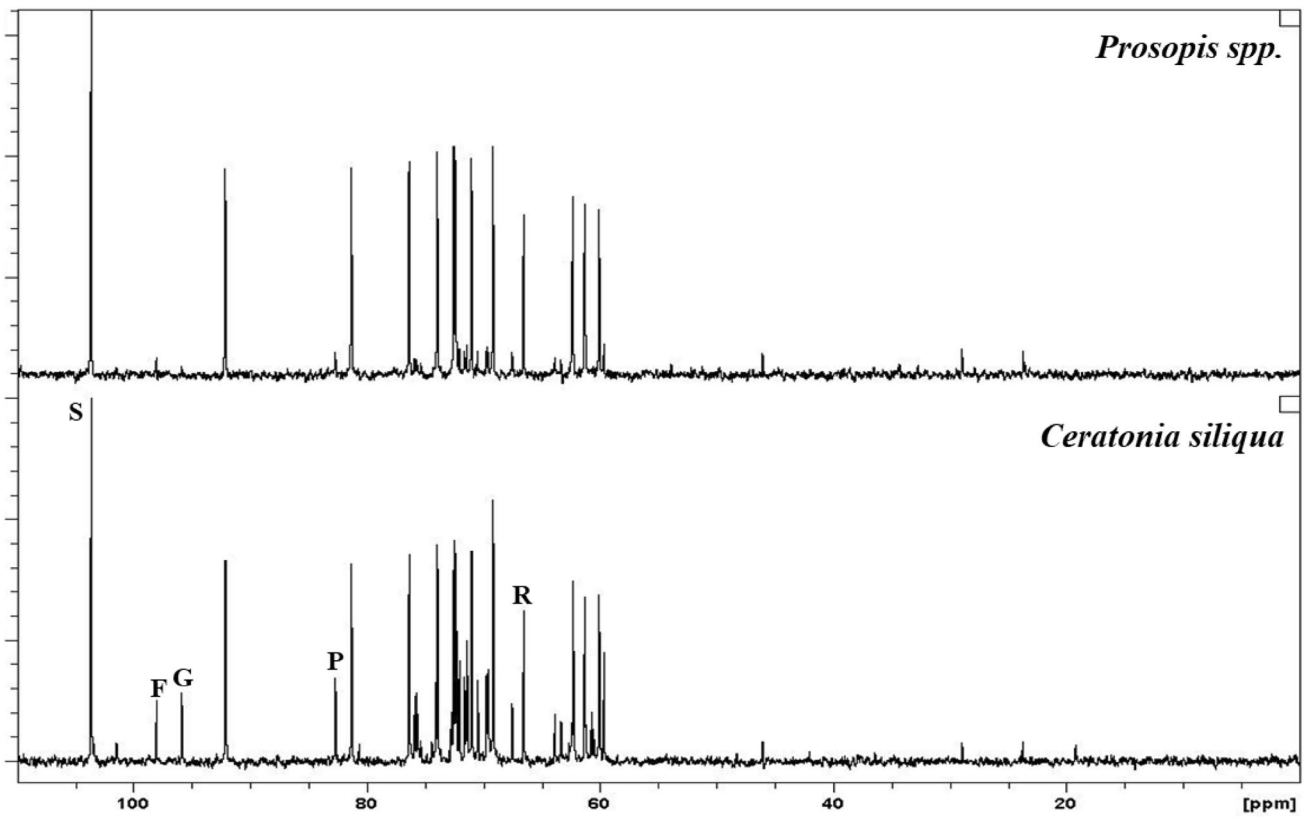

Fig. 2 ^{13}C-NMR spectra of water soluble metabolites from carob flours (*C. siliqua* and *Prosopis* spp.). The identified metabolites are marked in only one of their peaks as follows: *F* fructose, *G* glucose, *P* pinitol, *R* dioxan (as internal reference) and *S* sucrose

The total phenols content and the antioxidant activity of the carob flours extracts, evaluated by DPPH radical scavenging and FRAP assay, are presented in Table 2. The total phenols content for *Ceratonia* is 17.7 mg g^{-1} flour, which is significantly higher than the value of 0.5 ± 0.01 mg g^{-1} obtained for *Prosopis*. The same tendency was obtained for total phenol content of germ flours obtained from carob seeds [47]. Studies on carob flours polyphenolic compounds and antioxidant properties are still limited, but some authors [3, 10, 17] reported for *Ceratonia* values similar to those we observed. Petkova et al. [48] found similar values in the basic chemical composition of *C. siliqua* but report lower content of total phenols and antioxidant activity obtained by FRAP assay. For *Prosopis*, Felker [7] reported values in the range 0.06–0.14 mg g^{-1}, while Cardozo [6] indicated the higher value of 4 ± 0.01 mg g^{-1}. Part of the phenolic compounds of carob pods are in the form of condensed tannins, which were considered to exhibit negative nutritional properties (e.g. reduction of protein digestibility) [10]. However, this antinutritional aspect can be solved when the pods are processed [34, 49]. Nowadays it is assumed that the total phenolics content and antioxidant activity of carob flours have a positive effect both in nutritional aspect [19, 47] and in the shelf-life (2–3 years) of the carob products [50].

The antioxidant activity of *Ceratonia* flour values obtained by both FRAP and DPPH scavenging activity techniques are higher than those obtained for *Prosopis* (Table 2). This could be attributed to phenolic compounds since, according to Apak [51] and Serrano [34], antioxidants detected by the FRAP assay are mainly due to hydrosoluble phenolic compounds (e.g. flavonoids, phenolic acids and hydrolysable tannins).

Values for DPPH radical scavenging activity between the flours of the two carobs might be related to the structural conformation, number of hydroxyl groups and concentration of flavonoids and derivatives, which possess a strong ability to scavenge radicals.

Conclusion

Our studies of *Prosopis* and *Ceratonia* commercial flours showed that *Prosopis* has higher amounts of protein, starch, and fat, while *Ceratonia* showed higher contents in pinitol and antioxidant activity due to the high level of phenolic compounds. The results may be justified by the existence of more food products with great nutraceutical potential for *Ceratonia* commercial flour than for *Prosopis* flour.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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