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VALORIZATION OF CAROB SEEDS AS A FUNCTIONAL FOOD

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ABSTRACT

Our study aims to promote carob seeds as a functional food. For this; the nutritional value, the compounds with beneficial physiological effects and functional properties (fibers and gums), as well as the antioxidant potential were determined. Analysis of the chemical composition of carob seeds reveals their high protein, ash and fat content. However, the total sugars content was estimated to be moderate. The quantification of the compounds with a beneficial physiological effect shows that the seeds are rich in crude dietary fibers (8.39%). Regarding the gums, the yields are evaluated at 39.44% for the crude gums and at 4.026% for the purified gums. The phytochemical assays reveal a richness of the seeds in total polyphenols, in total flavonoids with a moderate content of flavonols and hydrolyzable tannins. The antioxidant potential was studied using two methods: reduction of the free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and the iron reduction method. According to the results, the carob seeds have a discreet antioxidant potential compared to the standards tested (gallic acid and quercetin).

1. Introduction

The carob tree (*Ceratonia siliqua* L.) is a fruit and forest tree, native to arid and semi-arid areas of the Mediterranean and the Arabian Peninsula. It is considered one of the best performing trees since all its parts (leaves, flowers, fruits, wood, bark and roots) are useful and have values in several areas (Benmahioul *et al.*, 2011).

The carob tree is used not only in animal feed but also in medicine and human food. Carob flour is used in the pharmaceutical industry, mainly against pulmonary tuberculosis, bronchial diseases and gastrointestinal disorders. The fibers contained in carob powder act as a natural laxative in case of constipation and tannins fight against diarrhea. These would retain the water present in the stool and act as a binder. Flour is also used above all in the food industry as an ingredient for the preparation of certain products, such as cakes, candies, ice creams, sauces and mayonnaise and as a cocoa substitute for the manufacture of chocolate (Serairi-Béji *et al.*, 2000).

Carob seeds are also a very good source of soluble and insoluble fiber, useful for regulating transit. They are rich in minerals (calcium, iron,

magnesium, phosphorus), vitamins (B3, A and E) and phenolic compounds. Another essential product is obtained from locust bean, which is gum, which is extracted from the albumen of the seeds. The latter is used in the food industry as a thickener known under the standardized code E 410, it replaces pectin and gelatin (Benmahioul *et al.*, 2011).

In Algeria, the carob tree remains much neglected and has not yet had the place, it deserves in reforestation programs, despite the socio-economic repercussions that this plant can have at the national and especially regional level and this despite the enthusiasm and interest shown in it for several decades by manufacturers. Few studies have been carried out so far on locust bean seeds, in particular on their nutritional and antioxidant values. The topics covered particularly concern the extraction and identification of gums, the analysis of the chemical composition, the determination of the energy value and the antimicrobial potential of the fruit pulp. Our interest in this product follows on from these observations and the present work consists in filling the lack of information on the nutritional, antioxidant and functional properties of carob beans. Our work aims to enhance the value of the carob tree through the valorization of its seeds.

2. Materials and methods

2.1. Collection and preparation of samples

The plant material, consisting of ripe carob tree pods, was collected in a forest in Bordj-Menail (region located in the Wilaya of Boumerdes, northern Algeria). The harvest was done randomly from several trees in August 2019. According to identification tests, this is the variety Belhessenat and Bouzegza widely responded to in the area. In the laboratory, we separated the pulps from the seeds. These were grinded and stored in dark glass bottles for subsequent analyzes.

2.2. Analysis methods

2.2.1. Determination of physicochemical parameters

Some physicochemical parameters were analyzed (moisture content and solids content (AOAC, 1995), pH and titratable acidity (AOAC, 2000)). The seed rate (SR %) is expressed as a percentage of mass, is given by the following formula:

$$SR\ \% = (m_2 / m_1)\ x100. \quad (1)$$

With: SR %: Seed rate in g per 100 g of whole fruit; m_1: the mass of whole fruits (in g) and m_2: the mass of seeds (in g).

2.2.2. Determination of biochemical composition

The total sugar content was determined according to the method of Dubois et al. (1956). In the presence of sulfuric acid and hot, carbohydrates are dehydrated to furfural compounds which combine easily with phenol and give a pinkish-salmon color. The reduction of Fehling's liquor by sugars makes it possible to determine the reducing sugars, the hydrolysis of the defecated solution in acidic and hot medium allowed us to determine the total sugars (reducing sugars + hydrolysable sugars) and to deduce indirectly the rate of non-reducing sugars (total sugars-reducing sugars) (Chidan-Kumar et al., 2014). The protein content is determined by the Kjeldhal method using the conversion factor of 6.25 (AOAC, 1995). The extraction of the fat was carried out by organic solvent (Hexane) with a Soxhlet type apparatus, according to the method of ISO 659 (1998). The ash content was determined by the destruction of any organic matter under the effect of the high temperature (550°C for 3 to 5 hours) to obtain a white or greyish-white powder (AOAC, 2002). The determination of mineral elements such as Na, Ca, Zn, Fe, Mn, Cd, Cu and Mg was carried out using atomic absorption spectrophotometry (AFNOR, 1986). The fiber content was determined according to method detailed by Henneberg and Stohmann (1860). It consists of treating the sample to be analyzed successively with sulfuric acid and potash. Acid / base

hydrolysis (hot) solubilizes almost all of the cellular content with the exception of dietary fiber and mineral salts. The extraction of the gums was carried out by acid decorticating followed by washing and soaking in water according to the protocol described by Dakia *et al.* (2007). Purification is carried out by precipitation in two solvents: ethanol and isopropanol.

The yield was determined by the following formula:

Yield of crude gum = (mass of crude gum / mass of seeds) X 100. (2)

Yield of purified gums = (mass of purified gum / mass of seeds) X 100 (3)

The extraction of phenolic compounds was performed according to Lagha-Benamrouche et Madani (2013). Total polyphenols are quantified according to Meyers *et al.* (2003), the condensed tannins were determined by the vanillin method described by Ba *et al.* (2010). The flavonoids were determined according to Bahorun *et al.* (1996) by direct dosing with aluminum chloride.

2.2.3. Determination of the overall energy value

The overall energy value is the energy released by the combustion of proteins, fats and carbohydrates contained in the diet, taking into account the digestibility of each of these macromolecules and their Atwater coefficients. The Atwater coefficients are defined as the metabolizable energy in kcal per 1g of nutrient. For carbohydrates and proteins, this coefficient is equal to 4 kcal, or 17 kJ and for lipids, it corresponds to 9 kcal or 38 kJ (AFNOR, 1987). The overall energy value is calculated from the relationship below.

$$E = (9 \times L) + (4 \times C) + (4 \times P) \quad (4)$$

With, E: global energy value in kcal, L: total lipid content in g per 100 g of sample, C: total carbohydrate content in g per 100 g of sample, P: total protein content in g per 100g of sample and 9, 4 and 4: the Atwater coefficients of lipids, carbohydrates and proteins.

2.2.4. Antioxidant activity

In this study the antioxidant activity was evaluated using both methods; the scavenging activity of the free radical 1, 1-diphenyl-1-2-picrylhydrazyl(DPPH) (Brand-Williams *et al.*, 1995) and the reducing power (Oyaizu, 1986). The presence of reducing agents in the extracts induces a reduction of ferric ions (Fe^{+3}) into ferrous ions (Fe^{+2}). This reduction is measured by the intensity of the resulting blue-green color. An increase in absorbance indicates high reducing power. In the presence of RLs scavengers, diphenyl picryl-hydrazyl (DPPH) having a violet color is reduced to a yellow compound, diphenyl picryl-hydrazine, the color intensity of which is inversely proportional to the capacity of the antioxidants present in the medium to donate protons

2.3. Statistical analysis

The statistical analysis of the results was carried out using the STATISTICA 5.5 software and the degree of significance is taken at the probability $p \leq 0.05$. We performed a one-way analysis of variance followed by a Tukey's test. All data represent the mean of the three tests ± standard deviation.

3.Results and discussions

3.1. Physicochemical parameters

The physicochemical characteristics of the analyzed carob seeds are illustrated in the Table 1.

Table 1. Physicochemical characteristics of carob seeds

pH	Acidity (g CAE/L)	Brix (%)	Moisture (%)	Seed rate (% of fruit)
6.453 ± 0.025	2.689 ± 0.665	19 ± 1	1.856 ± 0.016	8.79 ± 0.57

EAC : Equivalent Acide citrique

The pH of the seeds is estimated at 6.4. Our result is slightly higher than that found by Yousif and Alghzawi (2000). These report pH values of around 5.96 and 4.81 for unroasted and roasted carob flour, respectively. This difference in results can be explained by the nature of the part of the plant studied (pod or seed) and by the technological process applied (effect of the roasting process).

The titratable acidity tells us about the total acid concentration. From the results of Table 1, it can be seen that the acidity of the seeds is estimated at 2.69 g EAC/L. Our result is slightly higher than that found by Meziou-Chebouti *et al.* (2015) for carob pulp (2.1 g EAC/L).

The Brix generally tells us about the sugar content but the other components of soluble solids can however influence this rate if their proportion increases. The Brix rate of carob seeds is estimated at 19%. This rate is lower than those found by Gaouar (2011) (28.40% to 30.80%). The soluble solids content of carob seeds is much lower compared to the edible part (the pulp). Gaouar (2011) reports Brix levels in the order of 88.68% to 90.40% for the pulp against 28.40% to 30.80% for the seed.

The results illustrated in Table 1 show overall that the moisture of the seeds is estimated at 1.86%. The moisture contents of the seeds are compared to those of the pulp; it is observed that the seeds are less humid. Özcan *et al.* (2007), Youssef et al. (2013) and Loullis *et al.* (2018) report contents of 6%, 5.3% and 6 to 11% for the carob pulp, respectively.

The seed rate is estimated at 8.79% of the fruit. According to Yousif and Alghzawi (2000), the seeds represent 10 to 20% of the weight of the pod. This rate depends on the number of seeds contained in the seed which is estimated between 15 to 20 seeds (Sidina *et al.*, 2009).

3.2. Chemical composition

The chemical composition of the carob seeds studied is illustrated in the Table 2. From the obtained results, the protein content is estimated at 30.04%. Our result is more superior to those reported by Meziou-Chebouti *et al.* (2015) and Loullis *et al*. (2018) for carob pulp. These report protein contents in the range of 7% and 2 to 7.6%, respectively. From these results, it can be seen that the carob seeds are richer in protein than the pulp. The unequal proportion in pulp and seed depends on the biological activity of the two parts of the plant (Linden and Lorient, 1994).

Table 2. Chemical composition of carob seeds

Compounds	Contents
Proteins (g BSA E/100 g)	30.04±0.03
Total sugars (g G E /100g)	27.36±0.01
Reducing sugars (g/100 g)	06.36±0.03
Non-reducing sugars (g/100 g)	15.09±0.06
Fat (g/100 g)	9.74±0.06
Ash (g/100 g)	5.66±0.08
Total fibers (%)	8.39 ±0.77
Yield of crude gums (%)	39.44±0.11
Yield of purified gums (%)	4.03±0.00
Global energy value (Kcal / 100g)	317.21

BSA E: Bovine Serum Albumine Equivalent. GE: Glucose Equivalent.

Carob is a fruit rich in simple sugars which gives it its very sweet flavor and high energy value and which makes it a feed for cattle. According to the results of Table 2, the level of total sugars in carob seeds is estimated at 27.36% with 6.36% for reducing sugars and 15.09% for non-reducing sugars. By comparing our result with that found by Meziou-Chebouti

et al. (2015), we see that the carob pulp is richer in sugar compared to the seed (27.36% against 50.9%).

From the results of Table 2, it can be seen that the fat content of the seeds is estimated at 9.73%. This value is higher than those reported by Özcan *et al.* (2007) and Loullis *et al.* (2018) (0.4 to 1.3% and 0.2%, respectively). Multiple parameters influence the fat content such as particle size, humidity, the nature of the solvent and the extraction method used (Gaouar, 2011).

The ash content is estimated at 5.66%. This content is higher than that found by Bezzala (2005) (4%). This difference found can be explained by the cultivar, the nature of the soil, climatic and irrigation conditions and the edaphic characteristics of soils (Bezzala, 2005). The ash content of the seeds exceeds that of the pulp. El Batal *et al.* (2016) and Bezzala (2005) report contents between 2.4 to 3.9% and 2.1 to 2.4% for carob pulp. These findings were also confirmed by Gaouar (2011). The latter found that the mineral content of the seed is greater than that of the pulp (4% against 2.67%). The unequal proportion in pulp and seed depends on the biological activity of the two parts of the plant (Linden and Lorient, 1994).

Atomic absorption spectroscopy has allowed the determination of some minerals such as Ca, Zn, Na, Mg, Fe, Cd, Cu and Mn (Table 3).

Table 3. Mineral composition of the carob seeds studied.

Mineral	Contents (mg/g)
Manganese	0.055±0.003
Iron	0.089±0.003
Cadmium	Nd
Calcium	10.411±0.253
Copper	Nd
Magnesium	3.114±0.015
Sodium	2.215±0.024
Zinc	0.071 ± 0.0006

Nd: Not determined

From the results in the Table 3, the various minerals found in the carob seeds and which are in the dominant quantity are calcium at 10.41 mg/g, followed by Magnesium at 3.11 mg/g and sodium at 2.215 mg/g. Zinc, iron and manganese are found in trace form. By comparing our results to those found by Hafize *et al.* (2020), we see that our sample is richer in calcium (10.41 mg/g against 8.3 mg/g) and in magnesium (3.114 mg/g against 0.89 mg/g) but poor in zinc (0.071 mg/g against 0.12 mg/g) and manganese (0.055 mg/g against 0.19 mg/g). In comparison with the data obtained by for the pulp, it can be seen that the latter is richer in minerals than the seed. According to Gubbuk *et al.* (2010), Afoakwa *et al.* (2013), Khlifa *et al.* (2013) and Torres-Moreno *et al.* (2015), the mineral composition of the pulp is as follows: Ca (285.4 - 480 mg/g), Mg (54-170 mg/g), Zn (0.4-2.7 mg/g), Fe (1.8-5.1 mg/g) and Mn (0.2-2.7 mg/g).

Based on our results (Table 2), it is observed that the crude fiber content of the analyzed carob seeds is 8.39%. Our result exceeds twice that of Gaouar (2011). The latter reports a rate of 4% for carob seeds of Algerian origin. By comparing the raw fiber contents recorded for the analyzed samples with the bibliographic data, it can be seen that the seeds provide as much fiber as the pulp (8.01% according to Albanell *et al.* (1991) for varieties from Spain, 10.99% according to Yousif and Alghzawi (2000) for varieties from Jordan, 10 % according to Gaouar (2011) and 11% for Moroccan varieties according to Salih and Jilal (2020)).

The yields of crude and purified gums are estimated at 39.44% and 4.026%, respectively. The percentage of purification is estimated at 10.29%. According to Lopez Da Silva *et al.* (1990), locust bean gum constitutes one third of the total weight of the seed. It is mainly

composed of galactomannans (around 93%), protein (about 4-5%), lipids (1%) and minerals (1%). Purification removes cellulose, lignin and lipids, as well as considerably decrease the amounts of minerals and proteins. We compare our results to those found by Lopez da Silva *et al*. (1990) and Dakia *et al.* (2008) we find that our raw gum yield far exceeds that of Lopez Da Silva *et al.* (1990) (20%) and it is included in the range given by Dakia *et al.* (2008) (37% -61%). The yield has a direct relationship with the composition of the endosperm and the method used for extraction. The results of the Table 2, show that the overall energy value of carob seeds is estimated at 317,212 kcal/100g. This value is lower than that found by Kamal *et al.* (2013) (346.95 kcal/100g) for carob seed powder. This difference in results can be explained by the variability of the nutrient quantification methods, the degree of ripening of the fruit, the sweetness of the fruit and humidity of the sample, etc. (Dakia *et al*. 2007).

3.3. Antioxidant activity

The results of Table 4 show that the total polyphenol contents of the carob seeds analyzed are estimated at 283.68 mg GAE/g. The results also show that the content of flavonoids is four to five times higher than that of hydrolyzable tannins (12.65 mg QE/g against 2.79 mg TAE/g) and that the class of flavonols represents a quarter of total flavonoids.

Table 4. Levels of phenolic compounds in carob seeds

Total phenol (mg GAE/g)	Flavonoids (mg QE /g)	Flavonols (mg QE /g)	Hydrolyzable tannins (mg TAE/g)
283.68±6.89	12.65±1.01	3.23±0.10	2.79±0.45

EAG: Gallique acid Equivalent, QE: Qercetin Equivalent, TAE: Tannic acid Equivalent.

The results of the phytochemical assays obtained for our sample of carob seeds are far superior to those found by Mekhoukhe *et al.* (2018) and Ben Ayache *et al.* (2020). The latter report much lower contents of total polyphenols and flavonoids (12.24 mg/g, 1.33 mg/g, 9 mg/g and 1.76 mg/g, 0.30 mg/g, 6 mg/g, against 283.68 mg/g and 12.65 mg/g, respectively). While a minimal difference in results is seen with Mekhoukhe *et al*. (2018) for the flavonol contents in favor of our sample (3.23 mg/g and 2.97 mg/g, respectively). Our recorded result for hydrolyzable tannins far exceeds those of Avallone *et al.* (1997) and Gaouar (2011). The latter report contents of 0.95 mg/g and 0.04mg/g, respectively. However, the content of hydrolyzable tannins obtained for our analyzed sample (2.79 mg/g) was found to be low compared to that reported by Ben Ayache *et al.* (2020) (6 mg/g) and intermediate between those recorded for total and condensed tannins (4.29 mg/g and 0.53 mg/g, respectively) (Mekhoukhe *et al.,* 2018). This difference in results can be explained according to Lee *et al.* (2003), by several factors such as: the climate, the environment (the geographical area, drought, the nature of the soil, aggressions and diseases, .etc.), the genetic factor, the cultivar, the harvest period, the stage of development as well as conservation methods, extraction and quantification of these substances. According to Ydjedd *et al.* (2017), the solubility of phenolic compounds depends on their degree of polymerization, the solvent used and their interaction with other components of the cell matrix as well as the formation of insoluble complexes.

The antioxidant potential of the seeds was estimated using two methods: the potassium ferricyanide reduction method and the scavenging of the stable radical DPPH (Table 5).

Table 5. Reducing power and antioxidant activity against the DPPH radical of the aqueous organic extract of carob seeds and standards (quercetin and gallic acid)

Reducing power (Absorbances at 700nm)		Scavenger activity against the DPPH radical (%)	
Carob seeds (1 mg/mL)	0.12 ± 0.02^{c}	Carob seeds(1 mg/mL)	38.69 ± 2.66^{c}
Gallic acid (20 µg/mL)	0.29 ± 0.07^{b}	Gallic acid (40 µg/mL)	92.00 ± 0.55^{a}
Quercetin (20 µg/mL)	0.45 ± 0.17^{a}	Quercetin (40 µg/mL)	64.00 ± 0.27^{b}

DPPH: 1.1-diphenyl 1-2-picrylhydrazyl. The carrying values of the different letters for each analyzed parameter present significant differences (P≤0.05). The results are listed in descending order: a> b> c.

Analysis of the reducing power of the aqueous organic extract of carob seeds at a concentration of 1 mg/mL leads to an absorbance of 0.120 (Table 5). This absorbance is significantly low ($p\leq0.05$) compared to those of gallic acid and quercetin tested at 20 µg/mL. As can be seen from the results, it is quercetin which has significantly ($p\leq0.05$) the highest absorbance and therefore the most pronounced reducing power followed by gallic acid.

As indicated in Table 5, the anti-free radical activity for our studied extract (at a concentration of 1mg/mL) is estimated at 38.69%. The latter is significantly very lower ($p\leq0.05$) than those of the standards tested (gallic acid (92%) and quercetin (64%)).

4. Conclusions

Our study aims to promote carob seeds as a functional food source by determining their nutritional and energy value, extraction and purification of gums, determining the level of crude dietary fibers and studying their antioxidant potential.

Physicochemical analyzes show that the carob seeds have an estimated pH of 6.453 with a total acidity of 2.689 g EAC/L. An average moisture content of between 1.872% and 1.840% with a soluble solids content of around 19 ° Bx and an estimated seed rate of 8.79% of the fruit. Analysis of the chemical composition of the seeds indicates their high protein content (30.04 g BSA E/100 g) and fat content (7.74 g/100 g). However, the total sugars content was estimated to be moderate (27.36 g GE/100 g). Carob seeds are also found to be rich in minerals. The mineralogical analysis carried out on the ashes of the seed reveals a predominance of calcium with a content of 10.41 mg/g, followed by magnesium (3.11 mg/g) and sodium (2.215 mg/g). Manganese (0.055 mg/g), zinc (0.071 mg/g) as well as iron (0.089 mg/g) are found in trace form. According to the data, the carob seeds are very energetic; its overall energy value has been estimated at 317.21 kcal/100g. The gum yields are evaluated at 39.44% for the crude gums and at 4.026% for the purified gums. The percentage of purification is estimated at 10.29%. The results of the crude fiber assay show that the seeds are rich in these compounds with beneficial physiological effects. The crude fiber content was evaluated at 8.39%. The phytochemical assays reveal that the carob seeds analyzed are rich in total polyphenols (283.68 mg GAE/g), in total flavonoids (12.65 mg QE/g) with a moderate content of flavonols (3.23 mg QE/g) and hydrolyzable tannins (2.79 mg TAE/g).

The antioxidant potential was studied using two methods: reduction of the free radical1.1-diphenyl-2-picryl-hydrazyl (DPPH) and the iron reduction method. According to the results, the carob seeds have a discreet antioxidant potential compared to the standards tested (gallic acid and quercetin).

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