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The effects of boiling and freezing processing methods on the quality characteristics, bioactive compound content, and antioxidant capacity of Jijel region carob pods, *Ceratonia siliqua* L.

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Abstract: This is the first report to look at how processing affects the quality and phytochemical properties of a well-known and intriguing Mediterranean fruit called "*Ceratonia siliqua* L." Powders of carob bean pulp from two regions of Jijel (Djimla and El-Milia), located in the northeast of Algeria, were analyzed to determine the effect of two transformation processes (boiling and freezing) on proximate composition, physicochemical characteristics, bioactive compound content, and antioxidant capacity. Comparing to other studies, Djimla samples were high in trace minerals (Zn and Cu) and total polyphenols, while El-Milia samples were high in flavonoids and flavonols. However, boiling resulted in a decrease in titratable acidity (TTA), dry matter, ash, crude protein, total sugar, and bioactive compound contents while increasing pH, moisture content, and total antioxidant capacity. Freezing at -18°C had an effect on all parameters, but not as strongly as boiling. However, it significantly increases total antioxidant capacity, particularly in the El-Milia sample, with a percentage of 250%.

Keywords: *Ceratonia siliqua* L.; Boiling; Freezing; Physicochemical characteristics; Bioactive compound; Antioxidant capacity

1. Introduction

Plants are a major source of modern medicine and are of great interest in drug discovery. Only about 25% of modern medicines are derived from plants, and only about 5-15% of plants are being studied for their medicinal properties (Gurnani *et al.*, 2014). Medicinal plants have been used in traditional medicine and ethnomedicine all over the world (Bourhia *et al.*, 2021). Today, medicinal herbs and functional foods are being extensively researched, resulting in lucrative therapeutic potentials (Atanasov *et al.*, 2021). *Ceratonia siliqua* L., commonly known as carob, is one of these medicinal plants.

Carob is an evergreen, long-lived tree native to the Middle East that is primarily grown in Mediterranean countries. It is a member of the *Fabaceae* family and the *Caesalpinioideae* subfamily.

Its annual global production is estimated to be more than 315,000 tons of carob products (Baumel *et al.*, 2018). With an annual production of more than 135,000 tons, the Mediterranean basin is the primary carob production center (FAO, 2019). Algeria is a Mediterranean country with a wide range of bioclimatic zones. The northern climate is mild and rainy in winter but hot and dry in summer (Mahdad and Gaouar, 2016). Algeria has a rich and diverse flora due to its strategic location, with many endemic species such as the carob tree, which has always been associated with the olive, almond, and lentisk trees, all of which are considered useful plants (Benmahioul *et al.*, 2011). The carob tree is known in Algeria as "Kharroub", "Karrûba," "Taslighoua," "Tikharroubt," and "Tikida" (Baba Aissa, 2000). The majority of carob trees are wild and grow in the north and northwest of the country. The annual production of carob reaches 3000 tons per year (Nasar-Abbas *et al.*, 2016). Local farmers harvest the entire fruit by hand at the end of the summer, from August to October (Boublenza *et al.*, 2017).

Carob cultivation is currently expanding in response to increased demand for its compositional, functional, nutritional, and industrial value, making it an economically important crop (Tzatzani and Ouzounidou, 2023). The mature carob pod is brown and divided into two parts: the pulp and the seeds, which have a weight ratio of roughly 90/10 (Di Guardo *et al.*, 2019). This edible plant has recently received a lot of attention due to its health-promoting properties and potential use in value-added food production (Ioannou *et al.*, 2023). It has a diverse nutritional profile that includes sugars, dietary fibers, minerals, vitamins, and a variety of polyphenols, making it a functional food (Basharat *et al.*, 2023). Carob fruits are used in a variety of industries, including the food industry, which has the potential to be very important (Goulas and Georgiou, 2019; Ben Othman *et al.*, 2020; García-Díez *et al.*, 2022); cosmetics (Ayad *et al.*, 2022); and biotechnology (Azaizeh *et al.*, 2020). Numerous studies have shown that carob, in addition to its antioxidant, antimicrobial, and anti-inflammatory activities (Ayad *et al.*, 2022; El Haddad *et al.*, 2022), has anti-hyperlipidemic (Valero-Muñoz *et al.*, 2019), anti-endometriotic (Khodabandeh *et al.*, 2021), analgesic (Ben Ayache *et al.*, 2020), antidiabetic (Macho-González *et al.*, 2020), anti-diarrheal (Asgari *et al.*, 2012), corrosion inhibition (Ben Hammou *et al.*, 2012; Ghazi *et al.*, 2022), and anti-cancer effects (Gregoriou *et al.*, 2021). These significant biological effects and health benefits have been linked to the presence of polyphenols, which act as bioactive molecules (Câmara *et al.*, 2020).

Many factors influence the presence and concentration of antioxidant secondary metabolites in plant foods, including genetics, growing conditions (Lattanzio *et al.*, 2006), ripening, and post-harvest conditions (Kyriacou *et al.*, 2021). Another important factor influencing not only the antioxidant activity of plant foods but also their overall quality properties is processing (Arias-Rico *et al.*, 2020). Indeed, because of their perishability and seasonality, these products are consumed or used not only fresh but also as processed products or in complex and possibly processed food formulations (Neri *et al.*, 2020).

Over the last five years, research on the various parts of *Ceratonia siliqua* L., their properties, and applications has made remarkable progress in a variety of fields. Despite the fact that many studies have focused on its functional, physicochemical, and antioxidant properties (El Bouzdoudi *et al.*, 2016; Mahtout *et al.*, 2018; Fadel *et al.*, 2020; El Oumlouki *et al.*, 2021; Ayad *et al.*, 2022; Yatim *et al.*, 2022; Ioannou *et al.*, 2023); none of them have reported on the effect of processing treatments such as boiling and freezing on the polyphenol content, antioxidant potential, and physicochemical characteristics. In this light, the current study aims to highlight and discuss the effect of two transformation processes, freezing and boiling, on the physicochemical properties, phenolic compound content, and antioxidant activity of two samples of *Ceratonia siliqua* L. pod pulp from the Jijel region.

2. Methodology

2.1 Plant sampling and processing

Cultivated carob pods were harvested at maturity in the northeast of Algeria from the Jijel region (latitude: 36° 49′ 13″ north, longitude: 5° 46′ 00″ east, elevation from sea level: 9 m). The pods with slightly different morphologies were collected at random from the 2017 harvest in two different regions: El-Milia and Djimla (**Figure 1**). These samples were then washed under tap water to remove debris, rinsed with deionized water, patted dry, and processed in the laboratory as follows:

Nine pods were randomly chosen from each sample, three of which were frozen at -18 °C for 24 hours, three others were cooked in boiling water for 60 minutes, and three others served as controls.

Before use, the frozen pods were thawed at room temperature for a few hours, and the boiled ones were wrung dry with absorbent paper. The carob kibbles were ground in a grinder (Retsch, Grindomix GM 200) and passed through a 450-mm CISA sieve after the seeds were removed. The fine powders that resulted (**Figure 2**) were used in all experiments.

Figure 1. Mature carob pods from: (**1**) El-Milia, (**2**) Djimla

Figure 2. Powders from: (**A**) raw pods, (**B**) boiled pods, (**C**) frozen pods

2.2 Morphological characterization of carob pods

The morphological parameters measured for the pods of each sample were length, width, thickness, and weight.

2.3 Proximate composition and physicochemical characterization of carob powders

The chemical proximate composition and physicochemical properties of carob powders were investigated. All samples were tested for pH, total titratable acidity (TTA), moisture, dry matter, ash, and protein content using standard analytical procedures of the Association of Official Agricultural Chemists, AOAC. The analysis was performed in triplicate, and the average values were calculated and expressed as mean ± SD (Standard Deviation).

The pH evaluation of a solution containing 10% (w/v) of each carob powder was carried out using a Hanna Hi 2210 pH meter (Hanna Glass Works, Medfield, MA) (AOAC 943.02, 1995). Total titratable acidity (TTA) was determined as a percentage of acetic acid by titration against 0.1 N NaOH (AOAC 920.92, 2005). The moisture content was calculated by subtracting the fresh and dry weights of samples after drying them at 105 ± 1°C until they reached a constant weight (AOAC 925.10, 2005). While the dry matter was determined by dividing the fresh and dry masses of samples after drying them at 105 ± 1°C until weight stabilization (AOAC 925.10, 2005). The crude ash fraction was obtained by organic matter incineration at 550°C for about five hours until the sample was free of carbon particles (AOAC 923.03, 2005). Zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd) contents of the resulted ash fraction was determined by atomic absorption spectrometry (Shimadzu AA-6200) (AOAC 999.11, 2000).

Crude protein content was measured by the Kjeldahl method (AOAC 955.04D, 2000). The total sugar content was measured using the phenol/sulfuric acid method (DuBois *et al.*, 1956). Briefly, 0.125 g of the sample was mixed with 5 mL of 0.5 M sulfuric acid (H_2SO_4) and placed in an oven at 105 °C for 3 hours. After cooling, the solution was transferred to a 500-mL flask, and the volume was adjusted up to 500 mL with distilled water. After filtration, three dilutions at 1/3 were performed. A reaction mixture containing 1 mL of each dilution was added to 1 mL of 5% phenol and 5 mL of 98% sulfuric acid, H_2SO_4. These tubes were kept for 5 minutes at 105°C and then left to cool in the dark for 30 minutes. The absorbance at 485 nm was measured using a spectrophotometer (SPECORD 50) against a blank. The sugar content is expressed in mg/L of glucose based on a calibration curve.

2.4 Phenolic compound estimation

2.4.1 Extraction procedure

10 g of each powder sample was macerated in 100 mL of a methanol/water mixture (80:20 v/v) for one night at room temperature with gentle agitation. The resulting extract was filtered twice through sterile Whatman filter paper No. 1. The methanol was evaporated from the filtrate using a rotary evaporator (Buchi R-300) at 48 °C, yielding an extract with a dark brown color that is considered the dry crude extract.

2.4.2 Total polyphenol content

The Folin-Ciocalteu colorimetric method was used to determine the total phenolic content (Singleton and Rossi, 1965). 1.20 mL of Folin-Ciocalteu reagent was added to 0.30 mL of each extract. After 5 min, 1.5 mL of sodium carbonate solution (7.5%) was thoroughly mixed with the mixture. The obtained solutions were incubated in the dark at room temperature for 2 hours. The absorbance was then measured at 765 nm using a UV-Vis spectrophotometer (SPECORD 50). The calibration curve for sample quantification was created using various concentrations of gallic acid standard solution (0–250 µg/mL). The results were expressed in micrograms of gallic acid equivalents per milligram of extract dry weight (µg GAE/mg).

2.4.3 Total flavonoids content

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Woisky and Salatino (1998). A volume of 0.5 mL of 2% $AlCl_3$ methanolic solution was added to 0.5 mL of each extract. After 1 hour of incubation at room temperature, the absorbance at 420 nm was measured. The flavonoid concentration was determined using a calibration curve established with quercetin (0–50 µg/mL) as the standard. The results are given in micrograms of quercetin equivalents per milligram of dry extract (µg QE/mg).

2.4.4 Total flavonol content

The total flavonol content in the carob powder extracts was calculated as described by Kumaran and Karunakaran (2007). A mixture of 2 mL of 2% $AlCl_3$ methanolic solution and 3 mL (75 g/L) sodium acetate was added to 2 mL of methanolic extract. The absorption at 440 nm was read after 2 hours and 30 minutes of incubation at 20 °C. The flavonol concentration was calculated using the calibration curve with quercetin (0–50 µg/mL) as the standard. The findings were expressed in terms of micrograms of quercetin equivalents per milligram of dry extract (µg QE/mg).

2.5 Evaluation of the antioxidant activity of the extracts

The antioxidant effectiveness of carob kibble powder extracts was determined using two different tests: the DPPH radical scavenging assay and the phosphomolybdate method.

2.5.1 Radical scavenging activity by DPPH assay

The DPPH method with ascorbic acid as a standard antioxidant was used to assess the antioxidant activity of each carob kibble powder extract (Braca *et al.*, 2001). Briefly, 0.1 mL of each extract was mixed with 3 mL of a 0.004% DPPH methanolic solution. The mixture was vortexed and left at room temperature in the dark for 30 minutes. The absorbance was then measured at 517 nm with a visible UV-SPECORD 50 spectrophotometer. The free radical scavenging activity (FRSA) was calculated as a percentage using the following equation:

$$\text{FRSA} (\%) = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100.$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control without sample and $\text{Abs}_{\text{sample}}$ is the absorbance of the sample (extract). Results are expressed as inhibitory concentrations (IC_{50}). IC_{50} values denote the concentration of sample required to scavenge 50% DPPH free radicals.

2.5.2 Total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacity of the extracts was determined by the phosphomolybdate method using ascorbic acid as a standard (Prieto *et al.*, 1999). A 0.3-mL aliquot of each methanolic extract was combined with 3 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated for 90 minutes in a 95°C water bath. After the samples were cooled to room temperature, the absorbance at 695 nm was measured against a blank (methanol plus reagent) using a SPECORD 50 spectrophotometer. The total antioxidant capacity was expressed as micrograms of ascorbic acid equivalent per milligram of dry extract (µg AAE/mg DW).

2.6 Statistical analysis

The data were subjected to one-way analysis of variance and expressed as the mean ± the standard deviation (SD). All statistical analysis were performed using the Statistical Package for Social Sciences, 23.0 (*SPSS for Windows*; SPSS Inc., Chicago, IL). Significance of differences was defined at $p < 0.05$.

3. Results and Discussion

3.1 Morphological analysis of carob pods

Five pods from each carob sample were measured for their morphological characteristics (length, width, thickness, and weight). **Table 1** displays the results. Carob pods vary in size, shape, quality, color, and seed yield depending on the variety. These variations can be attributed to plant genotype, geographical origin, climatic conditions, and harvesting and storage methods (Batlle and Tous, 1997;

Naghmouchi *et al.*, 2009; El Bouzdoudi *et al.*, 2016). Pod size, as defined by the average value of its length, resulted in two categories of harvested pods: slightly long (14 < L ≤ 15 cm) and slightly short (10 ≤ L < 14 cm). This classification was based on the work of Tutin *et al.* (1993), Tous *et al.* (1996), and Batlle and Tous (1997), who reported that the average pod size could range from 10 to 30 cm. In our samples, the slightly long size (14.10 ± 2.76 cm) characterizes the pods of Djimla, while the slightly short one characterizes the pods of El-Milia (12.90 ± 0.36 cm). These values are higher than those reported by Boublenza *et al.* (2019) for the pods from the region of Bejaia, which are 10.30 ± 0.31 cm long; closer to those of the Tlemcen and Boumerdes regions with 13.64 ± 0.45 and 13.13 ± 0.28, respectively; and lower than those of the Ain Temouchent, Mostaganem, and Tipaza pods, which are 18.75 ± 0.47 cm, 18.21 ± 0.38 cm, and 18.02 ± 0.47 cm, respectively.

The width of a carob pod is an important agronomic indicator. It is independent of pod size and can provide data not only on its compressed or expanded state but also on the volume of seeds and pulp. According to Tous *et al.* (2009), pods that have a high pulp content produce a low seed yield. When compared to Djimla samples, the region of El-Milia has the widest pods, with an average width of 1.93 ± 0.11 cm. These findings agree with those of Tutin *et al.* (1993) and Batlle and Tous (1997), who found 1.5 to 2.5 cm and 1.5 to 3.5 cm, respectively. Boublenza *et al.* (2019) found Boumerdes and Bejaia carob pods to have values of 1.82 ± 0.03 and 1.91 ± 0.04, respectively.

The thickness of the pods varies greatly from sample to sample. It is used to differentiate between compressed and bulky pods. It can grow to be 1 cm long, especially in fleshy pods (Batlle and Tous, 1997). This variable distinguished fleshy and voluminous pods that are thicker than 0.61 cm, such as those in the El-Milia region (0.83 ± 0.15 cm), from those that are flattened or compressed with a value between 0.33 and 0.49 cm, such as Djimla pods (0.43 ± 0.05 cm). The carob pods of El-Milia are the thickest of any of the regions studied by Khlifa *et al.* (2013), Boublenza *et al.* (2019), and Fadel *et al.* (2020).

The previously measured variables, length, width, and thickness, all have a significant impact on the total weight of the pod and the amount of pulp contained within it. Indeed, samples with a higher fruit weight had pods that were sufficiently wide and thick. The sample from El-Milia yields the highest value (11.89 ± 0.77 cm). This value is higher than that reported by Boublenza *et al.* (2019) for the Bejaia region of Algeria (7.04 ± 0.44 cm) and closer to that found by Fadel *et al.* (2020) for El Hoceima and Chafchaouen in northern Morocco (11.22 ± 4.75 cm).

Table 1. Morphological characteristics of raw pods

Characteristics	**Samples**	
	Djimla	**El-Milia**
Length (cm)	14.10 ± 2.76^{a}	12.90 ± 0.36^{b}
Width (cm)	1.77 ± 0.05^{a}	1.93 ± 0.11^{b}
Thickness (cm)	0.43 ± 0.05^{a}	0.83 ± 0.15^{b}
Weight (g)	8.17 ± 0.72^{a}	11.89 ± 0.77^{b}

$^{a\text{-}c}$ Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

3.2 Boiling and freezing effects on the physicochemical characteristics of carob powders

3.2.1 pH and titratable acidity

Based on these findings, our raw samples have an acidic pH ranging from 5.26 ± 0.02 to 5.31 ± 0.02 (**Table 2**). These results are between the pH values published by Baston (2016) and Boublenza *et al.* (2017), which were 4.34 and 5.6, respectively. It should be noted that the pH values of the samples in

their various states—raw, frozen, and boiled—are very close. Boiling samples yielded the highest values, with averages of 5.45 ± 0.01 and 5.48 ± 0.02 for Djimla and El-Milia pod powders, respectively, while the raw samples yielded the lowest.

When compared to the control, the pH of the boiled powders was significantly higher ($p < 0.05$). The pH rise could be attributed to the loss of organic acids, which resulted in a decrease in acid content and, consequently, an increase in the pH of the boiled powders (Igual *et al.*, 2010). Similar to the changes in pH, the boiled samples had significantly lower titratable acidity ($p < 0.05$) when compared to the control (**Table 2**). The decrease in titratable acidity could be attributed to the leaching of hydrogen ions and sugars into the water during boiling (Ampofo-Asiama *et al.*, 2020). The composition of organic acids determines acidity, which varies between cultivars (Avallone *et al.*, 1997). According to Bhardwaj and Pandeya (2011), decreased acidity may be due to the acidic hydrolysis of polysaccharides, in which acid is used to convert non-reducing sugars to reducing sugars. Fathi *et al.* (2019) also explained this decrease in titratable acidity and increase in pH value during freezing by increasing the content of alcoholic compounds during storage. It should be noted that the acidity of the powders obtained by grinding Djimla pods was higher in all three states studied.

3.2.2 Moisture content

The results show that the moisture content of the boiled samples is very high, with a significant rate recorded for the sample from the Djimla region at 60.37 ± 0.002%. Fresh samples have the lowest rates, with very close averages of 13.11 ± 0.005% and 13.13 ± 0.003% (**Table 2**). These findings are comparable to those of Khlifa *et al.* (2013), who reported an average value of 10.2 ± 0.13%, and significantly higher than those of Youssef *et al.* (2013) and Boublenza *et al.* (2019), who found a percentage of 5.29% and 5.13 ± 0.02% of the Bejaia region, respectively. According to Baston (2016), the technique of preparation and storage is mostly responsible for moisture changes in carob powder. Heat could explain the greater moisture content in boiling samples by shattering the cell's inner lamella and allowing water from the surrounding area to be absorbed for starch gelatinization (Srivastava and Singh, 2017).

The moisture content of frozen pod powders increased slightly, especially in the Djimla region ($p > 0.05$). This increase resulted from the increased moisture content of the upper kernel surface during a freeze-thaw cycle. Moisture migrates to the frozen front during the freezing process, resulting in unequal moisture distribution in the frozen pod. It should also be highlighted that cell membranes and capillaries between cells are the principal barriers to free water. As a result, maintaining cell structural integrity and preventing myofibrillar protein denaturation during freezing is critical for retaining water immobilized and reducing drip loss (Li *et al.*, 2018).

3.2.3 Dry matter content

The highest dry matter content was found in raw carob powders with closer values. The samples from Djimla and El-Milia have nearly the same content, 86.88 ± 0.005% and 86.89 ± 0.003%, respectively (**Table 2**). These values are lower than those reported by Gaouar (2011), who found a very high dry matter content of 90.40% in Jijel carob pulp. El Batal *et al.* (2016) registered a dry matter content ranging from 86.53 to 87.84%.

The dry matter content of boiled samples decreased significantly ($p < 0.05$), with losses ranging from 11 to 27%, while there was no significant difference between fresh and frozen samples. According to Handayani *et al.* (2018), the decrease in dry matter content during the boiling process was caused by the dissolution of nutrients in water, such as water-soluble proteins, water-soluble vitamins, and

macrominerals. In this experiment, protein and mineral content decreased as crude protein and ash content decreased (**Table 2**). The fact that the dry matter content of the powders does not change during frozen storage implies that the water content of the powders does not evaporate. This also implies that no water escapes the system during the gelling process (Hidas *et al.*, 2020).

3.2.4 Ash content

The ash contents of the raw samples are very similar, with a slight increase recorded for the El-Milia sample, with an average percentage of 3.04 ± 0.001%. (**Table 2**). Our results are in line with those obtained by Mahtout *et al.* (2018), who found 3.0 ± 0.03% of ash content, and with the two limits of the variation interval (2.45 ± 0.02% – 4.52 ± 0.03%) found by Boublenza *et al.* (2019) for the ten Algerian regions studied. The variation in ash content of the pods may be due to the climatic conditions and soil edaphic characteristics.

The boiled samples have the lowest rates. According to Nzewi and Egbuonu (2011) and Handayani *et al.* (2018), this reduction in ash percentage may be due to the mineral compounds dissolving in the boiling water. As a result, boiling water becomes more mineral-laden as the boiling time increases, which is why it is best not to overwash certain foods and to avoid cooking them by steaming or boiling in a small amount of water.

The ash content of frozen powders was the same as that of fresh powders. This is due to the low temperature, which reduces the kinetics of many biochemical reactions, and the low water activity due to crystallization, which allows for minimal mineral compound leaching. As a result, slight reductions in mineral components may result in ash content stability. The work of Sahoré and Nemlin (2012) confirmed the fact that cold storage avoided significant reductions in chemical components, which is in line with our results.

Table 2. Physicochemical attributes of carob powders

Attributes \ Samples	Djimla			El-Milia		
	R	B	F	R	B	F
pH	5.26 ± 0.02^{a}	5.45 ± 0.01^{b}	5.28±0.01^{c}	5.31 ± 0.02^{a}	5.48±0.02^{b}	5.46 ± 0.02^{c}
TTA (% acetic acid)	1.16 ± 0.01^{a}	0.37 ± 0.01^{b}	0.46±0.007^{c}	0.85 ± 0.007^{a}	0.35 ± 0.007^{b}	0.40 ± 0.01^{c}
Moisture content (%)	13.13±0.003^{a}	60.37±0.002^{b}	13.67±0.005^{a}	13.11±0.005^{a}	36.99±0.002^{b}	17.83±0.003^{c}
Dry matter content (%)	86.88±0.005^{a}	39.64±0.004^{b}	86.33±0.005^{c}	86.89±0.003^{a}	63.02±0.002^{b}	82.18±0.002^{c}
Ash content (%)	2.72 ± 0.002^{a}	2.42 ± 0.002^{a}	2.72 ± 0.002^{a}	3.04 ± 0.001^{a}	2.22 ± 0.002^{a}	3.04 ± 0.002^{a}
Crude protein content (%)	4.65 ± 0.09^{a}	2.22 ± 0.50^{b}	3.02 ± 0.28^{c}	4.79 ± 0.04^{a}	2.65 ± 0.46^{b}	3.56 ± 0.36^{c}
Total sugar content (%)	26.8 ± 1.34^{a}	26.6 ± 1.40^{a}	22.7 ± 1.76^{b}	27.7 ± 3.86^{a}	24.6 ± 1.92^{b}	24.5 ± 0.98^{c}

R: Raw, B: Boiled, F: Frozen, TTA: Total Titratable Acidity.

$^{a-c}$ Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

3.2.5 Crude protein content

The highest protein levels were found in raw pod powders, with an average increase for pods from the El-Milia region (4.79 ± 0.04%) (**Table 2**). These results are lower than those reported by Youssef *et al.* (2013), who reported a content of 6.34%, but higher than those reported by Mahtout *et al.* (2018) and Fadel *et al.* (2020), who recorded contents of 3.0 ± 0.03% and 3.35 ± 0.79%, respectively. Many studies have shown that the amino acid composition of carob fruits varies depending on species, geographical origin, ripening stage, and cultivation method (El Batal *et al.*, 2016; Boublenza *et al.*,

2019; Fadel *et al.*, 2020). According to Baston (2016), the amino acid valine was the most abundant in carob pulp powder, followed by threonine. Cysteine and cystine, on the other hand, had the lowest levels, followed by glycine and tryptophan.

The lowest levels are found in boiled pod samples. This is due to the loss of soluble or proteinaceous components in the cooking water. According to Handayani *et al.* (2018), heating duration had a significant effect on crude protein content but had no effect on heating method. The freezing and thawing of the pods at room temperature results in a temperature change that causes the initial crude protein to gradually degrade to more volatile products such as total volatile bases (TVB), trimethylamine (TMA), hydrogen sulfide, and ammonia. The reduction in protein content may be associated with the leaching of some of the protein fractions into ice (Obemeata and Christopher, 2012).

3.2.6 Total sugar content

Sugars are some of the most important constituents of fruits and vegetables; they play an important role in determining nutritive value and maintaining quality (Ayaz *et al.*, 2007). In our study, raw pod samples had the highest total sugar contents, with an increase in average content for pods from the El-Milia region of 27.7 ± 3.86% (**Table 2**). Our results are very low when compared to those of Baston (2016) and Mahtout et al. (2018), who obtained percentages of 57.88 ± 2.33% and 65.00 ± 3.3%, respectively. Regarding the Algerian carob pulp, Boublenza *et al.* (2019) recorded a variation interval of 43-50% of total sugar content. It is worth noting that sugar content varies by country and is affected by species, physiological maturity, harvest season, climate, and storage conditions (Ayaz *et al.*, 2007). According to Boublenza *et al.* (2017) and Mahtout *et al.* (2018), the sugars found in carob pulp by percentage importance are sucrose, fructose, and glucose.

Boiling had a significant effect ($p < 0.05$) on the sugar content of powders derived from El-Milia pods. On the one hand, this could be attributed to gelatinization during boiling; one of the main reactions is the Maillard reaction, in which glucose and fructose react with amino acids to improve the sensory properties of the product, and sucrose is hydrolyzed during the heat treatment (Murniece *et al.*, 2011). On the other hand, the leaching of sugars in the water during processing. In the case of boiling Djimla pods, we stated the same values as for fresh ones. According to Nzewi and Egbuonu (2011), carbohydrates may absorb water to bulk up through a cross-linking reaction caused by the boiling process's heat. This may improve the stability of the carbohydrates, allowing them to withstand additional heat.

When frozen samples were compared to raw samples, the total sugar content was significantly reduced ($p < 0.05$). This decrease could be attributed to respiration and sugar conversion. Low temperatures typically cause starch to be converted to reducing sugars (Ji *et al.*, 2017).

3.2.7 Trace mineral elements and heavy metal contents

Table 3 summarizes the mineral and heavy metal levels of raw and processed carob powders. The highest mineral values were found in fresh Djimla samples. It should be highlighted that the Zn level was significant, with an average value of 70.79 ± 0.028 ppm (**Table 3**). This Zn content in fresh Djimla pods can help supplement our daily food intake. Zinc is necessary for immune system and different organism functions such as protein synthesis, wound healing, DNA synthesis, and cell division (Chasapis *et al.*, 2020). According to Ayaz *et al.* (2007) and Ben Othmen *et al.* (2019), the mineral composition of carob pulp varies depending on cultivars, geographical origin, harvest time, environmental factors, soil type, seasonal climate, and ripening stages. Our Zn and Cu content findings are higher than those of El Batal *et al.* (2016) and El Oumlouki *et al.* (2021), who reported ranges of

variation of 1.7–7.5 ppm and 18.0–26.1 ppm for Zn and 1.6–12.8 ppm and 4.2–5.6 ppm for Cu, respectively. There have been no studies on the cadmium and lead contents. The presence of these two heavy metals in our samples could be due to soil contamination.

After boiling, trace minerals decreased significantly ($p < 0.05$), with a loss of up to 71% in Zn. The outflow of minerals from pods is assumed to be the cause of mineral loss (Kimura and Itokawa, 1990). According to Puupponen-Pimiä *et al.* (2003), mineral losses during the boiling process are generated by leaching into the cooking water rather than destruction. When compared to boiling, the freezing technique resulted in reduced mineral loss. This could be because of drip loss and dehydration during frozen storage (Sikorski and Kolakowski, 2000).

Table 3. Values of trace mineral elements and heavy metals in carob powders

Samples / Elements (ppm)	Djimla			El-Milia		
	R	**B**	**F**	**R**	**B**	**F**
Trace mineral elements (ppm)						
Zinc (Zn)	70.79±0.028[a]	20.00±0.014[b]	54.55±0.134[c]	46.88±0.672[a]	20.52±0.233[b]	27.42±0.092[c]
Copper (Cu)	5.91±0.092[a]	2.96±0.057[b]	4.37±0.071[c]	4.08±0.092[a]	1.83±0.021[b]	2.53±0.007[c]
Heavy metals (ppm)						
Lead (Pb)	1.057±0.052[a]	0.453±0.069[b]	0.988±0.009[a]	0.384±0.006[a]	0.384±0.004[a]	0.467±0.007[a]
Cadmium (Cd)	0.095±0.025[a]	0.056±0.008[a]	0.068±0.007[a]	0.096±0.011[a]	0.051±0.004[b]	0.067±0.028[c]

R: Raw, B: Boiled, F: Frozen, ppm: parts per million.

[a-c] Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

3.3 Boiling and freezing effects on bioactive compound content and antioxidant power

Table 4 summarizes all of the findings regarding the effects of boiling and freezing on the concentrations of the various bioactive compounds studied and the antioxidant capacities assessed.

3.3.1 Total polyphenols, flavonoids, and flavonol contents

According to Nicoli *et al.* (1999), the total phenolic content of fruits and vegetables can increase, decrease, or remain constant after thermal processing. Raw and frozen Djimla samples contained the most polyphenols, with values of 127.05 ± 0.04 µg GAE/mg DW and 99.85 ± 0.04 µg GAE/mg DW, respectively. El-Milia had the highest concentrations of flavonoids and flavonols, 3.23 ± 0.03 µg GAE/mg DW and 53.74 ± 0.03 µg GAE/mg DW, respectively (**Table 4**).

Our total polyphenol compound results exceed those of Ben Othmen *et al.* (2019), who reported a variation interval of 20.72–40.92 mg GAE/g DW. In a separate study, Ayad *et al.* (2022) achieved a value of 23.375 ± 0.83 mg GAE/g DW with the goal of optimizing phytoantioxyadant compounds from carob pods from the Texenna region in Jijel. In terms of flavonoid content, our findings are higher than those of El Bouzdoudi *et al.* (2016) and Fadel *et al.* (2020), who found ranges of variation of 0.19–0.65 mg QE/g DW and 0.17–0.60 mg QE/g DW, respectively. There is no comparable data on the determination of flavonol content in brown carob pods, but Goulas and Georgiou (2019) reported that the most potent antioxidants in carob extracts were flavonol aglycones, specifically quercetin and myricentin.

Concerning the boiling effect, it was revealed that this cooking process significantly reduced total polyphenol content ($p < 0.05$), resulting in a 38% decrease in Djimla carob pods and a 15% decrease in El-Milia carob pods when compared to raw samples. We noticed the same significant loss in flavonol concentration up to 34% and 44% in Djimla and El-Milia pulp powders, respectively (**Table 4**). These losses could be attributed to the diffusion of these soluble compounds into boiling water or to their

degradation during the boiling process (Gunathilake *et al.*, 2018). Other research has found that food processing disrupts cellular structures such as lignin and polysaccharides, as well as the release of phenolic compounds in cooking water (Faller and Fialho, 2009). Furthermore, Mokrani and Madani (2016) have also found that flavonoids are more heat-sensitive, with the degradation of flavones and flavanols/flavanones occurring at lower temperatures. The differences in loss between the two samples are mainly due to the flavonoid composition variability; thus, the sample from Djimla is more likely to contain bioactive compounds that are more sensitive to temperature than that from El-Milia.

Moreover, freezing processing affected bioactive compound content in the same way as boiling, but at a slower rate. In this study, raw and frozen powder extracts produced very similar results but significative difference ($p < 0.05$) in flavonoid content, with only a 2% and 3% loss for El-Milia and Djimla samples, respectively. Plant foods are susceptible to damage during freezing and frozen storage, resulting in lower final quality and functional properties, particularly after thawing, than the corresponding fresh product (Neri *et al.*, 2020). In fact, cell rupture can result in the decompartmentalization of antioxidants like anthocyanins and other phenolic compounds, as well as their degradation through interactions with oxidative enzymes (Khattab *et al.*, 2015). Moreover, the formation of ice crystals can affect food texture and the content of some bioactive compounds, which may be lost due to leaching during thawing (van der Sman, 2020).

3.3.2 Antioxidant activity and total antioxidant capacity

Antioxidant activity or capacity evaluation of a food reflects not only its functional significance, but it can also be used as an index to predict its oxidative stability (Del Carlo *et al.*, 2004) or as an index of its changes or damage caused by processing (Nicoli *et al.*, 1999).

Two methods (the DPPH assay and the phosphomolybdete method) were used to assess the effectiveness of antioxidants in samples (**Table 4**). The DPPH test provided information about the activities of compounds containing stable free radicals; the DPPH effect was assumed to be due to their hydrogen-donating ability. Higher IC_{50} values indicate lower antioxidant activity, and vice versa (Boublenza *et al.*, 2017). The antioxidant activity of raw samples measured by DPPH had IC_{50} values of 0.09 ± 0.28 mg/mL and 0.35 ± 0.58 mg/mL for El-Milia and Djimla, respectively. Boublenza *et al.* (2017) found an IC_{50} between 0.05 and 0.06 mg/mL for ripe carob pods harvested in the Tlemcen region of western Algeria. According to Ben Othmen *et al.* (2019), the antioxidant activity level was origin- and ripening-stage-dependent.

In terms of antioxidant activity, the boiled sample from Djimla had the highest value, while that from El-Milia had the lowest. Although the bioactive compounds from the Djimla region were the most affected by boiling, they had higher antioxidant activity. This latter efficacy could be influenced more by the quality of bioactive chemicals than their quantity. However, this is most likely due to the neoformed molecules that resulted during the Maillard reaction, which may even have antioxidant properties (Gorinstein *et al.*, 2008). In contrast, the boiling process had a significant impact ($p < 0.05$) on the bioactive compounds in the El-Milia samples. In this case, the diminution may be directly linked to bioactive compound losses.

Regarding the total antioxidant capacity, we discovered an increase in both boiling and freezing samples. The latter resulted in an increase of up to 250% in the El-Milia sample over the raw sample. Because polyphenols and flavonoids were the least affected by freezing, we can conclude that they were responsible for this ability. Mullen *et al.* (2002) have also demonstrated that freezing operations can improve the quality and functional properties of plant foods by releasing bioactive compounds such as bound phenolic acids and anthocyanins, resulting in an increase in antioxidant activity. When

comparing the results of the two methods, we noticed that we were facing contradictory results. In the literature, studies evaluating the effect of freezing and frozen storage on the antioxidant activity of fruits and vegetables in general report conflicting results (Neri *et al.*, 2020).

Table 4. Effect of boiling and freezing on the bioactive compound content and antioxidant capacity of carob powder extracts

Assays \ Samples	Djimla			El-Milia		
	R	B	F	R	B	F
Total polyphenols (µg GAE/mg DW)	127.05±0.04[a]	78.05±0.03[b]	99.85±0.04[c]	97.75±0.04[a]	82.44±0.02[b]	96,04±0,05[c]
Total flavonoids (µg QE/mg DW)	2.14±0.03[a]	1.78±0.02[b]	2.07±0.02[c]	3.23±0.03[a]	3.16±0.02[b]	3.09±0.03[c]
Total flavonols (µg QE/mg DW)	20.72±0.02[a]	11.60±0.03[b]	14.14±0.02[c]	53.74±0.03[a]	35.58±0.02[b]	38.12±0.03[c]
Antioxidant activity (IC_{50} mg/mL)	0.35±0.58[a]	0.01±0.28[b]	0.44±0.32[c]	0.09±0.28[a]	0.70±0.33[b]	0.16±0.36[c]
Total Antioxidant capacity (µg AAE/mg DW)	20.05±0.42[a]	21.27±6.88[b]	25.42±8.65[c]	16.53±4.90[a]	19.77±9.00[b]	41.57±4.87[c]

R: Raw, B: Boiled, F: Frozen, GAE: Gallic Acid Equivalent, QE: Quercetin Equivalent, AAE: Ascorbic Acid Equivalent, IC_{50}: Half Maximal Inhibitory Concentration, DW: Dry Weight.
[a-c] Values in the same line and labelled with different letter differ significantly ($p < 0.05$).

Conclusion

Ceratonia siliqua L., a member of the *Fabaceae* family, is a medicinal plant with high therapeutic potential. The goal of this study is to highlight the effect of boiling and freezing processes on the proximate composition, physicochemical properties, phenolic compound level, and antioxidant power of two carob pod samples harvested from the Jijel region in Algeria's northeast.

The results revealed that the boiling process had a greater negative impact on the majority of the physicochemical quality properties studied than freezing. In terms of bioactive compounds and antioxidant activity, these samples are being studied for their phenolic compound concentrations as well as the antioxidant activity of their methanolic extracts, which was assessed using two different methods: the DPPH assay and the phosphomolybdate method.

Trace minerals (Zn and Cu) and total polyphenols were abundant in Djimla samples, while flavonoids and flavonols were abundant in El-Milia samples. Heat sensitivity was higher in polyphenols and flavonols than in flavonoids. When compared to raw samples, boiling resulted in a significant decrease in total polyphenol and favonol contents of up to 38% and 44% in Djimla and El-Milia carob pods, respectively. The antioxidant activity of the boiled Djimla sample was the highest. All parameters were affected by freezing at -18°C, though not as strongly as boiling. However, it significantly boosts total antioxidant capacity, particularly in the El-Milia sample, by 250%.

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