

Comparative study of the nutritional quality of two Tunisian carob varieties

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Abstract

Two varieties of Tunisian carob (*Ceratonia siliqua* L.), from different regions of Tunisia (Kairouan and Bizerte), have been characterized, to compare their different physico-chemical and antimicrobial properties as well as their dietary values, given their biological and dietary significance, and their potential for valorization. Hence, a comparative morphological characterization was conducted, followed by a quantitative analysis of total sugars in the mash. The results showed a significant difference between the two varieties. The first variety, coded J.S.14, which is local to Kairouan, contains a lower sugars contents (33.1%) compared to the other variety, J.S.15, which originated from Bizerte, and has a sugar content of 68.38. Using, the Kjeldahl method, J.S.14 was found to have 2.37% total protein content, whereas J.S.15 had 3.24%. The two carob varieties also exhibit a difference in their dry matter content. Specifically, S.14 and J.S.15 contain 91.15% and 85.91%, respectively. There is also a significant difference in fat content between the two samples, with J.S.14 being notably richer (7.504%) than J.S.15 (3.57%). A high fiber content was found in both samples, with J.S.14 containing 28.8% and J.S.15 containing 23.43%. An evaluation of the antioxidant activity of carob, was conducted by characterizing the secondary metabolites, such as polyphenols, flavonoids, and condensed tannins of the fluid extracts. The Folin-Ciocalteu, aluminum trichloride (AlCl₃) and vaniline methods were used to identify these metabolites. The results showed that, J.S.14 contains 70.39 mg EAG/g DM of polyphenols, 13.49 mg EC/g DM of condensed tannins and 8.22 mg ER/g DM of flavonoids. On the other hand, J.S.15, it contains 80.01 mg EAG/g DM of polyphenols, 55.66 mg EC/g DM of condensed tannins, and 18.78 mg ER/g DM of flavonoids. The antiradical activity of both samples was also evaluated, in which J.S.14 and J.S.15 showed activities of 98.88% and 99.03% respectively. The obtained results have confirmed the potential antioxidant activity of the two studied carob varieties. Additionally, the findings from the antimicrobial tests have established the microbiological stability and bactericidal activity of carob. Based on this study, we can establish that these two Tunisians carob varieties are rich in nutritious components and can be used in functional food products.

Keywords: Carob, nutritional values, antioxidant activity, microbiological stability

Introduction

The food market, both in Tunisian and globally, has expressed a growing interest in highlighting and promoting regional products that have been overlooked despite their nutritional and functional properties. One such product is carob, a legume, scientifically known as *Ceratonia siliqua* L. that belongs to the Fabaceae family and is indigenous to the Mediterranean regions. In Tunisia, the carob trees grow naturally and can be found in the wild, often in association with olive and lentisk trees. They are commonly found in humid, subhumid, and semi-arid regions with warm to temperate climates [3].

Carob production is primarily concentrated in the Northern region of Tunisia, accounting for (75% of total production). This species has been cultivated in Tunisia for a significant period of time and was included in the national list of property for the conservation and management of forest genetic resources in Tunisia [8].

The production area extends north to Nefza, Hdhil, Tebourba, Tebourouk, Bargou, Zaghouan, Serj, oueslatia, Lansarine [2]. The variety «Sfax» is particularly thanks to the quality of its fruits, and samples of this variety can be found in the Fahs and Souassi (Henchir Msatria) regions.

According to statistics provided by FAO, the area cultivated in carob is 414 ha, with an average annual quantity of 847 tons, with a yield of 2.07 t/ha [12].

Carob is a valuable source of sugars (Ayaz *et al.*, 2007), dietary fiber [5], and bioactive ingredients, such as polyphenols, flavonoids, and condensed tannins, that have been linked to various health benefits, including antioxidant, anti-inflammatory and cardiovascular properties, with a lack of theobromine and caffeine [1].

Its richness in these organic and nutritional components makes it a primary ingredient in some food products such as chocolate, yogurt, etc [13, 24].

It is worth noting that due to genetic, environmental, and maturation factors, the composition of bioactive and nutritional compounds can vary significantly among different carob varieties.

In this study, we conducted a detailed analysis to compare the chemical and biological composition of two carob varieties. Our goal was to better understand the differences in their nutritional, antioxidant, and microbiological properties and their potential implications for human health.

Material and methods

1. Preparation of samples

The research conducted in this study focuses on the plant materials from the species *Ceratonia siliqua* L. The materials were, and collected randomly from two different regions: Kairouan, coded as J.S.14, and Bizerte, coded as J.S.15. Following the collection, the carob pods were sorted, washed, and cleaned with water to remove any impurities.

The pulp was then separated from the seeds and crushed using a hammer. The matrices obtained were stored away from light for further analysis.

2. Morphological characteristics

20 carob pods of each variety were randomly selected to measure various morphological parameters of the pod and seeds such as shape, color, length, width, weight, thickness, size index (length/width) and yield per pod, number of seeds/pod and seed/pod mass ratio. The length in centimeters (cm) was determined by wire and then measured by a graduated ruler. Width and thickness (cm) were measured with a caliper, taking 3 measurements for each seed. The final width was considered to be the average of the 3 measurements made. The size index was determined as the ratio of length over width.

3. Extraction of carob flour

Once the fruit matrix is prepared (Cleaning, Separation, Crushing), the carob pulp is air-dried for three weeks. After that, it was grounded and sieved to obtain fine flour with a grain size less than 200 μm , as shown in Figure 1. The resulting flour is then stored in glass containers.

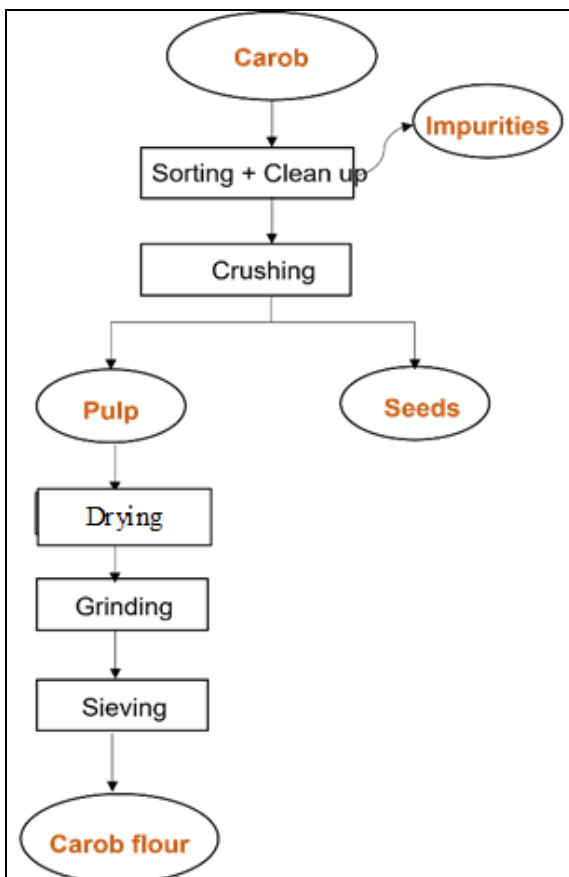


Fig 1: Diagram of carob flour extraction

4. Characterization of carob flour

4.1 Physico-chemical properties of the carob powder

In order to evaluate the physico-chemical properties of the carob flour, tests to determine the dry matter content and pH were conducted using established methods [4]. The titratable acidity was also measured by performing NaOH titration via the method of Katina *et al.*, (2007) [18]. Finally, the flour's electrical conductivity was measured using the Aubert method (1978) via a conductivity meter type ADWA AD3000 [6].

4.2 Determination of the nutritional composition

The protein content was measured using the Kjeldahl method [19], while the fat content was determined using the traditional Soxhlet method [15]. Total sugar content was determined using the phenol/sulfuric acid method introduced by Dubois *et al.* (1956) [11], and fiber content was determined using the method developed by De Pádua *et al.* (2004) [10].

4.3 Biological properties

For the determination of secondary metabolites, the method of Sharif *et al.* (2016) was used with some modifications [25]. Firstly, 20 g of carob flour maceration was carried out in 200 ml of solvent and stirred at a room temperature for 30 min. The mixture was then kept in the refrigerator for 15h in the dark. After that, the mixture was filtered using filter paper, and the filtrate was used to determine the secondary metabolites. Once this step was completed the total polyphenols content was measured using the colorimetric method with the folin-Ciocalteu reagent [26]. The flavonoid content was determined using the AlCl₃ method [21]. The condensed tannins were also quantitatively determined using the vanillin method with HCl [23].

After quantifying the different antioxidant components, the antiradical power of the two carob varieties against the free radicals of DPPH was evaluated. Through plotting the percentage reduction of DPPH against extract concentration, we were able to determine the IC₅₀, which represents the concentration of the antioxidant required to reduce/inhibit 50% of DPPH [9].

4.4 Evaluation of antimicrobial activity

The evaluation of the microbiological quality of our samples was conducted by following conventional methods. This involved identifying the presence of various microbial strains, such as Faecal coliforms on nutrient agar incubated at 44°C for 18 to 24 hours, Total coliforms on nutrient agar incubated at 37°C for 18 to 24 hours, Staphylococcus aureus on Chapman incubated at 37°C for 18 to 24 hours, and Salmonella on agar Salmonella-Shigella (S-S) incubated at 37°C for 18 to 24 hours. Additionally, the methods outlined in ISO 4833 (2003), Regulation (Ce) 2073 (2005), ISO 7218 (2007), ISO 6887-5 (2010), and Mutsch (2018) were employed to detect the presence of yeasts and molds after incubating on Sabouraud at 30°C for 3 days [14, 16, 17, 20, 22].

Results and discussion

1. Morphological characterization of carob

Through morphological characterization of carob pulp and seeds, we were able to identify the quantitative and qualitative characteristics of the two carob varieties, and create their descriptive profiles.

The irregular round shape and light brown color of both varieties were found to be similar based on the parameters.

Figure 2 presents the results of the morphological characterization of the carob pulp, revealing that variety 1 has the highest values for the studied criteria compared to variety 2.

Similarly, figure 3 displays the results of the morphological characterization of the seeds, where variety 1 had the highest values for length, width, thickness and weight, while variety 2 had the highest values for seed yield and seed numbers per pod.

The data was analyzed using ANOVA statistical test, and significant differences among different values were observed ($p < 5\%$).

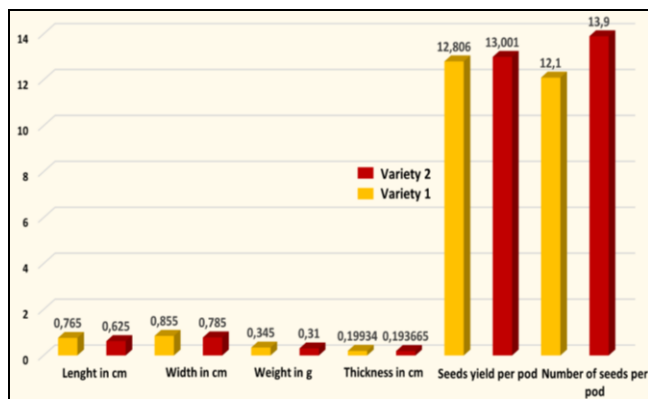


Fig 2: Morphological characterization of pulps

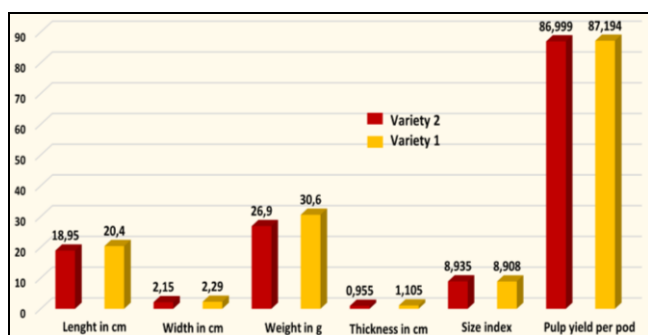


Fig 3: Morphological characterization of seeds

2. Result of physico-chemical characterization

The pH assessment study of the two varieties showed that they are acidic. However, it has been determined that the second variety (J.S.15) has a lower pH level of $pH = 5.16$ in comparison to the first variety with a pH level of $= 5.60$. Furthermore, the acidity test confirms that the second variety (J.S.15) has a higher acidity level of $0,84\%$ as opposed to the first variety with a level of $0,74\%$.

3. Result of nutritional composition

Table 1 displays the chemical characteristics of the locust bean of the two studied varieties. The ANOVA test was applied to the data and significant differences between the two varieties were found.

These findings align with previous research, which notes that carob pulp is characterized by its high level of total sugars. The results revealed a range of $33.1\% \pm 0.2$ for variety 1 to $68.38\% \pm 0.59$ for variety 2. This difference can be attributed to several factors, such species and variety, physiological maturity stage, harvest season, climate, and storage conditions. The decrease in sugar levels can also be explained by the formation of new products resulting from the Maillard reaction.

Variety 1 of carob boasts a low-fat content of approximately $7.504\% \pm 0.102$, whereas variety 2 has a lower fat content of $3.57\% \pm 0.036$.

This disparity may be attributed to a range of factors including genetics and, environmental factors such as climate, soil, sun exposure, and maturity. Thus, the hot and dry climate of the Kairouan region compared to that of Bizerte, which is a cooler and humid climate, influences the increase in fat content in the pulp. It's worth noting that the

presence of unsaturated fatty acids more than saturated ones, which gives carob the property of reducing the risk of cardiovascular diseases.

Varieties 1 and 2 exhibit protein contents of $2.37\% \pm 0.33$ and $3.24\% \pm 0.401$, respectively. Various factors such as harvest period, degree of maturation, and geographical distribution can influence protein content. The drying method of locust bean pods is also a significant factor as drying at a high temperature can lead to protein denaturation and coagulation. Thus, the richness of the carob in sugars can trigger protein complexation through Maillard reactions. Variety 1 boasts a higher total fibre content of $28.80\% \pm 2.7$ compared to that of the second variety of $23.43\% \pm 1.01$. The variance can be explained by the fluctuations in environmental conditions. Thus, the fibers present at the level of carob powder are responsible for increasing the metabolism of fatty acids oxidation, which impacts ingested energy and body weight. Furthermore, these fibers not only reduce postprandial blood glucose and insulinemia for type II diabetics, but also lower cholesterol and blood triglyceride levels. In addition, these fibers contribute to the proper functioning of the intestinal transit by providing prebiotic power, making them a valuable asset for weight loss diets.

The dry matter content of carob varieties 1 and 2 differ significantly. Variety 1 has a dry matter content of $91.15\% \pm 0.17$, while variety 2 has a dry matter content of $85.91\% \pm 0.586$. this difference can be explained by a variety of factors such as pedoclimatic factors, changes in carob varieties, and duration between harvest and treatments (during preparation and storage).

Additionally, the results showed that carob variety 2 (J.S.15) has higher carbohydrate and protein content when compared to variety 1 (J.S.14), which contains higher fat, fiber, and dry matter contents.

Based on our findings we were able to estimate the calories per 100 g of carob of each variety. The first (J.S.14) contains $209.42 \text{ cal}/100\text{g}$, while the second, J.S.15, contains $318.61 \text{ cal}/100\text{g}$.

Table 1: Chemical characteristics of carob varieties

Characteristic	Variety 1	Variety 2
DM (%)	91.15 ± 0.17	85.91 ± 0.586
Sugars (%)	33.1 ± 0.2	68.38 ± 0.59
Fat (%)	7.504 ± 0.102	3.57 ± 0.036
Protein (%)	2.37 ± 0.33	3.24 ± 0.401
Fiber (%)	28.80 ± 2.7	23.43 ± 1.01

4. Result of biological composition and properties

Table 2 presents the biological composition and properties of the locust bean for the two studied varieties. The data was analyzed using ANOVA and significant differences were found.

Upon conducting an analysis of secondary metabolites, it has been determined that both variety 1 and 2 exhibit a significant abundance of total polyphenols. Specifically, variety 1 boasts $70.39 \pm 20.53 \text{ mg EAG/ g DM}$, while variety 2 demonstrates $80.01 \pm 7.61 \text{ mg EAG/ g DM}$. These compounds are essential in defining the nutritional and functional quality of fruits, as they provide crucial antioxidant, anti-inflammatories, cardiovascular, sugar regulators and digestive benefits.

For flavonoids, the results showed a content of $8.22 \pm 1.06 \text{ mg ER/ g DM}$ for variety 1 and $18.78 \pm 2.41 \text{ mg ER/ g DM}$

for variety 2. Such a rich concentration of flavonoids in carob can offer numerous health advantages, including as antioxidants, anti-inflammatory, and cardiovascular properties by reducing the oxidation of LDL cholesterol. Additionally, they can serve as neuroprotectors.

In terms of the tannin dosage, we found a concentration of 13.49 ± 6.67 mg EC/ g DM in variety 1 compared to 18.78 ± 2.41 mg EC/ g DM in variety 2. While the high content of condensed tannins present in carob can offer antioxidant and anti-inflammatory health benefits, it may also affect digestion and nutrient availability.

Based on these results, we conclude that carob variety 2 (J.S.15) is richer in secondary metabolites composition than the first variety.

Thus, this difference can be explained by geographical provenance, cultivar, and fruit ripening. Similarly, drying techniques and treatments influence the rate of secondary metabolisms. In addition, subjecting the material to a high temperature may cause the breakdown of delicate compounds. The presence of these components in carob promotes the reduction of bad cholesterol and blood triglyceride, and increases its prebiotic power.

Antioxidant activity was evaluated by the reduction percentage of DPPH. Remarkably, variety 1 (J.S.14) demonstrated a maximum inhibition of this radical 98.88% at the concentration of 100 mg/ml, while variety 2 (J.S.15) achieved 99.01% inhibition. This important antiradical activity is due to the high total polyphenols and flavonoid content.

To further assess their antioxidant potential, the IC50 parameter was graphically calculated. Variety 1 (J.S.14) exhibited an IC50 of 11.86 mg/ml, whereas variety 2 (J.S.15) had an IC50 of 0.18 mg/ml.

This difference in radical activity can be explained by the abundant bioactive compounds and antioxidants in variety 2, which enhance its antiradical capacity.

Table 2: Biological composition of carob varieties

Characteristic	Variety 1	Variety 2
Total polyphenols (mg EAG/ g DM)	70.39 ± 20.53	80.01 ± 7.61
Flavonoid (mg ER/ g DM)	8.22 ± 1.06	18.78 ± 2.41
Condensed tannins (mg EC/ g DM)	13.49 ± 6.67	18.78 ± 2.41
Antioxidant activity (%)	98.88	99.01

5. Results of microbiological analyses

Table 3 presents the different microbiological properties of the locust bean of the two studied varieties.

The strains studied did not multiply in the optimal conditions, except for total coliforms in the 1st variety (J.S.14), and yeasts and molds for both varieties, with a multiplication rate that remains within the acceptable international standard. This compliance is important to ensure that carob samples are safe for consumption and recovery.

As a result, carob samples do not present microbiological risks to consumer health, such as food infections or food poisoning. These findings demonstrate the microbiological stability and the bactericidal activity of both varieties.

Other factors that have contributed to ensuring the microbiological safety of carob samples are good agricultural practices, hygienic processing, appropriate storage conditions, and rigorous quality controls.

Table 3: Microbiological properties of carob varieties

Germ studied	Variety 1	Variety 2	Standards
Total coliforms	Abs	Abs	10 UFC/ ml
Fecal coliforms	1	Abs	1 UFC/ ml
<i>Staphylococcus aureus</i>	Abs	Abs	10 UFC/ ml
Salmonella	Abs	Abs	Abs
Yeasts and molds	2	1	100 UFC/ ml

Conclusion

Based on the findings of our study, it can be concluded that variety 2 (J.S.15), originating from Bizerte, is richer in bioactive compounds, such as antioxidants, as well as nutritional compounds, including sugar and proteins, compared to the variety 1 (J.S.14), originating from Kairouan.

This study highlighted the valuable Tunisian carob varieties, comparing their nutritional, biological, and microbiological properties, and confirming their abundance in bioactive and nutritional compounds. This data confirmed that carob is an excellent candidate for the formulation of functional and dietary food products.

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