

ORIGINAL ARTICLE

Food Chemistry, Engineering, Processing and Packaging | Functional and Novel Foods

Comparative Study of The Physicochemical, Phenolic, Mineral, and Antioxidant Profiles of Wild and Cultivated Carob (*Ceratonia siliqua*) Pulp from MoroccoSoumaya Atouife¹ , Abdelghani Aboukhalaf^{1,2} , Kaoutar Naciri¹ , Jamila Elbiyad¹
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ABSTRACT

Background: The carob tree (*Ceratonia siliqua* L.) is an emblematic and ecologically significant woody species of the Mediterranean basin that has attracted increasing scientific and industrial interest, owing to the considerable nutritional value and versatile functional properties of its fruit.**Aims:** The present study aimed to evaluate and comparatively characterize the nutritional and functional attributes of wild and cultivated carob varieties sourced from Morocco.**Material and Methods:** Carob Pulp Samples were collected from two geographically distinct sites within the Guelmim-Oued Noun region of southern Morocco. The following analytical parameters were assessed: physicochemical properties (ash, moisture, total sugars, and crude protein content), mineral composition (macro- and microelements), bioactive compound content comprising total polyphenols, flavonoids, and condensed tannins, and antioxidant activity, evaluated employing three complementary assays—the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the ferric reducing antioxidant power (FRAP) assay, and the β -carotene antioxidant activity test (CAT assay).**Results:** Cultivated carob pulp exhibited significantly higher ($p < 0.05$) total sugar content (65.85 ± 0.07 vs. $58.40 \pm 0.26\%$), crude protein content (3.47 ± 0.22 vs. $2.35 \pm 0.29\%$), potassium concentration (9351.0 ± 4.4 vs. 6812.0 ± 3.8 mg/kg), and calcium concentration (2564.0 ± 1.2 vs. 1339.0 ± 2.0 mg/kg) relative to wild carob pulp. Conversely, wild carob pulp displayed significantly lower IC_{50} values ($p < 0.05$) across all three antioxidant assays—DPPH (127.09 ± 0.41 vs. 162.17 ± 0.06 $\mu\text{g/mL}$), FRAP (9.08 ± 0.18 vs. 12.61 ± 0.33 $\mu\text{g/mL}$), and CAT (62.19 ± 0.56 vs. 78.94 ± 0.80 $\mu\text{g/mL}$) assays, indicating superior radical scavenging and reducing capacity relative to the cultivated variety.**Conclusions:** The findings reveal complementary and distinct nutritional and functional characteristics between wild and cultivated Moroccan carob pulp, with both botanical forms representing valuable sources of nutrients and bioactive compounds for food and nutraceutical applications. The cultivated form demonstrated a superior macronutrient and mineral profile, whilst the wild form exhibited greater antioxidant potency, suggesting differential valorization potential according to intended application. Further systematic characterization of individual phytochemical constituents is recommended to deepen understanding of their functional properties and support their potential applications in functional food product development and sustainable agroforestry systems.**Keywords:** *Ceratonia siliqua* L.; Antioxidant Activity; Phenolic Compounds; Phytochemicals; Carob pulp; Morocco.

Article Information

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Tel. (+212) 523 34 2325**Received:** February 01, 2026**Revised:** August 03, 2026**Accepted:** August 03, 2026**Published:** August 15, 2026Cite this article as: Atouife, S., Aboukhalaf, A., Naciri, K., Elbiyad, J., El Habazi, A., & Belahsen, R. (2026). Comparative Study of The Physicochemical, Phenolic, Mineral, and Antioxidant Profiles of Wild and Cultivated Carob (*Ceratonia siliqua*) Pulp from Morocco. *The North African Journal of Food and Nutrition Research*, 10 (22): 64–75. <https://doi.org/10.51745/najfnr.10.22.64-75>

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1 INTRODUCTION

The carob tree (*Ceratonia siliqua* L.) is a thermophilic, predominantly dioecious leguminous tree belonging to the family Fabaceae, although it may occasionally exhibit monoecious or hermaphroditic reproductive strategies. The species thrives in mild, semi-arid environments on poor and shallow soils, demonstrates marked drought tolerance, and exhibits pronounced sensitivity to extreme cold. Although characterized by slow growth, the carob tree is distinguished by its exceptional longevity, which contributes to its ecological stability and agroforestry value (Batlle & Tous, 1997; Kassout *et al.*, 2024; Şahin & Taşlıgil, 2016).

The carob tree has been recognized since antiquity as a native species of the Mediterranean basin; however, its precise geographical origin remains a subject of scientific debate. Whilst some investigations have proposed southern Arabia or Indo-Malaysia as the center of origin, the prevailing scientific consensus points to the eastern Mediterranean region (Batlle & Tous, 1997; Dahmani *et al.*, 2023). Through domestication processes closely associated with the development and dissemination of grafting techniques, the carob tree has been successfully introduced to other regions characterized by a Mediterranean-type climates, including Argentina, California, and Mexico (Batlle & Tous, 1997; Dahmani *et al.*, 2023).

Among the distinct anatomical tissues constituting the carob fruit, the pulp (mesocarp) constitutes the largest fraction, accounting for approximately 90% of the fruit's total dry weight. The pulp is traditionally processed into flours, cocoa substitutes, and syrups and has attracted increasing scientific and industrial interest owing to its richness in bioactive compounds, including polyphenols, flavonoids, and condensed tannins, as well as its significant mineral content, and dietary fiber. These compositional characteristics confer upon carob pulp well-documented nutritional and functional properties, including antioxidant, hypoglycemic, and anti-inflammatory activities (Bissar & Özcan, 2022; Laaraj et al., 2024).

In Morocco, the carob tree holds considerable agroecological and socioeconomic significance, with a widespread natural distribution across the mountainous and coastal areas of the southern, center, and norther regions of the country. The species constitutes a rapidly developing local value chain, contributing to soil erosion control, climate change mitigation, biodiversity preservation, and the enhancement of agricultural system resilience (Kassout et al., 2024). Its importance has been documented in several ethnobotanical studies conducted across diverse Moroccan regions, highlighting its multiple applications — encompassing nutritional, medicinal, and agroforestry applications (Naciri et al., 2022).

Notwithstanding the established significance of this species in Morocco, in-depth investigations remain limited in certain regions of the country, such as Guelmim-Oued Noun, located in southern Morocco. Furthermore, direct comparative studies between cultivated and wild carob populations are scarce in the literature, and wild populations are considered relatively uncommon attributable to the extensive domestication and widespread cultivation of selected and improved genotypes (Batlle & Tous, 1997;

Dahmani et al., 2023). The present study therefore aimed to characterize the pulp of both cultivated and wild *C. siliqua* populations from this underexplored region, with the specific objectives of comparatively evaluating their physicochemical, mineral, and bioactive compound composition, and of assessing their antioxidant activity using three complementary and validated assays: DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and the catalase activity assay (CAT).

2 MATERIALS AND METHODS

2.1 Plant Materials

Samples of mature carob (*Ceratonia siliqua* L.) fruits were harvested manually during September 2024 from the Guelmim-Oued Noun region of southern Morocco, at two geographically and ecologically distinct collection sites (Figure 1). Fruit maturity was determined on the basis of morphological and visual criteria, including complete pod development, uniform dark brown coloration, firm texture, and the complete absence of residual green areas — all indicative of physiological maturity.

- **Laqsabi Tagoust** (29.001787° N, 10.222262° W; altitude: 300 m above sea level): collection site for the cultivated carob variety.
- **Timoulay** (29.142276° N, 9.559521° W; altitude: 792 m above sea level): collection site for the wild carob variety.

Following collection, the pods were carefully cleaned and rinsed with distilled water. The pulp was mechanically separated from the seeds and subsequently oven-dried at 50°C for 48 hours to ensure uniform and complete moisture removal while minimizing the thermal degradation of bioactive compounds. The dried pulp was reduced into a fine powder employing a laboratory electric grinder and stored in hermetically sealed opaque containers, protected from

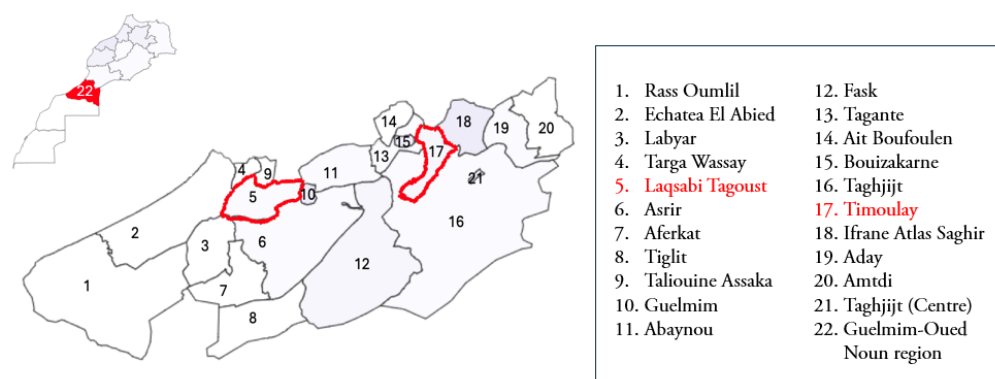


Figure 1. Localization of the Study Areas (Sites 5 and 17) in Guelmim Oued-Noun region

Note: The map shows the administrative division of Guelmim Province and indicates the two carob sampling sites. Zone 5 corresponds to the cultivated variety collected in Laqsabi Tagoust, while zone 17 corresponds to the wild variety collected in Timoulay. Source: RGP 2014.

humidity, light, and heat, until further analysis. A schematic flow diagram summarizing the overall experimental procedure is presented in Figure 2.

2.2 Physicochemical Characterizations

2.2.1 Moisture Content

Moisture content was determined gravimetrically by drying carob pulp samples in a thermostatically controlled oven at 105°C until constant mass was attained, in accordance with the standard method prescribed by the Association of Official Analytical Chemists (AOAC) (Horwitz, 2006).

2.2.2 Ash Content

Ash content was determined by incineration of dried samples in a muffle furnace at 600°C for 6 hours, until complete combustion of organic matter was achieved and only white ash residue remained, in accordance with the AOAC method (Horwitz, 2006).

2.2.3 Total Sugar Content

Total sugar content was quantified utilizing the phenol-sulphuric acid colorimetric method described by Dubois *et al.* (1956), based on the condensation reaction of carbohydrates with phenol in the presence of concentrated sulfuric acid,

producing a stable chromophore measurable by spectrophotometry.

2.2.4 Soluble Proteins Content

Soluble protein content was determined through the Bradford colorimetric method (Bradford, 1976), based on the binding of Coomassie Brilliant Blue G-250 dye to protein molecules, resulting in a measurable shift in the dye's absorbance maximum. All physicochemical analyses were performed in three independent replicates.

2.3 Mineral Composition Analysis

Carob pulp samples from both wild and cultivated varieties were dried, ground to a fine powder, and then incinerated at 550°C for 6 hours in a muffle furnace. The resulting ash residues were dissolved in concentrated hydrochloric acid under gentle heating, filtered, and quantitatively transferred to 50 mL volumetric flasks and diluted to volume with distilled water. The concentrations of potassium (K), calcium (Ca), iron (Fe), and magnesium (Mg) were determined by flame atomic absorption spectroscopy (AAS), whilst zinc (Zn) was analyzed by electrothermal atomization (graphite furnace), in accordance with AOAC recommendations (Horwitz, 2006). All mineral concentrations are expressed as milligrams per kilogram of dry weight (mg/kg DW).

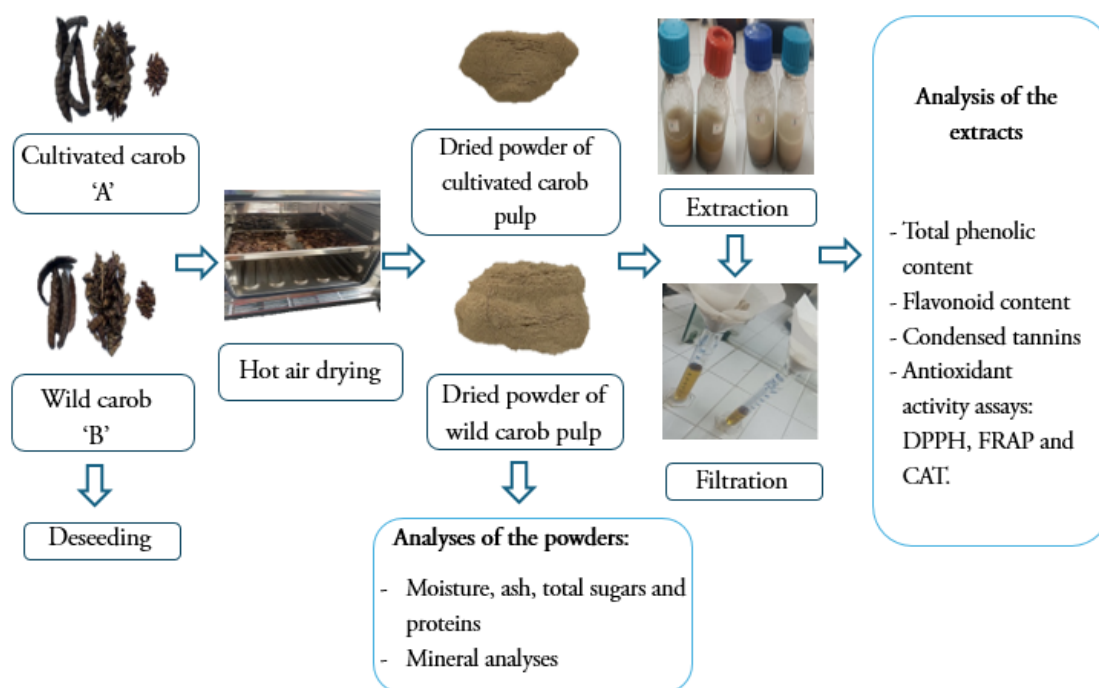


Figure 2. Schematic Flowline of the Study

Note: The figure summarizes the main steps of the study, including fruit collection (wild and cultivated carob), deseeding, drying, grinding, approximate and mineral analyses, extraction and filtration, and finally the evaluation of phenolic content and antioxidant activity (DPPH, FRAP, CAT)

2.4 Extraction of Bioactive Compounds

Bioactive compounds were extracted from both wild and cultivated carob pulp samples using two distinct and complementary extraction methods, enabling a comparative evaluation of polyphenolic content and antioxidant activity between the two carob varieties.

Aqueous extraction: Aqueous extraction was performed according to previously established protocols for carob research (Ayaz *et al.*, 2007; Yalimkaya & Özdemir, 2022). For each sample, 10 g of finely ground dried powder were combined with 500 mL of ultrapure water in amber glass vessels. The mixtures were placed on a magnetic stirrer at 40°C and 150 rpm for 24 hours, followed by continued stirring at ambient temperature for a further 12 hours. The resulting suspensions were filtered and centrifuged at 3,000 rpm for 15 minutes. The supernatants were collected, frozen at -24°C for 24 hours, freeze-dried, and stored in light-protected containers until analysis.

Methanolic Solvent Extraction: Methanolic extraction was performed in accordance with the procedure described by Caccagnese and Azzolina (1979). For each variety, 20 g of dried powder were combined in triplicate with 150 mL of methanol, and macerated at ambient temperature in the dark under continuous stirring for 48 hours. The resulting mixtures were filtered to separate the liquid phase from the solid residue, and the methanolic extracts were subsequently concentrated by vacuum evaporation in a rotary evaporator to yield crude extracts, which were stored at 4°C until further analysis.

2.5 Determination of Polyphenolic Compound Content

2.5.1 Total Phenolic Compounds (TPC)

Total phenolic content (TPC) was quantified using the Folin–Ciocalteu colorimetric method, as described by Tikent *et al.* (2024). An aliquot of 100 µL of extract was transferred to a hemolysis tube and combined with 1 mL of distilled water and 200 µL of 20% (w/v) aqueous sodium carbonate (Na_2CO_3) solution. After 3 minutes, 1 mL of Folin–Ciocalteu reagent was added, and the mixture was subsequently homogenized and incubated at 40°C for 30 minutes in the dark. Absorbance was measured spectrophotometrically at 725 nm against a reagent blank. TPC was calculated from a gallic acid standard calibration curve and results are expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

2.5.2 Total Flavonoids Content (TFC)

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method described by Dehpour *et al.* (2009). A volume of 500 µL volume of extract

from each sample was mixed with 1.5 mL of 95% methanol, 100 µL of 10% (w/v) aluminum chloride solution, 100 µL of 1 M sodium acetate, and 2.8 mL of distilled water. The mixture was thoroughly agitated in the dark at room temperature for 30 minutes. A blank was prepared by substituting the extract with an equal volume of 95% methanol. Absorbance was measured spectrophotometrically at 415 nm. TFC was calculated from a catechin standard calibration curve, and results are expressed as milligrams of catechin equivalent per gram of extract (mg CE/g).

2.5.3 Condensed Tannins Content (CTC)

Condensed tannin content (CTC) was determined employing the acid vanillin method described by Mohti *et al.* (2020). A volume of 200 µL of each extract was combined with 1,000 µL of vanillin reagent and thoroughly agitated. The mixture was incubated at 30°C for 20 minutes in the dark. Absorbance was measured spectrophotometrically at 500 nm against a blank prepared with equal volumes of 37% (v/v) methanol and 8% (v/v) hydrochloric acid, subjected to identical incubation conditions. CTC was calculated from a catechin standard calibration curve, and results are expressed as milligrams of catechin equivalent per gram of extract (mg CE/g).

2.6 Antioxidant Activity Assessment

The antioxidant potential of the wild and cultivated carob pulp extracts was evaluated through three complementary assays — DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and the catalase activity assay (CAT) — to provide a comprehensive and multi-mechanistic assessment of antioxidant capacity.

2.6.1 DPPH Radical Scavenging Assay

DPPH radical scavenging activity was assessed according to the method described by Brand-Williams *et al.* (1995). A freshly prepared DPPH solution (2.4 g/100 mL of methanol) was employed. A volume of 100 µL of extract at concentration ranging from 5 and 30 mg/mL was supplemented to 2 mL of the DPPH solution. Following incubation at ambient temperature in the dark for 30 minutes, absorbance was measured at 517 nm. Ascorbic acid served as the positive reference standard.

2.6.2 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed in accordance with the method described by Ksouri *et al.* (2009). A volume of 1 mL of extract at concentration ranging from 5 and 30 mg/mL was combined with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide solution. The mixture was incubated at 50°C for 30 minutes, followed by the addition of 2.5 mL of 1% (w/v) trichloroacetic acid.

Following centrifugation at 3,000 rpm for 10 minutes, 2 mL of the supernatant was collected and combined with 2 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride solution. Absorbance of the mixture was measured at 700 nm against an appropriate reagent blank.

2.6.3 Catalase Activity Assay (CAT)

The catalase activity assay was performed according to the method described by Boulaaba et al. (2019). A volume of 300 μ L of extract at concentration ranging from 5 to 30 mg/mL was combined with 3 mL of a reagent solution containing sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM) in hermetically sealed tubes, and incubated at 95°C for 90 minutes. Following return to ambient temperature, absorbance was measured at 695 nm. A blank was prepared by substituting the extract with 300 μ L of methanol and subjected to identical incubation conditions. Results for all three antioxidant assays are expressed as milligrams of ascorbic acid equivalent per gram of extract or dry weight (mg AAE/g).

2.7 Statistical Analysis

All results are expressed as mean values $\pm$ standard deviation (SD) of three independent replicates. Statistical differences between cultivated and wild carob samples were evaluated through the independent samples Student's t-test at a 95% confidence level ($\alpha = 0.05$). A p-value below 0.05 was considered indicative of statistical significance. All statistical analyses were performed employing SPSS version 25 (Inc., Chicago, IL, USA).

3 RESULTS & DISCUSSIONS

3.1 Physicochemical Characterization

The results of the physicochemical analyses of cultivated and wild carob pulp are presented in Table 1. The cultivated pulp revealed significantly higher moisture, ash, crude protein, and total sugar contents than the wild pulp ($p < 0.05$). The moisture contents measured in the present study are comparable to those reported for wild Moroccan carob pulp by Laaraj et al. (2024). Differences in the physicochemical composition of carob pulp among studies may be attributed to geographical origin and variability

among sampled populations (Erraleb et al., 2024; Fadel et al., 2020).

With respect to ash content — a widely accepted global indicator of the total mineral load of a food material — the values recorded in the present study are consistent with those reported by Oziyci et al. (2014), who obtained ash values ranging from 2.04% to 4.37% for cultivated carob pulp and from 1.38% to 5.21% for wild pulp. The ash content of carob pulp in general reported to range between 1% and 6% across the published literature (Avallone et al., 1997; Azab, 2022), and the findings of the present study fall within this established range. It should additionally be underlined that the ash content measured in the wild pulp in the present study falls within the range reported for Algerian carob pulp by Kocherane (2021) (2.15%–3.13%). The ash content of the cultivated pulp in the present study was slightly higher than the values reported for three Cypriot carob pulp cultivars by Papaefstathiou et al. (2018) (2.46%–2.63%).

Regarding crude protein content, carob pulp is characteristically recognized as a low-protein food material; however, it contains a proportion of nutritionally significant essential amino acids of interest for the development of functional food products and pharmaceutical applications (Arribas et al., 2019; Hussain et al., 2024; Simsek et al., 2017). It should be noted, however, neither carob pulp nor its processed derivatives can be legitimately designated as a "source of protein" within the regulation framework established by Regulation (EC) No. 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods (European Union [EU], 2006), which stipulates that such a designation requires that at least 12% of the energy value of a food be provided by protein. Furthermore, the nutritional significance of any protein source must be evaluated not solely on the basis of quantity but with due consideration of protein digestibility and amino acid bioavailability *in vivo* (Fatima et al., 2024).

It is well established in the literature that carob pulp is characterized by a high carbohydrate content, predominantly in the form of simple sugars — most notably sucrose, glucose, and fructose (Goulas et al., 2016). This compositional profile confers upon the pulp a naturally sweet organoleptic character and renders it highly suitable for use in cocoa substitute

Table 1. Chemical Composition of Cultivated & Wild Carob Pulp Powder

	Cultivated pulp	Wild pulp	t-value	p-value
Moisture (%)	10.22 $\pm$ 0.35	9.32 $\pm$ 0.19	3.98	0.016
Ash (%)	3.20 $\pm$ 0.01	2.80 $\pm$ 0.02	31.0	< 0.001
Protein (%)	3.47 $\pm$ 0.22	2.35 $\pm$ 0.29	5.38	0.006
Total sugar (%)	65.85 $\pm$ 0.07	58.40 $\pm$ 0.26	47.7	< 0.001

Note: Moisture content is expressed on a fresh weight basis. Ash, protein, and total sugar contents are expressed on a dry weight basis. Data are presented as mean $\pm$ standard deviation (SD) for three independent replicates (n = 3). Statistical differences between cultivated and wild carob pulps were assessed using the independent Student's t-test, and the corresponding t-values and p-values are reported.

formulations and as a substrate for lactic acid fermentation (Loullis & Pinakoulaki, 2018). In the present study, both carob varieties exhibited elevated total sugar concentrations, with the cultivated variety demonstrating a notably higher content than the wild variety. These observations are broadly consistent with the published literature: Biner *et al.* (2007) similarly reported higher total sugar content in cultivated pulp (53.1%) relative to wild pulp (43.7%), whilst Baston (2016) documented a value of 58% for wild pulp. In contrast, certain studies have revealed opposing trends: Simsek *et al.* (2017) recorded a total sugar content of 88.53% in wild carob samples, substantially exceeding the 46.84% recorded for cultivated samples in the same investigation — a finding that directly contradicts the pattern observed in the present study.

These considerable variability in total sugar content documented across studies can be attributed to a range of well-recognized determinants, including prevailing climatic conditions, agricultural management practices, the physiological maturity stage of the fruit at harvest, and the extent of inter-individual genetic variation among trees. These factors are well documented as exerting a significant influence on the biochemical composition of carob fruits, particularly with respect to the accumulation of sugars and nitrogenous compounds.

3.2 Mineral Composition of Carob Pulp

Mineral composition analysis carried of wild and cultivated carob pulp powders confirmed the presence of a range of nutritionally essential mineral elements, specifically potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), and zinc (Zn). The quantitative results for each mineral are presented in Table 2.

Statistically significant differences ($p < 0.05$) were observed between cultivated and wild carob pulps for all mineral elements analyzed. Potassium and calcium exhibited the most pronounced inter-varietal differences, with markedly higher concentrations recorded in the cultivated pulp. Magnesium and phosphorus were also significantly more concentrated in the cultivated samples, although the magnitude of these differences was comparatively less pronounced. With respect to microelements, the cultivated pulp exhibited higher concentrations of both iron and zinc relative to the wild variety: iron concentration was

approximately 2.6-fold higher in the cultivated pulp, whilst zinc concentration was about 1.3-fold higher.

Among all the macronutrients analyzed, potassium was confirmed as the predominant mineral constituent in carob pulp — a finding consistent with the established literature on carob mineral composition (El Bouzdoudi *et al.*, 2017; Hussain *et al.*, 2024). The potassium concentrations recorded in the present study are within the ranges previously documented, with values spanning 6,400 to 27,200 mg/kg DW reported across studies. Similarly, published ranges for calcium (2,070–9,600 mg/kg DW), magnesium (4,100–6,900 mg/kg DW), and iron (4.7–1,390.82 mg/kg DW) are consistent with the values obtained in the present investigation (El Bouzdoudi *et al.*, 2017). The higher levels of K, Ca, Mg, and Zn in cultivated relative to wild carob pulp observed in the present study are corroborated by the findings of Oziyici *et al.* (2014).

However, the mineral profile observed in the present study differs from that reported by Oziyici *et al.* (2014) for wild and grafted carob fruits from Turkey. Although potassium was the most abundant macromineral in their samples, fruit type was not a significant factor affecting mineral concentrations. Differences between studies may be related to geographical origin and the characteristics of the sampled populations, as mineral composition has also been reported to vary among carob provenances in Morocco (Ben Amar *et al.*, 2025; Ettaleb *et al.*, 2024).

The inter-study variability in mineral composition may be attributed to a complex interplay of factors, encompassing cultivar and variety differences, the analytical methods employed, or post-harvest drying conditions (Hussain *et al.*, 2024), the physiological maturity stage of the fruit at the time of sampling (Nechchadi *et al.*, 2023), and environmental determinants including irrigation regime, drought stress, soil type, and fertilization practices — all of which are well documented as exerting a significant influence on the chemical and mineral composition of carob fruit (Ayaz *et al.*, 2007; Oziyici *et al.*, 2014).

3.3 TPC, TFC, and CTC

Carob pulp, the sweet and fibrous mesocarp of the fruit of *Ceratonia siliqua* L., is widely recognized as a rich source of polyphenolic compounds, particularly gallic acid, catechins, and hydroxybenzoic acids. These phytochemical constituents

Table 2. Mineral Composition (mg/kg DW) of Cultivated & Wild Carob Pulp.

	K	Ca	Mg	P	Fe	Zn
Cultivated carob pulp	9351.0±4.4	2564.0±1.2	1286.7±1.8	1190.0±2.0	25.2±0.5	12.4±0.4
Wild carob pulp	6812.0±3.8	1339.0±2.0	1013.3±2.5	790.0±1.8	9.8±0.6	9.7±0.1
p-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.005

Note: Mineral contents of cultivated and wild carob pulp (*Ceratonia siliqua* L.) pulp expressed on a dry weight basis (mg/kg DW). Values are expressed as mean ± standard deviation (SD) (n = 3). Statistical differences between cultivated and wild carob pulps were evaluated using the independent Student's *t*-test, and the corresponding *p*-values are presented in the table.

have been extensively associated with a range of beneficial health effects, including antidiabetic, hypolipidemic, anticancer, antibacterial, and antifungal activities. Therefore, carob pulp is considered a highly promising functional food ingredient with substantial potential for application in the prevention and dietary management of various chronic non-communicable diseases (Dahmani *et al.*, 2023).

Total Phenolic Content (TPC)

Statistically significant differences in TPC were observed between the two varieties ($p < 0.05$) as presented in Table 3. Cultivated carob pulp exhibited a higher TPC value of 23.69 ± 0.48 mg GAE/g of extract, compared with 20.75 ± 0.36 mg GAE/g of extract recorded for the wild variety. This inter-varietal difference may be attributable to factors including domestication-associated selection pressures, differences in prevailing climatic conditions between the two collection sites, and the metabolic specificities of each botanical variety.

Contextualization within the Moroccan literature reveals a degree of variability in TPC values across published studies. For instance, Fadel *et al.* (2020) reported concentrations of 7.35 to 9.41 mg/g. Furthermore, Hussain *et al.* (2024), employing three distinct drying techniques, reported a markedly higher TPC of 52.34 ± 0.20 mg GAE/g DM, substantially exceeding the values obtained in the present study.

At the broader Mediterranean scale, the TPC values recorded in the present study are broadly consistent with, or in certain instances exceed, published reference values. Avallone *et al.* (1997) reported a mean TPC of 19 ± 3 mg GAE/g for carob pulp from Sicily (Italy); Ayaz *et al.* (2007) obtained 13.5 mg/g for Turkish carob; El-Haddad *et al.* (2022) recorded 21.3 mg/g for Egyptian carob; and Djebbari *et al.* (2024) reported 13.8 mg/g for Algerian carob pulp. The considerable variability across these studies reflects the well-documented influence of fruit maturity stage, geographical origin, botanical variety, and experimental parameters, including drying method, extraction solvent, and analytical protocols (Hussain *et al.*, 2024).

Total Flavonoid Content (TFC)

Flavonoids constitute a major and structurally diverse subclass of polyphenolic compounds that play a central role in plant defense mechanisms and are widely recognized for their antioxidant, anti-inflammatory, and cardioprotective properties, as well as their potential protective effects against metabolic diseases (López-fernández *et al.*, 2020). TFC was accordingly evaluated in methanolic preparations extracts of both cultivated and wild carob pulp, with results presented in Table 3.

A statistically significant difference ($p < 0.05$) in TFC was observed between the two varieties. The cultivated variety exhibited a marginally higher TFC (7.18 ± 0.82 mg CE/g extract) compared to the wild variety (6.58 ± 0.33 mg CE/g extract). Whilst the magnitude of this difference was relatively modest, it may reflect differences in flavonoid biosynthetic pathway activity, attributable to genotypic differences or differential responses to contrasting environmental conditions at the two collection sites.

Compared with the available literature, the TFC obtained in the present study fall within the range previously reported for carob pulp, although certain investigations have documented higher values. El-Haddad *et al.* (2022) reported a TFC of 12.51 ± 0.01 mg CE/g for cultivated carob pulp, exceeding the values obtained in the present study (7.18 ± 0.82 mg CE/g for cultivated pulp and 6.58 ± 0.33 mg CE/g for wild pulp). The observed discrepancies may be attributed to differences in genotype, fruit maturity stage, geographical origin, climatic conditions, soil characteristics, and extraction procedures, all of which are established determinants of flavonoid accumulation in carob fruits.

Condensed Tannin Content (CTC)

A statistically significant difference ($p < 0.05$) in CTC was observed between cultivated and wild carob pulps (Table 3). The cultivated variety displayed a higher CTC value (4.63 ± 0.97 mg CE/g extract) compared with the wild variety (3.81 ± 0.62 mg CE/g extract), consistent with the pattern observed for TPC and TFC.

Table 3. Total Phenolic Contents (TPC), Total Flavonoids Contents (TFC), Condensed Tannins Contents (CTC) of Cultivated and Wild Carob Pulp

	TPC (mg GAE/g of extract)	TFC (mg CE/g of extract)	CTC (mg CE/g of extract)
Cultivated pulp	23.69 ± 0.48	7.18 ± 0.82	4.63 ± 0.97
Wild pulp	20.75 ± 0.36	6.58 ± 0.33	3.81 ± 0.62
<i>p</i> -value	<0.001	0.031	0.042

Note: Data are expressed as mean $\pm$ standard deviation (SD) (n=3). TPC is expressed in mg gallic acid equivalents per g of extract (mg GAE/g), TFC and CTC in mg catechin equivalents per g of extract (mg CE/g).

The condensed tannin contents obtained in the present study were broadly consistent with previously published values for carob pulp and comparable inter-varietal trends have been described by El Bouzdoudi *et al.* (2016), Ettaleb *et al.* (2024), Mekhoukhe *et al.* (2018), and Djebbari *et al.* (2024). Direct quantitative comparisons across studies should, however, be interpreted with appropriate caution, as differences in analytical procedures, extraction conditions, and reference standard materials are recognized to influence reported tannins values. The overall variability in CTC documented across the published literature underscores the

significant and well-established influence of genotype, geographical origin, fruit maturity stage, and post-harvest processing conditions on condensed tannin accumulation in carob pulp.

3.4 Antioxidant Activities

The antioxidant potential of carob pulp was analyzed through three complementary tests: DPPH, FRAP, and CAT. Results were expressed as the concentration required to inhibit 50% of oxidative activity (IC_{50} , $\mu\text{g/mL}$) with lower IC_{50} values indicating higher antioxidant activity.

The data presented in Table 4 reveals that the two varieties differed significantly ($p < 0.05$). Wild carob pulp exhibited a slightly higher antioxidant capacity than cultivated carob in all three tests. In the DPPH assay, the IC_{50} was $127.09 \pm 0.41 \mu\text{g/mL}$ for the wild variety, compared with $162.17 \pm 0.06 \mu\text{g/mL}$ for the cultivated variety. Similarly, in the FRAP assay, the wild variety yielded a lower IC_{50} ($9.08 \pm 0.18 \mu\text{g/mL}$) than the cultivated variety ($12.61 \pm 0.33 \mu\text{g/mL}$). The CAT assay likewise revealed a lower IC_{50} for the wild variety ($62.19 \pm 0.56 \mu\text{g/mL}$) than for the cultivated one ($78.94 \pm 0.80 \mu\text{g/mL}$). These results indicate that the wild variety possesses a greater free radical-scavenging and reducing capacity, which may be attributed to a greater diversity or specificity in antioxidant compounds.

The heterogeneity of the units utilized in the literature to

Table 4. Antioxidant Activity of Cultivated and Wild Carob Pulp

	DPPH ($IC_{50} \mu\text{g/mL}$)	FRAP ($IC_{50} \mu\text{g/mL}$)	CAT ($IC_{50} \mu\text{g/mL}$)
Cultivated pulp	162.17 ± 0.06	12.61 ± 0.33	78.94 ± 0.80
Wild pulp	127.09 ± 0.41	9.08 ± 0.18	62.19 ± 0.56
p-value	< 0.001	0.002	< 0.001

Note: The antioxidant activity was evaluated using DPPH radical scavenging, ferric reducing antioxidant power (FRAP), and catalase (CAT) activity assays. Data are expressed as mean $\pm$ standard deviation (SD) for three replicates ($n = 3$). Lower IC_{50} values indicate higher antioxidant activity.

express antioxidant activity complicates the comparison between the present findings and those reported elsewhere. DPPH results may be expressed as percentage inhibition, ascorbic acid equivalents (AAE) or Trolox equivalents (TE), whereas FRAP values are frequently reported as mmol $\text{FeSO}_4/100 \text{ g}$ of extract. Consequently, quantitative comparisons are difficult in the absence of standardized conversion (Dammak et al., 2024; Hussain et al., 2024).

By way of illustration, Dammak et al. (2024) reported a DPPH IC_{50} of $22.24 \pm 0.31 \text{ mg/mL}$, indicating markedly lower activity than that of the wild-type extract in the present study (IC_{50} of $127.09 \mu\text{g/mL}$, i.e., 0.027 mg/mL). However, their FRAP value ($0.39 \pm 0.04 \text{ mmol Eq. FeSO}_4/100 \text{ g}$) cannot be directly compared with the IC_{50} values ($\mu\text{g/mL}$) reported here. Likewise, Hussain et al. (2024) reported a

DPPH value of $41.6 \pm 0.2 \text{ mg Trolox/g}$, which cannot be compared directly with the results owing to the difference in units.

Nevertheless, despite these differences in units, the present results indicate that wild carob pulp exhibits a more effective antioxidant profile than cultivated carob, which is consistent with the observed richness of the wild pulp in certain secondary metabolites, notably tannins and polyphenols.

3.5 Relationships among Physicochemical Characteristics, Bioactive Compounds and Antioxidant Activity

The results obtained for both carob pulp varieties (wild and cultivated) highlight several relationships among physicochemical properties, bioactive compound profiles, mineral content, and antioxidant capacity. The differences in antioxidant capacity between the two varieties suggest a possible relationship with differences in flavonoid composition. Although the wild variety contained a slightly lower concentration of total polyphenols, it exhibited a more pronounced antioxidant capacity. This is generally attributed to the structural quality of secondary metabolites, which may reduce the synergistic effects (Dammak et al., 2024; Medda et al., 2021).

Moreover, higher contents of condensed tannins, proteins, and polyphenols were observed concurrently, suggesting a possible association among these parameters. This observation agrees with the findings of Mutabaruka et al. (2007) and Campbell et al. (2022), who reported that an increase in organic matter leads to a concomitant enrichment in proteins and phenolic compounds. Furthermore, condensed tannins are recognized to interact directly with proteins, reinforcing this association (Ma et al., 2016).

In the present study, the higher concentrations of macroelements (K, Ca, Mg) and microelements (Fe, Zn) in the cultivated carob pulp were not accompanied by higher antioxidant activity. Several studies suggest that several minerals, including zinc and iron, may participate indirectly in polyphenol biosynthesis by acting as enzymatic cofactors (Hänsch & Mendel, 2009); however, this relationship is complex and depends upon the physiological context of the plant. Similarly, total sugar content did not appear to be associated with higher antioxidant capacity, as evidenced by the cultivated variety, which contained a considerable quantity of sugar. Indeed, several studies have demonstrated that sugar content is influenced by irrigation and fruit maturity than by the biosynthesis of secondary metabolites (Rahmati et al., 2014).

These findings highlight the complex interrelationships among mineral nutrition, secondary metabolism, and

functional properties, that are shaped by both genetic factors (cultivated vs. wild varieties) and environmental conditions (soil, climate, and cultivation practices). The adoption of interdisciplinary approaches would contribute to a better understanding of the bioactive potential of carob fruits and guide their agri-food development and valorization in line with nutritional and functional objectives.

4 CONCLUSIONS

The comparative study of cultivated and wild carob pulps revealed differences in their physicochemical properties, mineral profiles, bioactive composition, and antioxidant activity. The cultivated variety exhibited higher levels of macroelements (particularly potassium, calcium, and magnesium), proteins, and sugars, whereas the wild variety displayed slightly stronger antioxidant activity despite a marginally lower total phenolic content. This difference may be attributable to variations in the qualitative composition of bioactive compounds and to possible synergistic interactions among them.

These findings emphasize the potential of both cultivated and wild carob pulps as valuable sources of nutrients and phytochemicals. Further investigations, particularly those involving the characterization of individual bioactive compounds, are required to better elucidate their functional properties and potential applications in food and sustainable agricultural systems.

These results additionally provide a basis for future research on the influence of cultivation practices and environmental conditions on the nutritional and antioxidant characteristics of carob fruits.

Acknowledgment: There are no acknowledgments.

Source of funding: There was no support or funding for this study.

Previous submissions: Not applicable.

Authors' Contribution: The authors contributed collectively to the conception, development, and completion of this manuscript. **Atouife S.:** Conceptualization, Resources, Writing - Original Draft & Manuscript approval. **Aboukalaf A.:** Design of the study, Methodology & Manuscript approval. **Naciri K.:** Methodology and Manuscript approval. **Elbiyad J.:** Methodology & Manuscript approval. **El Habazi A.:** Discussion of the result, Validation & Manuscript approval. **Belahsen R.:** Supervision, Result discussion, Validation, Writing - Review & Editing and Manuscript approval.

Artificial Intelligence (AI) Use Statement: The authors declare that no artificial intelligence (AI)-assisted tools, large language models (LLMs), or AI-generated content were employed in any stage of the preparation of this manuscript, including the conception and design of the study, data collection and analysis, interpretation of results, or the writing, editing, and revision of the manuscript text. All intellectual content presented in this article is the sole and original work of the listed authors,

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Conflicts of Interest: The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Preprint deposit: None.

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