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Nutritional composition and antioxidant capacity study of moroccan carob pulp

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ABSTRACT

In this study, we aimed to determine whether geographical origin has a significant influence on population-level variation in the nutritional value and antioxidant potential of Moroccan carob pulp. Pulp samples were collected from twelve natural *Ceratonia siliqua* populations distributed across distinct biogeographical and bioclimatic regions of Morocco. Phenolic and flavonoid contents were extracted using Ultra-Turrax-assisted homogenization and quantified by spectrophotometric methods. Antioxidant capacity was evaluated using the DPPH and FRAP assays, while nutritional quality was assessed by determining total sugars, dietary fiber, moisture, ash, and mineral composition. Results showed significant inter-population variability for all measured parameters ($p < 0.005$). Total phenolic contents ranged from 0. 77 to 24.27 mg GAE g^{-1} DW, while flavonoid contents varied between 7.45 and 12.42 mg RE g^{-1} DW. Antioxidant activity varied significantly among populations, with DPPH IC_{50} values ranging from 0. 02 to 0. 60 mg mL^{-1} and FRAP EC_{50} values from 0. 33 to 3. 15 mg mL^{-1}. Populations from semi- arid and high- altitude regions exhibited higher phenolic levels and stronger antioxidant activity than those from more humid environments, and nutritional traits also showed clear geographical differentiation. The obtained results provide a scientific basis for the targeted selection of Moroccan carob populations with superior nutritional and antioxidant qualities for functional food and nutraceutical applications. Nevertheless, the study focused on a single harvest season, and seasonal or interannual phytochemical variability should be evaluated. This work provides one of the most comprehensive evaluations of geographical variability in Moroccan carob pulp, highlighting its biochemical compositional diversity and reinforcing its potential role in a sustainable plant-based food system.

Keywords: *Ceratonia siliqua L*, geographical origin, biochemical composition, antioxidant activity, Ultraturrax extraction

INTRODUCTION

Ceratonia siliqua L., commonly known as the carob tree, is a perennial evergreen species native to the Mediterranean basin (Kocherane et al. 2019; Naghmouchi et al. 2009). The species exhibits remarkable ecological plasticity, enabling it to thrive under a wide range of pedoclimatic conditions, from arid and semi-arid inland areas to humid coastal environments (Baumel et al., 2020). In Morocco, carob populations are distributed across a broad ecological gradient, extending from the Rif Mountains in the north to the Anti-Atlas range in the south (Sbay 2008). This adaptability, combined with a high tolerance to water scarcity, marginal soils, and temperature fluctuations, makes carob an important plant resource in the context of climate change and sustainable food systems (Saïdi et al., 2016). Beyond its ecological importance, carob is widely recognized for its nutritional and food-related value. Carob pods consist of approximately 90% pulp and 10% seeds, with the pulp being traditionally consumed and increasingly exploited as a plant-based food ingredient (Jdaidi and Hasnaoui 2016; Skalli et al., 2019). Carob pulp is characterized by high levels of natural sugars, dietary fiber, essential minerals, and phenolic compounds, which contribute to its antioxidant capacity and nutritional quality. In contrast, the seeds are primarily exploited for the extraction of locust bean gum, a natural hydrocolloid extensively used as a thickening and stabilizing agent in the food industry (Richane et al., 2022).

Previous studies have highlighted the presence of phenolic compounds in carob pulp and their contribution to antioxidant-related properties, which are relevant for food quality and stability (Nasar-Abbas et al. 2016). However, despite growing interest in carob as a functional plant food, limited information is available on how geographical origin and environmental variability influence the nutritional and biochemical composition of carob pulp in Morocco. Most existing studies have focused on morphological traits, seed-derived products, or biochemical characterization of single or a few populations (El Batal et al., 2016; Elfazazi 2017; Elbouzidi et al., 2023; Laaraj et al., 2023).

Understanding the extent of intra-specific variability in pulp composition is essential for the selection of high-quality plant material, the valorization of local populations, and the development of sustainable food applications. Therefore, the present study aims to investigate twelve Moroccan populations of *Ceratonia siliqua* L., sampled across contrasting ecological zones. The specific objectives were to: (i) quantify total phenolic and flavonoid contents in carob pulp; (ii) evaluate antioxidant capacity using DPPH and FRAP assays; (iii) assess nutritional and mineral composition; and (iv) explore relationships between biochemical traits and environmental factors using multivariate statistical analyses. This integrated approach provides new insights into the nutritional relevance and compositional diversity of Moroccan carob pulp and identifies populations with potential interest for food and nutrition-oriented valorization.

MATERIAL AND METHODS

Plant material

Fresh pods from twelve naturally occurring populations of *Ceratonia siliqua* L. were harvested during the summer of 2022. Sampling sites were selected to represent Morocco's main phytoecological zones, including humid, sub-humid, semi-arid, and arid regions (Fig. 1, Table 1). Pods were cleaned and air-dried (Fig. 2a), then manually separated into pulp and seed fractions (Fig. 2b). Only pulp samples were retained for analysis; they were further dried, ground into powder (Fig. 2c), and stored at 4 °C until use

Preparation of the extracts

For the determination of phytochemical content and antioxidant activity, ethanolic extracts were prepared according to the protocol of El Chami et al. (2025), with minor modifications. Specifically, 1 g (Fig. 3a) of dried carob pulp powder was dispersed in 20 mL of ethanol/water (80:20, v/v) and then homogenized using an Ultra-Turrax (Ultra-Turrax T18 digital, IKA) for 5 minutes (Fig. 3b). The mixture was centrifuged (6000 rpm, 20 min) (Fig. 3c), and the supernatant was collected. The extract was filtered through Whatman paper (Fig. 3d), and the solvent was evaporated at 40 °C under reduced pressure using a Rotary Evaporator R-205 (Fig. 3e). The concentrated extract was dried in a ventilated oven at 35 °C for 3 hours, as outlined by Bihaoui et al. (2024). Subsequently, all extracts were stored at 4 °C until further analysis.

Biochemical assessment

Total phenolic content (TPC)

The measurement of total phenolic content (TPC) was conducted using the Folin-Ciocalteu assay, as described by Amrani et al. (2022). An aliquot of 100 µL from each sample extract was mixed with 0.5 mL of Folin-Ciocalteu reagent (1:9 v/v) and 2 mL of 7.5% (w/v) sodium carbonate. The mixture was incubated in the dark at room temperature for 20 minutes, and then the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. All assays were performed in triplicate, and the results of the total phenolic content were reported in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Total of flavonoids (TFC)

The flavonoid content in the pulp extract of *Ceratonia siliqua* L. was quantified using the aluminum trichloride method as described by Amrani et al. (2022). A volume of 1 mL of the extract

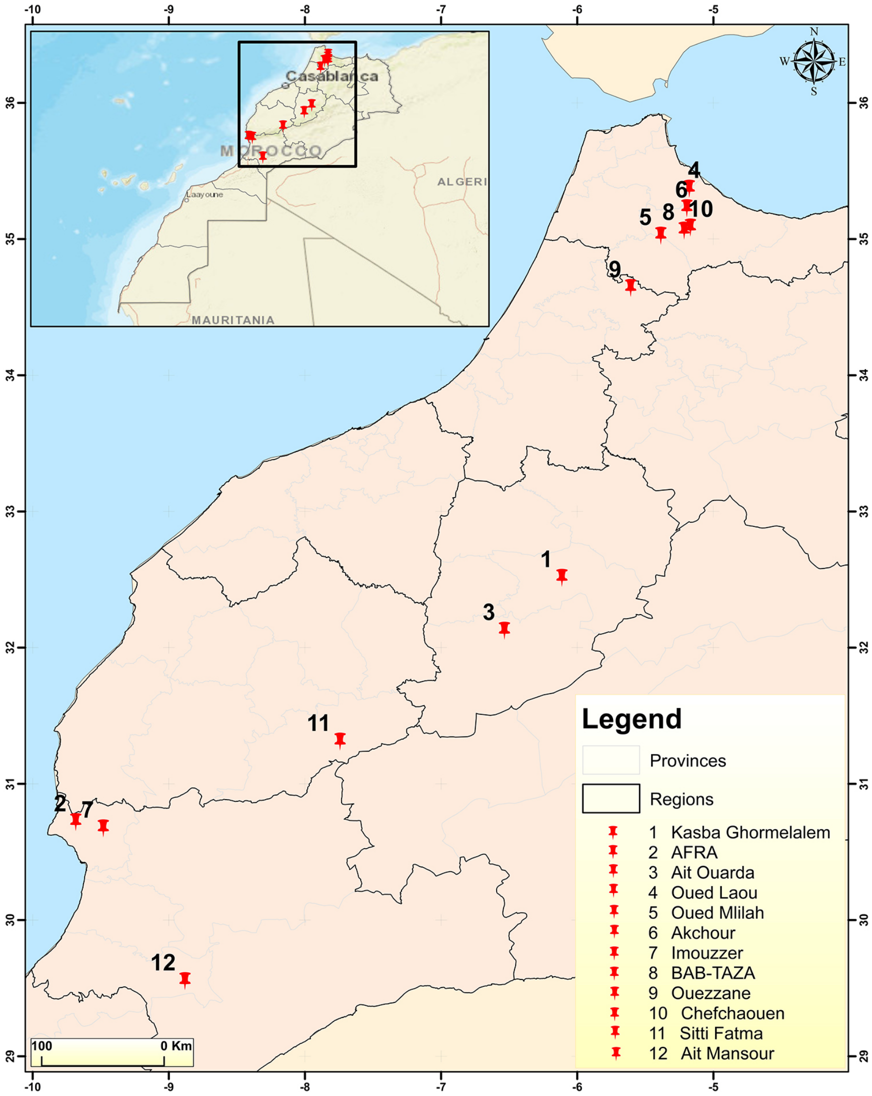
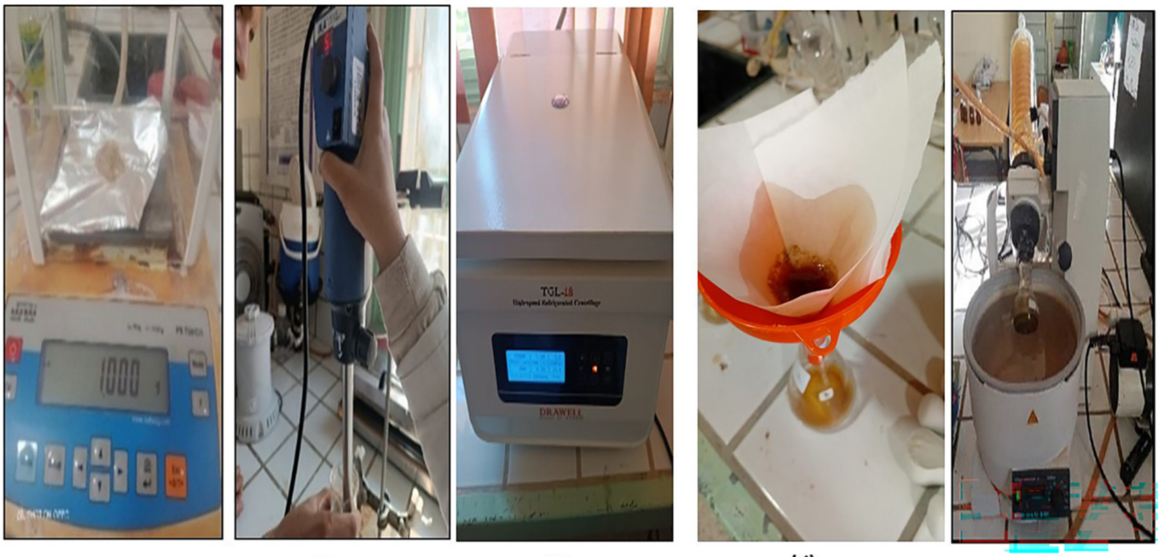


Figure. 1. Map of Morocco showing the locations of the 12 *Ceratonia siliqua* L. populations collected from different regions

Figure. 2. Preparation of carob pulp samples for analysis: (a) fresh pods, (b) dried pulp fragments, (c) powdered pulp



(a) (b) (c) (d) (e)

Figure. 3. Sample preparation and extraction steps of carob pulp: (a) weighing, (b) Ultra-Turrax homogenization, (c) centrifugation, (d) filtration, and (e) rotary evaporation

was combined with 1 mL of a 2% aluminum trichloride solution. Following a 20-minute incubation period, the absorbance was measured at 430 nm using a UV-Vis spectrophotometer. Total flavonoid content was expressed as milligrams of rutin equivalents per gram of dry weight (mg RE/g DW).

In vitro antioxidant activity

DPPH radical scavenging assay

Antioxidant activity was evaluated using the DPPH assay as described by Minarti et al. (2024). Briefly, 150 µL of 0.004% methanolic DPPH solution was mixed with 50 µL of each sample at various concentrations (500 to 3.9 µg/mL) in a 96-well microplate. The plate was incubated in the dark for 30 minutes, and absorbance was subsequently measured at 517 nm. The percentage of radical scavenging activity was calculated using the following formula:

$$\textit{Activity of scavenging}\ (\%) = $$
$$= [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

where: A' – indicates the sample's absorbance values and A_{DPPH} reflects the absorbance of the DPPH solution.

Table 1. Geographical and ecological information of the carob populations analyzed in this study

Population	Bioclimate	Mountains range	Altitude (m)	Longitude W/°	Latitude N/°	Rainfall average (mm)	Temperature average (°C)
Kasba Ghormelalem	Semi-arid	Middle Atlas	885	6°6	32◦30	476	18
AFRA	Semi-arid	Middle Atlas	883	5°48	32◦42	104	20.8
Ait Ouarda	Semi-arid	High Atlas	1226	6°31	32°8	406	14
Oued Laou	Humid	Western Rif	24	5°10	35°22	634	18.3
Oued Mlilah	Humid	Western Rif	171	5◦23	35°1	978	15.3
Akchour	Humid	Western Rif	685	5°18	35° 23	627	16.8
Imouzzer	Semi-arid	High Atlas	1227	9°28	30°40	335	18.2
BAB-TAZA	Sub-humid	Western Rif	825	5°12	35°3	864	14.9
Ouezzane	Sub-humid	Pre-Rif	318	5°36	34°38	751	17.5
Chefchaouen	Sub-humid	Western Rif	573	5°15	35 °10	878	15.3
Sitti Fatma	Semi-arid	High Atlas	1074	7°44	31°18	288	16.17
Ait Mansour	Arid	Anti-Atlas	1290	8°52	29°33	109	19.49

The antiradical activity was expressed as IC_{50}, which represents the extract concentration required to reduce 50% of the initial DPPH radical content. This value was determined graphically by the inhibition percentage (IP%) versus the extract concentration curve. Ascorbic acid was used as the reference antioxidant.

Reducing power assay

The reducing power (FRAP) was measured according to the method described by Kil et al. (2009). Briefly, 200 µL of each extract was mixed with 500 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 500 µL of 1% (w/v) potassium ferricyanide, then incubated at 50 °C for 20 minutes. The reaction was stopped by adding 500 µL of 10% (w/v) trichloroacetic acid. After centrifugation (650 rpm, 10 minutes), 500 µL of the supernatant was mixed with 500 µL of distilled water and 100 µL of 0.1% (w/v) ferric chloride. The absorbance was then measured at 700 nm. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation. The extract concentration inducing an absorbance of 0.5 (EC_{50}) was determined from the absorbance versus concentration curve. Ascorbic acid was used as the reference standard.

Total sugars extraction

Extraction procedure for total soluble sugars

Soluble sugars were extracted following the protocol described by Babu et al. (2002). Briefly, 0.5 g of the sample was homogenized with 10 mL of 80% ethanol (v/v), and the mixture was transferred into centrifuge tubes. Centrifugation was performed at 2000 rpm for 40 minutes. The resulting supernatant was collected, transferred into Eppendorf tubes, and stored at 4°C until further analysis.

Dosage of total soluble sugars

Total soluble sugars were quantified according to the method described by Dubois et al. (1956). A volume of 50 µL of the extract was combined with 0.5 mL of 5% phenol solution, then 1.5 mL of concentrated sulfuric acid (H_2SO_4) was added. The mixture was incubated in a water bath at 100 °C for 5 minutes, subsequently rapidly cooled using an ice bath. The absorbance was measured at 485 nm. A standard curve was prepared using glucose solutions derived from a 1 mg/mL stock solution.

Determination of moisture content

The moisture content of the samples was determined on a fresh-weight basis following the loss-on-drying method outlined by AOAC (2002). Approximately 2 g of each sample was placed in a hot-air oven at 105°C (Fig. 4a) and dried until a constant weight was achieved. The samples were then cooled in a desiccator (Fig. 4b) and weighed using an analytical balance. The moisture content was calculated using the formula:

$$Moisture\ content = \frac{We}{Ws} \times 100$$

where: W_e – the weight loss during drying,
W_s – the initial weight of the sample.

(a) (b)

Figure. 4. Moisture content determination: (a) oven drying at 105 °C, (b) cooling in a desiccator

Determination of crude fiber content

The crude fiber content was determined on a fresh weight basis using the acid-base digestion method. Five grams of homogenized sample powder (Fig. 5a) were boiled in 100 mL of 1.25% H_2SO_4 for 30 min using a reflux condenser (Fig. 5b). After this first extraction, the mixture was filtered to retain the carob particles (Fig. 4c). The resulting residue was washed with hot distilled water and then subjected to a second digestion using a 1.25% sodium hydroxide (NaOH) solution for 45 minutes at boiling temperature. After cooling, the mixture was vacuum-filtered through a crucible. The residue was further rinsed with acetone, oven-dried at 105°C to a constant weight (Fig. 5a), and weighed. Subsequently, it was ashed at 550 °C for 8 h in a muffle furnace (Fig. 5d). The crude fiber content was estimated based on the weight loss after incineration (Lesten and Kingsley 2020).

$$Crude\ fiber\ (\%) = (M1 - M2) / M \times 100$$

where: M1 represents the mass of the residue after drying at 105 °C, M2 is the mass of the residue after incineration at 550 °C, and M is the mass of the sample in grams.

The minerals composition

The mineral composition of carob pulp was determined following the method described by Kabiri et al. 2019 with minor modifications. A small amount (0.5 g) of dried pulp was digested with 2 mL of sulfuric acid (H_2SO_4), 6 mL of nitric acid (HNO_3), and 6 mL of hydrogen peroxide (H_2O_2). The mixture was heated for 30 minutes, and the resulting mineral residue was cooled and filtered using ash-free Whatman filter paper. The filtrate was then brought to a final volume of 25 mL with 0.1 M nitric acid. The prepared mineral solution was analyzed to quantify the concentrations of magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), aluminum (Al), arsenic (As), phosphorus (P), lead (Pb), silicon (Si), and sodium (Na) using an inductively coupled plasma–mass spectrometer (ICP-MS, iCAP Q, Thermo Fisher Scientific, Germany).

STATISTICAL ANALYSIS

All analyses were performed in triplicate, and the obtained data were subjected to comprehensive statistical analyses to assess the variability in the phytochemical and nutritional composition of carob pulp fruits collected from natural populations in Morocco. Results were expressed as mean ± standard deviation (1) of three independent replicates. Prior to analysis, data normality was evaluated using the Shapiro-Wilk test (1965), and homogeneity of variances was cheked using Levene's test (1960), respectively at significance value of 0.05. To evaluate the population effect, mean comparisons of the studied traits were performed using one-way analysis of variance (ANOVA). When significant differences were detected ($p < 0.05$), post-hoc comparisons were conducted using Tukey's honestly significant difference (HSD) test (2). All univariate statistical

(a) (b) (c) (d)

Figure. 5. Crude fiber determination procedure: (a) sample preparation, (b) acid digestion, (c) filtration and washing, (d) furnase incineration analyses were carried out using SPSS software (version 20). Correlations between biochemical, nutritional, and environmental variables were assessed using Pearson's correlation coefficient (3) implemented in Python program (version 3.13). In addition, all analyzed traits of carob pulp were subjected to multivariate analyses, including principal component analysis (PCA) and hierarchical clustering analysis using Euclidean distance (4) (Anderson, 1984), performed using PAST software.

Standard deviation: σ

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2} \quad (1)$$

where: σ is the standard deviation, N is the number of observations, x_i is each individual data point, and μ is the mean of the data.

Tukey's honestly significant difference (HSD) test:

$$HSD = q\sqrt{\frac{MSw}{nk}} \quad (2)$$

where: q is the studentized range statistic, MSw is the mean square within groups from ANOVA, and nk is the sample size per group.

Pearson correlation coefficient:

$$r_{xy} = \frac{cov\,(x,y)}{\sqrt{var(x)}.\sqrt{var(y)}} \quad (3)$$

where: cov (x, y) is the sample covariance of x and y; var(x) is the sample variance of x; and var(y) is the sample variance of y.

Euclidean distance (D):

$$D(x,y) = \frac{[\sum_i (xi - yi)\ 2]1}{2} \quad (4)$$

where: xi, yi are the Cartesian coordinates of two points.

RESULTS AND DISCUSSION

Phenolic compounds and antioxidant activity in carob pulp

The total phenolic content (TPC) and total flavonoid content (TFC) of carob pulp extracts from twelve Moroccan populations showed significant variation among populations (ANOVA, p < 0.001; Fig. 6).

For TPC, the Sitti Fatma population from the High Atlas region exhibited the highest value (24.27 ± 2.54 mg GAE g^{-1} DW), whereas the Ouezzane population from the Pre-Rif region showed the lowest value (0.77 ± 0.10 mg GAE g^{-1} DW). Regarding TFC, the highest content was also observed in the Sitti Fatma population (12.42 ± 1.27 mg RE g^{-1} DW), followed by the Aït Mansour population (7.45 ± 1.02 mg RE g^{-1} DW). In several populations, including Chefchaouen, Akchour, Oued Laou, and Ouezzane, flavonoid contents were below the quantification limit of

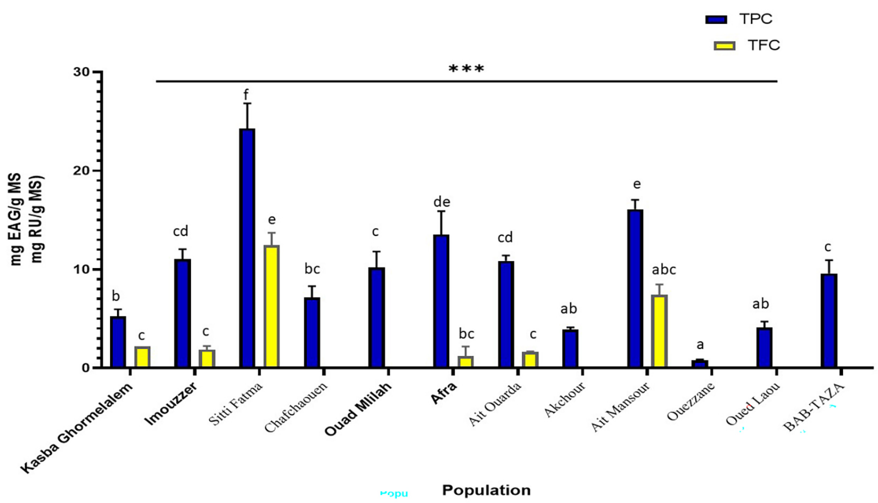


Fig. 6. Total polyphenol (TPC) and flavonoid (TFC) content in carob pulp (*Ceratonia siliqua*) from Moroccan populations. Data are presented as mean ± SD. Values sharing the same letter are not significantly different (p < 0.05) according to Tukey's test

the $AlCl_3$ assay and were therefore not detected. These results are in agreement with those reported by Cegledi and Dobroslavi (2024). However, the TPC levels recorded in the present study are higher than those previously reported for carob pulp by Custódio et al. (2015) and Makris and Kefalas (2004), while the TFC values are comparable to those reported by Fadel et al. (2020).

The observed variability in phenolic and flavonoid contents may be attributed to differences in environmental conditions and genetic background among populations, as well as to factors related to plant material and extraction conditions, as previously reported by Salim et al. (2024).

Overall, populations originating from semi-arid and arid regions exhibited higher levels of TPC and TFC than those from humid regions. This pattern suggests that site-specific environmental factors may promote the accumulation of secondary metabolites in carob pulp, thereby influencing its nutritional and functional quality, as highlighted by Richane et al. (2022).

The antioxidant capacity of pulp extracts from twelve carob (*Ceratonia siliqua* L.) populations, prepared using Ultra-Turrax-assisted homogenization, was evaluated using DPPH radical scavenging and FRAP assays (Table 2). Significant differences in antioxidant capacity were observed among populations from different geographical origins ($p < 0.001$).

In the DPPH assay, the IC_{50} values of pulp extracts ranged from 0.02 ± 0.00 to 0.60 ± 0.00 mg/mL. The Aït Ouarda and Afra populations, originating from semi-arid climatic conditions, exhibited the strongest radical scavenging activity, as reflected by the lowest IC_{50} values (0.02 ± 0.00 mg/mL). In contrast, the Chefchaouen population, collected from a humid region, showed the weakest activity, with the highest IC_{50} value (0.60 ± 0.00 mg/mL), indicating a lower antioxidant capacity. The IC_{50} values obtained in the present study are comparable to those reported by Amrani et al. (2022) and higher than those previously reported for carob pulp by Custódio et al. (2011). Similarly, in the FRAP assay, the Chefchaouen population exhibited the highest EC_{50} value (3.15 ± 0.01 mg/mL), indicating a lower reducing power, whereas the Aït Mansour (0.33 ± 0.01 mg/mL) and Aït Ouarda (0.39 ± 0.01 mg/mL) populations showed the lowest EC_{50} values, reflecting a stronger electron-donating capacity. These values are lower than those reported by Custódio et al. (2011), indicating a relatively higher reducing potential of the pulp extracts analyzed in this study.

Overall, the antioxidant capacity measured by the FRAP assay was lower than that determined by the DPPH assay, in agreement with previous observations (Ayache et al., 2020; Custódio et al., 2011). The mean IC_{50} values were 0.33 mg/mL for DPPH and 1.50 mg/mL for FRAP, both higher than those of ascorbic acid (0.03 mg/mL and 0.04 mg/mL, respectively), which was used as a reference antioxidant. Nevertheless, the results highlight carob pulp as a valuable plant-based food source of natural antioxidants, with antioxidant-related attributes that may contribute to its nutritional quality and functional properties.

In addition, all pulp extracts exhibited significant antioxidant activity, although at varying levels, reflecting the high chemical diversity among Moroccan carob populations. The results also suggest that climate has a strong influence on the antioxidant activity of the carob tree. Samples from humid regions generally showed lower antioxidant activity than those from semi-arid areas, likely due to environmental stresses such as drought and high solar radiation that stimulate the synthesis of protective bioactive compounds.

Consequently, carob populations with higher antioxidant activity may offer greater nutritional and metabolic benefits when included in the diet. Populations from semi-arid regions, such as Aït Ouarda, Afra, and Aït Mansour, appear to be the most suitable for nutraceutical and pharmaceutical applications due to their high antioxidant potential and high levels of phenolic compounds.

Physicochemical composition of carob pulp (*Ceratonia siliqua* L.) from Morocco

The results of the physicochemical composition of carob pulp (*Ceratonia siliqua* L.), including total sugars, moisture, ash, and dietary fiber contents in twelve Moroccan populations, are presented in Table 3. One-way analysis of variance (ANOVA), followed by Tukey's post hoc test, revealed significant differences among carob pulp samples from different populations for all analyzed parameters ($p < 0.05$), indicating that variations in sugar, moisture, ash, and fiber contents are likely influenced by geographical origin**.**

For moisture content, values ranged from 3.44 ± 0.50% for the Kasba Ghormelalem population to 7.72 ± 0.82% for the Sitti Fatma population, with an average of 6.32 ± 1.67%. These findings

Table 2. Antioxidant activity of twelve carobs (*Ceratonia siliqua* L.) pulp extracts from different populations, prepared using Ultra-Turrax and assessed by DPPH and FRAP assays

Population	IC_{50}DPPH (mg/ml)	EC_{50}FRAP (mg/ml)
Kasba Ghormelalem	0.19 ±0.006[bc]	2.32 ± 0.02[h]
Imouzzer	0.04 ± 0.02[ab]	0.87 ± 0.0001[c]
Sitti Fatma	0.12 ± 0.0006[ab]	0.76 ± 0.001[b]
Chafchaouen	0.69 ± 0.03[e]	3.15 ± 0.01[i]
Ait Ouarda	0.03 ± 0.01[a]	0.39 ± 0.01[a]
Akchour	0.37 ± 0.12[d]	1.88 ± 0.05[f]
Ait Mansour	0.09 ± 0.001[ab]	0.33 ± 0.01[a]
Afra	0.03±0.008[a]	1.70±0.0455[e]
Ouezzane	0.64 ± 0.09[e]	1.59 ± 0.004[d]
Oued-mlilah	0.316±0.009[cd]	2.36±0.079[h]
Oued Laou	0.67 ±0.04[e]	1.89 ± 0.004[f]
BAB-TAZA	0.46 ± 0.07[d]	2.05 ± 0.005[g]
Acide galique	0.03	0.048
Mean	0.33 ± 0.26	1.52 ± 0.88
Signification	p < 0.001***	p < 0.001***

Note: values are expressed in mg/mL as mean ± SD. Values sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's test.

align more closely with those reported by El Chami et al. (2025) for natural populations across the Mediterranean region (4.36% and 6.40%) and are lower than the values documented for Sudanese pods (8.8%) (Ishag et al., 2024) and other Azilal Moroccan pulp populations (9.3%) (Laaraj et al., 2024). This difference indicates that genotype plays a secondary role compared to microclimatic factors and post-harvest practices. Additionally, notably dry pulp is reflected in the lowest values (<8%), which fall below the critical water activity threshold (≈ 8–9%) where microorganisms typically grow (Pomeranz & Meloan 1994). In fact, low moisture content reduces the likelihood of microbial growth, offering a significant benefit for preservation in powder or flour form. It is crucial for maintaining quality and extending shelf life (Kabiri et al., 2019). Overall, most samples meet the maximum moisture requirements recommended for high-fiber functional flours (Spizzirri et al., 2024), making them suitable for nutraceutical or technological applications without the need for additional intensive drying.

Regarding ash content, significant differences were observed among the studied populations ($p < 0.05$). The Oued Laou population recorded the highest ash content (4.45 ± 0.85%), whereas the lowest value was observed in the Akchour population (0.75 ± 0.17%). The range of ash contents obtained in the present study is comparable to those reported for carob pulps from Morocco, Spain, and Lebanon (2.52–3.28% DW) (El Chami et al., 2025), as well as to values reported for Moroccan pulp from Azilal (4.16 ± 0.02%) (Laaraj et al., 2024) and Sudanese pulp from Khartoum (3.55 ± 0.27%) (Ishag et al., 2024).

Dietary fiber content also varied significantly among the twelve carob populations. The Akchour population shows the highest fiber content at 24.2%, while the Oued Laou population has the lowest at 9.62%. Most other populations range from 11% to 14%. Our results exceed those reported in the studies by Khlifa et al. (2013) and Korkmaz et al. (2020). This variability may be attributed to genetic diversity and differences in cultivation conditions (Kabiri et al., 2019). Even at the lower end, the values exceed those of wheat or soybeans (≤ 8%) and are similar to whole oats (≈ 10%), confirming the pulp's potential as a fiber-rich ingredient

Regarding total sugar concentration, significant differences were observed among the studied populations ($p < 0.05$). The Kasba Ghormelalem and Oued Laou populations exhibited the highest total sugar content (TSC), with values of 33.13 ± 0.05 g/100 g DM, whereas the lowest TSC was recorded in the BAB-Taza population (31.83 g/100 g DM). These values are consistent with those reported by El Batal et al. (2016). However, they are higher than the range of 13.7–28.1 g/100

Table 3. Composition of carob pulp (*Ceratonia siliqua* L.) in terms of sugar, moisture, ash, and fiber

Population	Sugar g/100gMS	Moisture (%)	Ash (%)	Fiber (%)
Kasba Ghormelalem	33.13 ± 0.05b	3.44 ±0.50[a]	2.75±0.15[bc]	13.77±0.47[e]
Imouzzer	32.34 ± 0.37[ab]	6.77 ± 1.37[ab]	3.85±0.65[cd]	11.12±1.77[abcde]
Sitti Fatma	32.77 ± 0.07ab	7.72 ± 0.82[b]	3.25±0.25[bcd]	11.40±2.50[abcde]
Chafchaouen	32.06 ± 0.31[ab]	6.34 ± 0.54[ab]	2.36±0.35[b]	9.79±0.30[abc]
Ouad Mlilah	32.26±0.96ab	7.70±0.65b	3.8±0.7[cd]	8.35±0.05a
Afra	32.34±0.37ab	5.22±1.67ab	3.47±0.42[bcd]	13.22±0.12[cde]
Ait Ouarda	32.52 ± 0.07[ab]	6.77 ± 0.52[ab]	3.45±0.25[bcd]	12.27±1.22bcd
Akchour	32.12 ± 0.15[ab]	5.60 ± 0.150[ab]	0.72±0.17[a]	9±25[ab]
Ait Mansour	33.13 ± 0.15b	6.67 ± 0.42[ab]	3.35±0.05[bcd]	13.64±0.04[de]
Ouezzane	32.57 ± 0.12[ab]	7.39 ± 7.39[b]	2.82±0.32bc	10.15±0.75[abcd]
Oued Laou	33.13 ± 0.05b	7.47 ± 1.37[b]	4.45±0.85d	9.62±0.67[ab]
BAB-TAZA	31.83 ± 0.76[a]	5.07 ± 3.37[ab]	3.37±0.37b[cd]	9.82±2.07[abc]
Average	32.56 ± 0.52	6.32 ± 1.67	3.25 ± 0.42	12.58±5.69
probability	0.04*	0.014*	p < 0.001***	0.013*

g DM reported for wild carob pulps from Spain, Morocco, and Lebanon (El Chami et al., 2025), while remaining lower than the sugar levels reported for fully ripe pods from Cyprus (53.59–54.83%) (Papaefstathiou and Agapiou, 2018).

The relatively limited variability observed among populations suggests that sugar accumulation in carob pulp may be more strongly influenced by fruit maturity stage and post-harvest processing conditions than by genetic factors, as also reported in European carob studies (Spizzirri et al., 2024). Overall, these findings confirm the high sweetening potential of Moroccan carob pulp, supporting its suitability for use as a natural sweetening ingredient and as a partial substitute for sucrose in nutraceutical and functional food formulations.

Values are expressed in mg/mL as mean ± SD. Values sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's test.

Mineral composition

Mineral composition is considered a key factor in evaluating the nutritional value of foods, as minerals are essential nutrients required for numerous physiological functions in the human body. In this context, the mineral composition of carob pulp from 12 populations of *Ceratonia siliqua* L. was analyzed (Table 4). The results revealed substantial inter-population variability in both macro- and microelement contents, corroborating the patterns previously described by Oumlouki et al. (2021). Overall, the mineral composition observed in our study is consistent with previously reported values (El Bouzdoudi et al., 2017; Oumlouki et al., 2021), confirming that carob pulp is a valuable source of essential minerals despite natural variation linked to genetic and environmental factors.

Potassium (K) was consistently the most abundant mineral across the studied populations, with concentrations ranging from a maximum of 1204 mg/100 g in Kasba Ghormelalem to a minimum of 130 mg/100 g in Afra. Calcium (Ca) was the second most prevalent element, reaching its highest level in Oued-Laou (308 mg/100 g), while the lowest concentrations (245 mg/100 g) were observed in the Ouazzane and Ait Ouarda populations. Magnesium (Mg) also displayed marked variability, ranging from 150 mg/100 g in Oued-Laou to 64 mg/100 g in Imouzzer. Regarding sodium (Na) and phosphorus (P), Kasba Ghormelalem again presented the highest levels (101 and 20 mg/100 g, respectively), while Imouzzer recorded the lowest (94 and 13 mg/100 g, respectively). Iron (Fe) is an essential element that serves as a cofactor for numerous enzymes. Although it is detected at relatively low concentrations, its levels vary considerably. For instance, Kasba Ghormelalem has the highest content at 8.1 mg/100 g, while Sitti Fatma has the lowest at 3.5 mg/100 g.

Overall, potassium was identified as the predominant mineral, followed by calcium and magnesium, whereas sodium and phosphorus were present at low concentrations. Among the

Table 4. Mineral composition of carob pulp from 12 Moroccan populations

Location	K(mg/100g)	Ca(mg/100g)	Mg(mg/100g)	Na(mg/100g)	P(mg/100g)	Fe(mg/100g)	Al(mg/100g)	As(mg/100g)	Pb(mg/100g)	Si (mg/100g)
Afra	130± 0.2a	258.5± 0.5c	87.5± 0.0C	94±0.05a	14± 0.1ab	7.83± 0.85	0.7± 0.01a	nd	0.01	<0,01
Ait Mansour	1090± 0.73b	248± 3bc	70± 0.01b	97±3a	18± 0.1cde	5.5± 0.5bc	0.6± 0.01a	nd	<0,01	<0,01
Ait Ouarda	1280± 0.5^{e}	245± 5ab	87.5±c	94±3a	17± 0.1bcd	5.7± 0.2bc	0.4± 0.01a	nd	<0,01	<0,01
Akchour	1120± 0.3bc	238± 3ab	76± 2b	84±4a	13.9± 0.17ab	4.5± 0.5ab	0.07± 0.05a	nd	<0,01	<0,01
Bab-Taza	1180± 0.5cd	250± 5bc	150.5± 2.5^{e}	95±2a	18.5± 1.5de	7.5± 0.5de	0.533± 0.057a	nd	<0.01	<0,01
Chefchaouen	1200± 5d	250± 3.4bc	127 ± 1.12d	9±0.14a	18± 1cde	5.2± 0.2bc	2± 0.2a	nd	<0,01	<0,01
Imouzzer	1187± 0.3d	234± 4a	64.± 1.15a	94±2a	13± 0a	7.5± 0.7de	0.5± 0.1a	nd	<0,01	<0,01
Kasba Ghormelalem	1204± 6d	296.66± 0.8de	140± e	101.6± 0.3a	20± 2e	8.1± 0.1e	1± 0.02a	nd	<0,01	<0,01
Ouad Mlilah	1167± 0.9d	287± 0.18d	153± 3^{e}	96±0.3a	15± 1ab	6.5± 0.5cd	0.4± 0.1a	nd	0.01	<0,01
Ouazzane	1152± 0.10d	245± 5ab	82± 2bc	90±3a	15± 2ab	7.5± 0.4de	2.9± 1.3a	nd	<0,01	<0,01
Oued-Laou	1200± 0.19d	308± 3^{e}	150.5± 2.5^{e}	84±2a	16.5± 1.5bcd	6.5± 0.7cd	0.1± 0.05a	nd	<0,01	<0,01
Sitti Fatma	1320± 0 .1e	294± 4d	64± 2a	87±2a	18.5± 1.5de	3.5± 0.5a	0.4± 0.1a	nd	<0,01	<0,01

microelements, iron was the most prevalent. This mineral hierarchy is broadly consistent with previous studies on carob. For instance, Hajaji et al. (2013) reported that in the leaves and bark of carob trees from Chefchaouen (Morocco), calcium was the most abundant mineral, followed by potassium, magnesium, sodium, and phosphorus. Similarly, Ayaz et al. (2009) found that Anatolian carob pods exhibited a mineral profile dominated by potassium, with calcium and magnesium also present at substantial levels, while iron, manganese, and copper appeared in lower concentrations. These convergent findings suggest that potassium, calcium, and magnesium are consistently the main minerals in both the fruits and other tissues of the carob tree. Likewise, the relatively high levels of calcium (Ca) and magnesium (Mg) highlight carob powder as a rich source of Ca, Fe, Na, K, and P, providing significant nutritional benefits through their mineral content. Although the specific physiological roles are not detailed in these sources, the consistent report of high mineral levels suggests that carob could be a valuable dietary mineral source.

Overall, the mineral profile of Moroccan carob fruits reinforces their nutritional value, while the observed variability among populations highlights the influence of both genetic background and environmental conditions on mineral composition (Ait Bella et al., 2023). However, the detection of trace amounts of Al, Pb, and As, even at low concentrations, raises concerns regarding environmental contamination. Nevertheless, the levels of these elements in all 12 studied populations remained below international food safety thresholds, in agreement with El Youssfi et al. (2023), thereby confirming that the carob pulp from these regions is safe for human consumption.

Values are expressed in mg/mL as mean ± SD. Values sharing the same letter are not significantly different ($p < 0.05$) according to Tukey's test.

Correlation among traits

The relationships among the measured variables were assessed using bivariate correlation analysis based on the Pearson correlation coefficient (Fig. 7). A strong positive correlation was observed between total phenolic content and total flavonoid content ($r = 0.79$). In addition, total phenolic content was negatively correlated with the IC_{50} values obtained from the DPPH assay ($r = -0.63$) and the FRAP assay ($r = -0.53$). Similarly, total flavonoid content showed negative correlations with DPPH IC_{50} ($r = -0.56$) and FRAP IC_{50} ($r = -0.58$). These correlations are consistent with previous reports by Salim et al. (2024) and Ioannou et al. (2023), indicating that flavonoids represent a major class of phenolic compounds in *Ceratonia siliqua* pulp and play a key role in its antioxidant capacity. The negative correlations between phenolic compounds and IC_{50} values further support the contribution of polyphenols and flavonoids as important chemical indicators of antioxidant activity.

Additionally, numerous previous studies have highlighted a strong association between the reducing power of extracts and their content of antioxidant compounds (El Kettabi et al., 2025; Salim et al., 2024). This antioxidant fingerprint is further supported by the strong inter-assay concordance observed between DPPH and FRAP (r = 0.75). Such correlations suggest that a common metabolic core, primarily composed of phenolic compounds, is responsible for both radical scavenging and ferric-reducing activities. This coherence highlights the pivotal role of polyphenols in shaping the antioxidant profile of carob pulp, providing the rationale for their recent use in the formulation of a prebiotic-antioxidant food product (Amoedo et al., 2025). The strong correlations observed, particularly between total flavonoid content (TFC), total phenolic content (TPC), and antioxidant capacity (as measured by DPPH and FRAP), suggest that these traits could serve as potential selection markers for carob. In addition, fiber content is negatively correlated with IC_{50} values of DPPH (r = -0.57) and FRAP (r = -0.42) (Fig. 3), suggesting that extracts with higher fiber content exhibit stronger antioxidant activity. Furthermore, calcium and magnesium displayed a positive correlation (r = 0.54), indicating that their levels tend to vary together. The same association pattern was revealed by Oumlouki et al. (2021). Iron is negatively correlated with total polyphenol content (r = -0.45) and flavonoid content (r = -0.54), which may suggest a potential antagonistic effect of iron on the accumulation of these antioxidant compounds. Environmental factors exerted a strong influence on the biochemical composition of *Ceratonia siliqua* fruit pulp. Rainfall is strongly negatively correlated with polyphenol content (r = -0.56), flavonoid content (r = -0.63), and fiber content (r = -0.72), indicating that drier sites promote higher levels of these compounds (De Araújo et al., 2017). In contrast, rainfall is positively correlated with magnesium (r = 0.67) and the IC_{50} values of DPPH (r = 0.75) and FRAP (r = 0.72), suggesting reduced antioxidant activity in wetter conditions (Antunes et al., 2016). Altitude positively correlates with polyphenols (r = 0.59), flavonoids (r = 0.59), and fiber (r = 0.61), while it negatively correlates with IC_{50} DPPH (-0.78), EC_{50} FRAP (-0.66) (Fig. 3), and magnesium (-0.61), highlighting a beneficial effect of higher altitudes on antioxidant content (Papanov et al., 2021). Furthermore, temperature shows positive correlations with fiber (r = 0.45), sugar (r = 0.37), and iron (r = 0.34), and a negative correlation with potassium (r = -0.65). The data collectively highlight the interaction of a central phenolic core with the matrix composition and ecogeographical conditions. This insight guides the selection of low-altitude, warm-growing

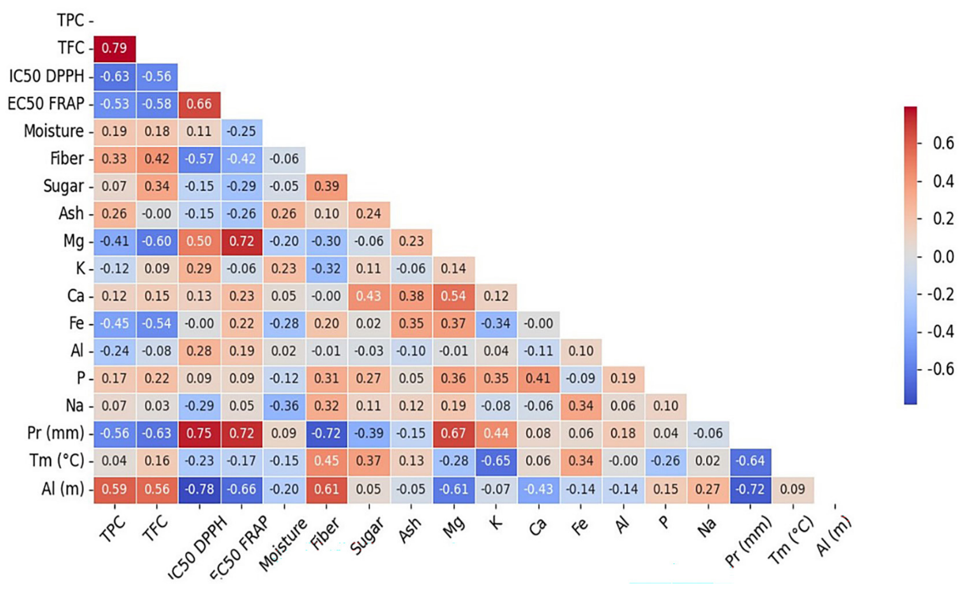


Fig. 7. Pearson correlation between biochemical, antioxidant, mineral, and nutritional parameters of carob pulp and climatic conditions across Moroccan populations

regions and appropriate stabilization processes to enhance the value of carob pulp, ensuring optimal retention of bioactive phenolic compounds.

Discrimination of carob pulp populations (*Ceratonia siliqua*)

The variation in bioactivity, chemical composition, and nutritional profiles among the 12 population extracts under study was assessed using a multivariate analytical approach. Principal component analysis (PCA), hierarchical clustering on principal components (HCPC), and hierarchical cluster analysis (HCA) were conducted using the quantitative dataset from this study, revealing pronounced differences among the populations and enabling the identification of distinct groups based on their phytochemical, antioxidant, and mineral characteristics. The principal component analysis (PCA), based on the mean values of the studied variables, revealed that the first two components together explained 41.77% of the total variation among populations (Fig. 8). The first component (PC1) accounted for 25.2% of the variation and was primarily associated with polyphenol content (TPC), flavonoids (TFC), sugar, and fiber, while showing a negative correlation with IC_{50} DPPH, EC_{50} FRAP, and magnesium. The second component (PC2) explained 17.08% of the variation and was positively correlated with sodium, iron, fiber, phosphorus, and magnesium, and negatively correlated with moisture and potassium.

Based on the PCA, three distinct population clusters were identified, as illustrated in Figure 8. The first group (G1) comprises populations from the Anti-Atlas and High Atlas regions, specifically Sitti-Fatma, Aït Mansour, Aït Ouarda, and Imouzzer. These populations are characterized by various bioclimates, including arid and semi-arid conditions, and are situated at altitudes ranging from 1,074 m to 1,290 m. The second group (G2) consists of the two populations of Kasba Ghormelalem and AFRA, located in the Middle Atlas region. These populations are characterized by a shared bioclimatic stage and exhibit similar altitudes, which range from 883 to 885 meters. The third group (G3) is represented by four populations under different subhumid and humid bioclimates: Oued Laou, Oued Mlilah, Akchour, and BAB-TAZA from the western Rif, and one population, Ouezzane, from the pre-Rif western region (Table 1).

Analysis of the hierarchical cluster analysis (HCA) dendrogram revealed two main groups of carob tree populations (Fig. 9). The first group (A) comprised only the Afra population, which was clearly separated from all other populations. This population was characterized by a low potassium content and relatively high levels of ash and iron. The second group (B) included the remaining populations and exhibited overall biochemical similarity. Within this group, four subgroups were distinguished:

- Subgroup B1: Siti Fatma and Ait Ouarda, showing a high degree of similarity.
- Subgroup B2: Kasba Ghormelalem, Chefchaouen, Bab Taza, Oued Mlilah, and Oued Laou, forming a distinct subcluster with close similarity.
- Subgroup B3: Ouazzane and Imouzzer, showing moderate similarity.
- Subgroup B4: Akchour and Ait Mansour, clustered together with a slightly greater distance, indicating moderate similarity.

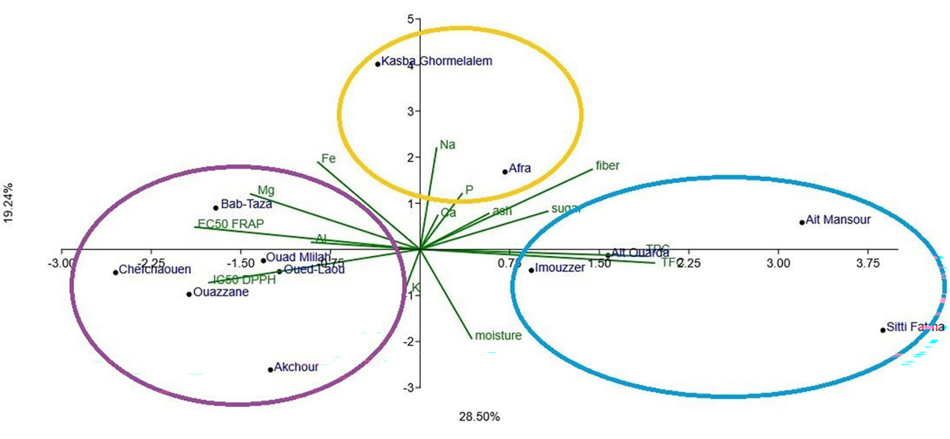


Fig. 8. Principal component analysis of variability in phytochemical composition, mineral content, and sugar and fiber levels of carob (Ceratonia siliqua L.) pulp from Moroccan populations

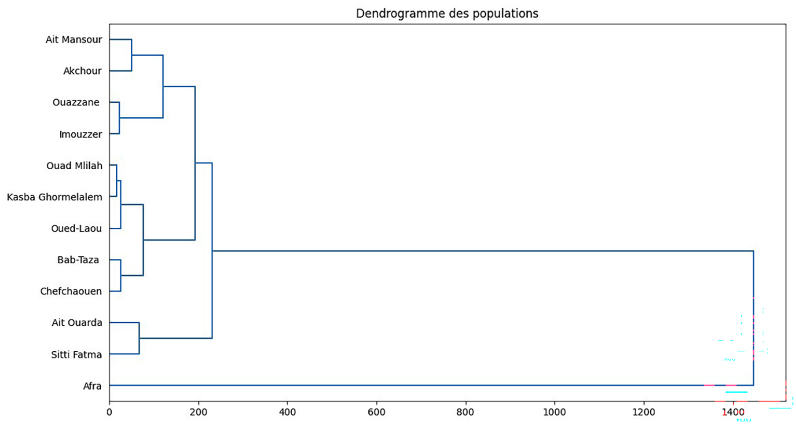


Fig. 9. Hierarchical clustering of carob pulp from 12 Moroccan populations using Euclidean distance based on nutritional and phytochemical parameters

The integration of PCA with hierarchical clustering (HCPC) is crucial for providing a clearer visualization of the grouping patterns among *Ceratonia siliqua* populations' pulps (Fig. 10). This method highlights that geographically proximate populations tend to exhibit similar biochemical profiles. The results suggest that the biochemical composition of carob fruits is flexible and influenced by environmental factors. As a result, populations from different regions may show varying metabolic profiles in response to different climatic and geographical conditions. The first group consists solely of the Kasba Ghormelalem population, while the second group, marked in blue, includes the Siti Fatma, Ait Mansour, Ait Ouarda, Afra, and Imouzzer populations. The third group comprises populations from the pre-Rif western region and western Rif, such as Oued Laou, Oued Mlilah, Akchour, Bab-Taza, and Ouezzane.

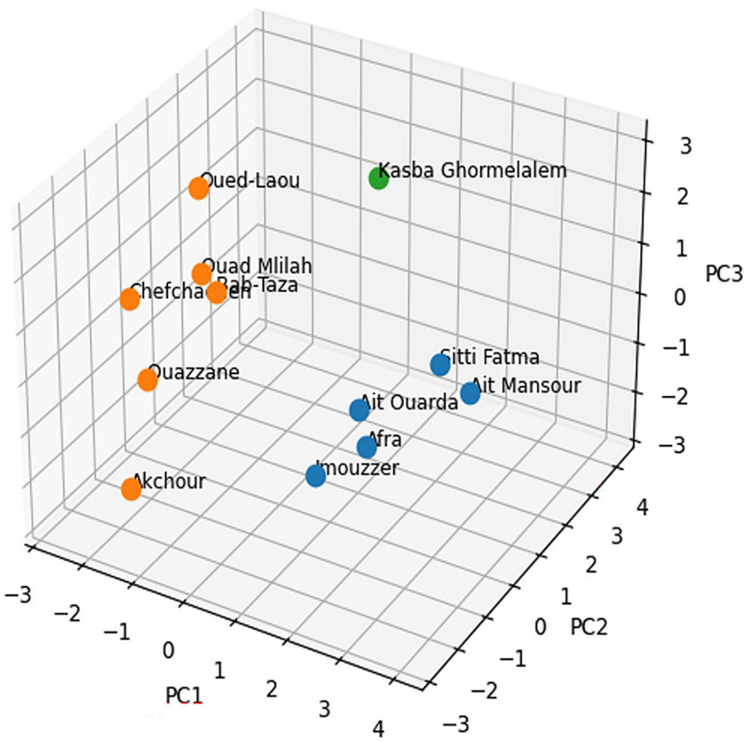


Fig. 10. 3D hierarchical clustering of *Ceratonia siliqua* L. populations based on biochemical, nutritional, and antioxidant data (PC1:29 %, PC2:19 % and PC3: 17 %)

CONCLUSIONS

The present study successfully demonstrated a marked biochemical and nutritional variability among Moroccan *Ceratonia siliqua* pulp extracts originating from different natural populations. These findings confirm that the objectives of the study were fully achieved. The results revealed notably high levels of total polyphenols and flavonoids, which were closely associated with substantial antioxidant activity, highlighting their strong capacity to scavenge free radicals and reduce oxidative processes.

The observed interpopulation variability reflects the complex interplay of genetic, environmental, and geographical factors, including local climate and altitude. In addition to their rich phytochemical profile, the carob pulp samples were shown to be an excellent source of essential minerals, particularly potassium, calcium, and magnesium, as well as significant amounts of sugars and dietary fiber. Importantly, the combined evaluation of phytochemical composition and nutritional traits enabled the identification of populations with particularly high functional potential, which had not been clearly distinguished in previous studies. This work therefore fills an important gap in the literature by providing an integrated, population-scale assessment of the biochemical and nutritional value of Moroccan carob pulp. The co-occurrence of high antioxidant capacity with a favorable mineral and macronutrient composition suggests that certain populations possess elite biochemical characteristics. These populations may be prioritized for nutritional applications (e.g., functional foods and dietary supplements) and industrial uses (e.g., antioxidant-rich extracts and fiber-based ingredients), reinforcing their relevance for both health-related and commercial valorization.

Overall, these results open new perspectives for sustainable exploitation of Moroccan carob pulp as a natural source of bioactive compounds for food, nutraceutical, and pharmaceutical applications. Future research should focus on identifying the specific phenolic compounds responsible for the observed bioactivities and on evaluating their stability and efficacy in real food systems and biological models.

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