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RESEARCH ARTICLE

A comparative morphometric and phytochemical analysis of the calabrian endemic treasure *Crepis aspromontana* Brullo, Scelsi & Spamp. with alimentary potential

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Abstract *Crepis aspromontana,* an endemic species from Calabria (Southern Italy), is a traditional nutritional source in the ethnobotany of the Graecanic area. This study compares the morphometric and chemical properties of wild and cultivated specimens, also evaluating the phytochemical response to abiotic stress. The results highlight a clear growth-defense trade-off: cultivated plants exhibit superior morphometric characteristics (larger rosettes, greater leaf number), whereas wild specimens produce significantly higher concentrations of beneficial phytochemicals, including polyphenols and flavonoids, and possess markedly greater antioxidant activity. Notably, cultivated specimens, when subjected to heat stress, showed a dramatic increase in polyphenol content, reaching levels well above those of wild samples. This research reveals that domestication optimizes yield but reduces phytochemical quality, which can be restored or enhanced through stress management. *Crepis aspromontana* is confirmed as a valuable, nutrient-rich food source, and this study balances biodiversity safeguarding with its potential as a typical Calabrian product.

Keywords *Crepis aspromontana* · Asteraceae · Ethnobotany · Food plant · Heat stress · Southern Italy · Wild plant domestication · Calabria endemic species

Abbreviations

a	Red-green coordinate
AA	Antioxidant activity
a_W	Water activity
$ABTS^+$	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
b	Yellow-blue coordinate
CFR	Cultivated fresh
CFRs25	Cultivated fresh stressed at 25 °C
CFRs29	Cultivated Fresh stressed at 29 °C
CFZ	Cultivated frozen
CFZs25	Cultivated frozen stressed at 25 °C
CFZs29	Cultivated frozen stressed at 29 °C
C. aspromontana	*Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae)
DPPH·	2,2-Diphenyl-1-picrylhydrazyl
GAE	Gallic acid equivalent

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IUCN	International union for conservation of nature
l	Lightness
LC	Least concern
QE	Quercetin
PCA	Principal component analysis
RH	Relative humidity
TA	Titratable acidity
TFC	Total flavonoid content
TPC	Total polyphenol content
TSS	Total soluble solids
WFR	Wild fresh
WFZ	Wild frozen

Introduction

The Mediterranean diet, recognized for reducing the risk of cardiovascular disease and diabetes (Willet et al. 1995; Abenavoli et al. 2021; Perrino 2022), prominently features the use of wild plants. These plants enhance nutritional variety and offer a rich source of bioactive compounds, antioxidants, and phytonutrients (Danesi and Bordoni 2008; Hadjichambis et al. 2008; Marrelli et al. 2018; Piątkowska et al. 2022). The consumption of wild edible plants is a tradition being rediscovered for its nutritional and cultural value (Łuczaj 2012; Dimitriadis et al. 2024; Musarella et al. 2024), providing nutrients that enhance the human diet while supporting agricultural biodiversity and food security (Hunter et al. 2019; Panfili et al. 2020; Garcia-Oliveira et al. 2021). Furthermore, recent research demonstrated that ingesting these plants can confer health benefits, including anti-inflammatory and anti-diabetic properties (Leonti 2011).

From this point of view, ethnobotanical research of territories characterised by widespread rurality and small settlements in areas of high natural quality can provide useful information. Numerous investigations (Nebel et al. 2006; Musarella et al. 2019; Patti et al. 2024a) have highlighted that regions of southern Italy, such as Calabria, hold a rich heritage of knowledge concerning the traditional uses of plants in cuisine. For instance, plants are used to prepare side dishes or baked desserts (Mattalia et al. 2020; Gentile et al. 2022; Patti et al. 2025a). Therefore, the search for new food plants with high nutraceutical value benefits from ethnobotanical research. Previous surveys on the traditional plant use in the Graecanic area of the Metropolitan City of Reggio Calabria revealed that the basal rosettes of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae), an endemic Southern Calabrian species, are consumed as a side dish when boiled or sautéed with seasonings. The leaves are also used in traditional medicine, with a decoction prepared from them said to facilitate digestion (Patti 2024; Patti et al. 2024b, 2025b). However, *C. aspromontana* is a potentially threatened species, according to The International Union for Conservation of Nature (IUCN) criteria, falls into the Least Concern (LC) category of the Red List of the Italian Flora (Rossi et al. 2020), indiscriminate collection could compromise the population of this species, which is exclusive to a limited territory. It is, however, important to protect this plant, not only to ensure the preservation of local biodiversity, but also to maintain the associated culinary traditions and ethnobotanical knowledge. One strategy for ensuring the protection of the species is domestication, which can reduce the risk of extinction due to indiscriminate harvesting and preserve biodiversity by integrating domesticated plants into agricultural systems and improving their characteristics for human use (Tanksley et al. 1997; Gepts 2003).

The nutritional use of *C. aspromontana* underscores the necessity for further investigation into its chemical and nutritional characteristics. Previous research conducted in the same region on Asteraceae family plants, such as *Hypochaeris laevigata* (L.) Ces., Pass. & Gibelli and *Hyoseris radiata* L., has demonstrated a high concentration of phenolic compounds and antioxidant activity, emphasizing the nutritional potential of the Asteraceae family (Sicari et al. 2021). Moreover, research on the genus *Crepis*, including species like *C. leontodontoides* All. and *C. vesicaria* L., revealed excellent contents of compounds beneficial to human health (Badalamenti et al. 2022). Further investigations have also been carried out on other species of the genus, such as *Crepis cameroonica* Babc. ex Hutch. & Dalziel (Ndom et al. 2006) and *Crepis crocea* (Lam.) Babc. (Li et al. 2021). However, no specific investigations have addressed *C. aspromontana*, highlighting not only its cultural relevance but also its nutritional value.

This study aims to fill the existing gap in the morphometric and phytochemical knowledge of *C. aspromontana*, an endemic Calabrian species that has

been scarcely investigated. To date, no comparative analysis between wild and cultivated populations has been conducted, nor have the effects of heat stress on cultivated plants been evaluated. This research represents a novelty as it examines the impact of two high-temperature treatments (25 °C and 29 °C) on the morphometric characteristics and phytochemical profiles of cultivated *C. aspromontana*. These analyses provide valuable insights into its potential as a domesticated edible plant and contribute to understanding how cultivation practices and environmental stressors influence the quality and nutritional value of this endemic species, promoting both its conservation and sustainable use.

Materials and methods

The species

Crepis aspromontana is an endemic plant, known locally as *'prìcomaruḍḍha'*, belongs to the Asteraceae family. It is a hemicryptophyte that reaches a height of 12–35 cm and is characterised by a robust woody root. The stem branches from the base. The basal leaves are spatulate or oblanceolate-spatulate with toothed margins and a blunt or rounded tip. They are arranged in basal rosettes. Cauline leaves are amplexicaul, lanceolate, and irregularly toothed. The cymose inflorescence has numerous, medium-sized flower heads with yellow ligules. This species differs from *Crepis vesicaria* L. in its robust stem and greater glandular hairiness (Brullo et al. 1995, Patti 2024). Ecologically, *C. aspromontana* grows on the Ionian slopes of Aspromonte in calcareous or conglomeratic rocky habitats under xeric conditions. According to Brullo et al. (1995), this area is the driest part of Calabria and is home to a unique collection of rare, drought-resistant Mediterranean species. Its close association with rocky habitats suggests an ancient origin and adaptation to extreme environmental conditions.

Plant material, cultivation, and experimental design

The germplasm of C. aspromontana was collected in February 2023 in Pentidattilo (37°57′11.0″ N 15°45′43.0″ E; 250 m a.s.l.), a district of Melito di Porto Salvo, a southern Calabrian municipality located in the Graecanic Area of the Metropolitan City of Reggio Calabria (southern Italy). Two samples were collected from the municipalities of Bova and Condofuri and stored at the herbarium of the Mediterranean University of Reggio Calabria (REGGIO) under IDs 8683 and 8684 (Online Resources 1 and 2). The acronym of the cited herbarium is in accordance with Thiers (2025).

In total, we collected about 4000 cypselae during the flowering period (February 2023). After collection, the cypselae were cleaned, sorted and were kept in sterile plastic containers in a refrigerator at 5 °C in the dark for 2 months to maintain viability. The germplasm was then used for in vivo germination in a climate cell set at 15 °C with a 12-h photoperiod and 50% relative humidity. The plants were grown in neutral pH clay soil (6.8–7.2) enriched with organic compost to improve nutrient availability and water retention, with the aim of replicating optimal natural conditions and promoting the desired morphological traits. The seeds were sown directly in pots placed under controlled conditions (inside the climate cell). During the growing period, manual watering was carried out twice a week to maintain adequate soil moisture and to avoid water stagnation. No chemical treatments were applied as the aim was to maintain natural growing conditions.

In total, 70 plants were grown. After three months of growth, leaves from the basal rosettes were randomly collected from different samples, either from plants grown under controlled conditions (within the climate cell) (Cultivated Fresh), or from plants grown in the field at the original collection site (in September 2023) (Wild Fresh). Leaves were collected at the same phenological stage and included different shapes and sizes to ensure a representative sample of the plant population. Some of the harvested leaves were frozen for 15 days at − 20 °C so that the samples could also be analyzed in frozen conditions.

All analyses were carried out for Cultivated Fresh (CFR) and Cultivated Frozen (CFZ) specimens, for Wild Fresh (WFR) and Wild Frozen (WFZ) specimens, and, in addition, for some of the plants grown in climate cell (Cultivated), heat stress treatments were applied during the cultivation phase in climate chambers to evaluate plant responses to elevated temperatures (25 °C and 29 °C). The tests were conducted over a period of 20 days to assess the species' behaviour in response to a possible abrupt

climatic change. The treatments included Cultivated Fresh stressed at 25 °C (CFRs25), Cultivated Fresh stressed at 29 °C (CFRs29), Cultivated Frozen stressed at 25 °C (CFZs25), and Cultivated Frozen stressed at 29 °C (CFZs29). Heat stress experiments were performed in two different climate chambers under artificial light with a 12 h photoperiod.

The different matrices with the various treatments are illustrated in the experimental plan in Fig. 1.

Summary of morphometric and phytochemical analyses

The study encompassed a comprehensive array of morphometric analyses, including the measurement of rosette diameters and number of leaves, as well as analytical techniques such as colorimetric analysis and determination of relative humidity (RH), ash content (%), water activity (aW), pH, total soluble solids content (TSS), chlorophyll, and carotenoid content, in accordance with established methodologies.

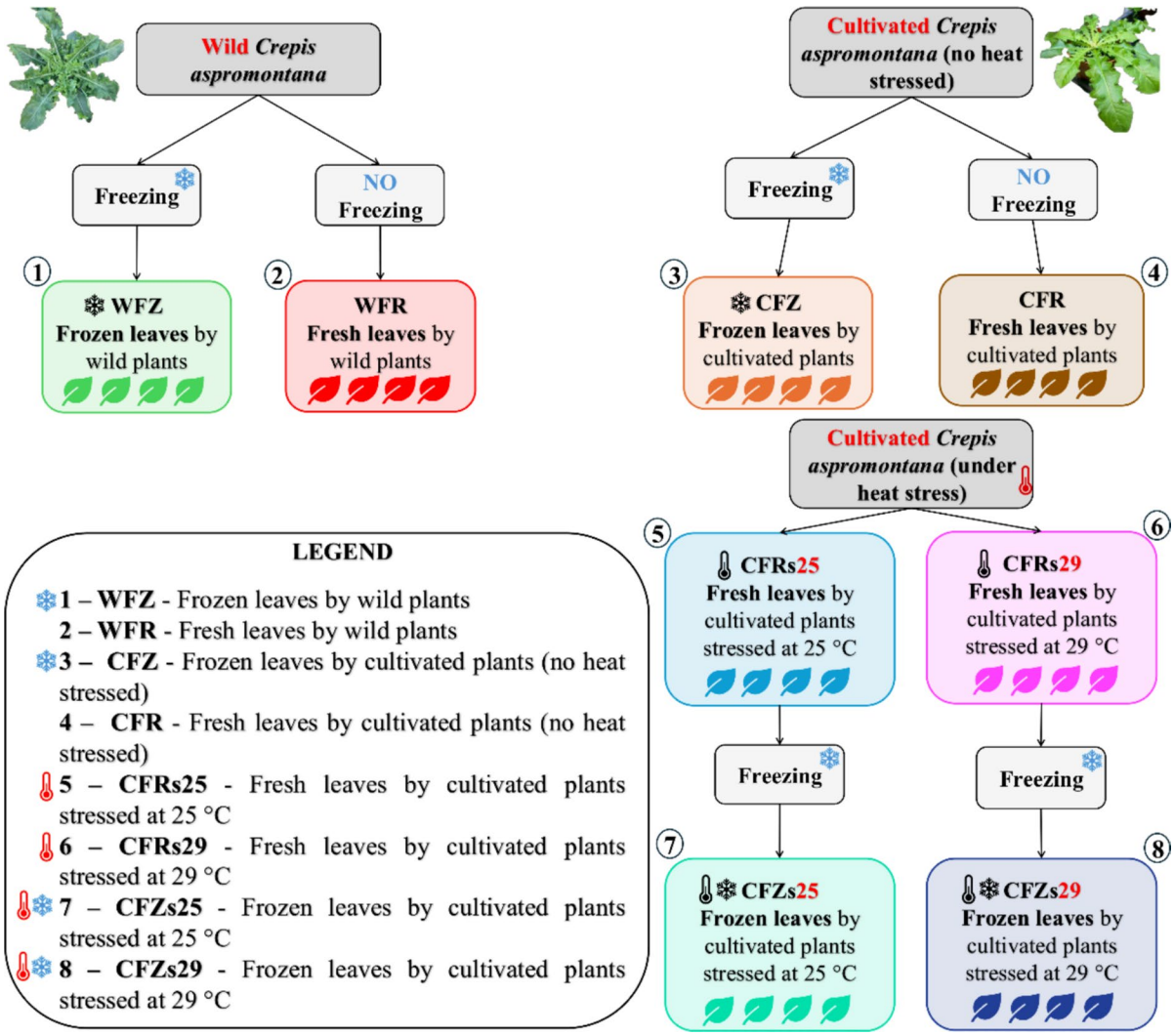

Fig. 1 Experimental plan of the analysis on *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) on wild, cultivated and treated plants. WFZ: Wild Frozen; WFR: Wild Fresh; CFZ: Cultivated Frozen; CFR: Cultivated Fresh; CFZs25: Cultivated Frozen stressed at 25 °C; CFRs25: Cultivated Fresh stressed at 25 °C; CFZs29: Cultivated Frozen stressed at 29 °C; CFRs29: Cultivated Fresh stressed at 29 °C

Additionally, Total Phenolic content (TPC) and Total Flavonoid content (TFC) were analyzed, and antioxidant activity were quantified using the percentage of inhibition for the radicals DPPH· and $ABTS^{+}$.

Morphometric measurements

Before collecting the leaf samples, morphometric measurements were made on 20 plants under cultivated conditions (CFR) and 20 plants under wild conditions (WFR), assessing the rosette diameters and the number of leaves. This ensured accurate comparison between the two groups.

Colorimetric analysis

Colorimetric analysis was conducted using a calibrated colorimeter (Tristimolo CR 300, Minolta), adopting the CIE L a b system as a reference. For each growth condition, four leaves of varying size and shape were analysed, with 15 measurements taken on each leaf surface (upper and lower). The analysis was performed exclusively on fresh leaves from wild and cultivated samples (CFR, WFR, CFRs25, CFRs29) to provide an overview of the species' characteristics (Lee et al. 2022). Results were expressed in terms of lightness (L), red-green coordinate (a), and yellow-blue coordinate (b).

Determination of chemical parameters: % ash, pH, relative humidity (RH), titratable acidity (TA) total soluble solids (TSS) and water activity (a_W)

To determine the Ash content, 20 g of the fresh sample were weighed and placed in a platinum cup, which was then transferred to a muffle furnace at 550 °C until a constant weight was achieved. The ash content was calculated as a percentage of the fresh weight. Analyses were conducted in duplicate on fresh leaves of wild and cultivated samples (CFR, WFR, CFRs25 and CFRs29). The ash percentage was calculated using the following formula, as outlined by Harris and Marshall (2017):

$$\%\ \text{Ash} = \frac{\text{weight of ashes}}{\text{weight of fresh sample}} * 100 \quad (1)$$

Relative Humidity (RH) was measured by breaking five grams of fresh sample and placing them in a thermobalance (MA160, Sartorius, California, US). The analysis was performed in duplicate on fresh leaves of wild and cultivated samples (CFR, WFR, CFRs25 and CFRs29). The Relative Humidity was expressed as a %.

Water Activity (a_W) was assessed using a hygrometer (Aqualab LITE Decagon) on fresh leaves of wild and cultivated samples (CFR, WFR, CFRs25 and CFRs29), with duplicate analyses performed.

For the determination of Titratable Acidity (TA), pH and Total Soluble Solids (TSS), the same extract was prepared on all fresh matrices (CFR, WFR, CFRs25 and CFRs29). Ten grams of leaf sample were taken, and 50 mL of distilled water was added; the mixture was homogenised with Ultra-Turrax (T 25 digital, IKA, Staufen, Germany) for 30 s at medium speed and centrifuged (NF 1200R, Nüve, Ankara, Türkiye) for 5 min at 5000 rpm at 5 °C. The supernatant was filtered through qualitative filter paper (Whatman ®, grade 4) into a 100 mL flask, and the solid residue was re-extracted with another 50 mL of water. A second centrifugation was performed, re-filtered and finally made up to volume.

Titratable Acidity (TA) was determined by titrating 10 mL of extract with 0.05 N NaOH using 1% phenolphthalein as an indicator. All analyses were conducted in duplicate on the different samples. The results were expressed as % citric acid.

For the pH determination, a sample aliquot was subjected to pH measurement by immersing the electrode in the sample. All analyses were conducted in duplicate using a Crison basiC 20 pH meter, equipped with a 52–60 electrode.

Total Soluble Solids (TSS) were measured using a digital refractometer (DBR 047 SALT, Giorgio Bormac s.r.l, Carpi (MO), Italy) and expressed in degrees Brix (°Bx). The analysis was carried out in duplicate. The TSS content was expressed in percent.

Phytochemical analysis: chlorophylls and carotenoids content

The extraction of pigments, including chlorophylls and carotenoids, was performed according to the method described by Nagata and Yamashita (1992). Two grams of leaves were combined with 10 mL of an acetone/hexane solution (2:3, *v/v*) and placed in a Falcon conical centrifuge tube. The mixture was homogenized using an Ultra-Turrax (T 25 digital,

IKA, Staufen, Germany) to ensure thorough mixing and efficient pigment extraction.

Following homogenization, the sample was centrifuged using a refrigerated centrifuge (NF 1200R, Nüve, Ankara, Türkiye) at 5 °C for 10 min at a speed of 6000 rpm. This step facilitated the separation of the liquid phase, containing the extracted pigments, from the solid residues. After centrifugation, the supernatant was carefully collected and filtered through qualitative filter paper (Whatman®, grade 4) to eliminate any remaining particulate matter.

The absorbance of the clarified extract was then measured using a UV–Vis spectrophotometer (UV–Vis k2, PerkinElmer Inc., Waltham, MA, USA) at four specific wavelengths: 453, 505, 645, and 663 nm. The acetone/hexane solution (2:3, *v/v*) used for the extraction served as the blank to calibrate the spectrophotometer.

This extraction procedure was applied to all variables, including fresh and frozen samples from both wild and cultivated plants (CFR, CFZ, WFR, WFZ, CFRs25, CFZs25, CFRs29, CFZs29), ensuring consistency in the analysis. The results were expressed as milligrams per 100 mL of extract, calculated using the following formulae:

$$Chlorophylla = 0.999A663 - 0.0989A645 \quad (2)$$

$$Chlorophyllb = -0.328A663 + 1.77A645 \quad (3)$$

$$\beta - Carotene = 0.216A663 - 1.22A645 - 0.304A505 + 0.452A453 \quad (4)$$

Phytochemical analysis: total polyphenol content (TPC), total flavonoid content (TFC) and antioxidant activity (AA) (ABTS.$^+$ and DPPH·)

Extract preparation

The extraction of polyphenols and flavonoids was carried out following the method described by Sicari et al. (2021). To prepare the extract, ten grams of the plant sample were mixed with 50 mL of an ethanol/water solution (80:20, *v/v*). The mixture was then homogenized using an Ultra-Turrax at medium speed for 30 s to ensure efficient extraction of bioactive compounds.

Following homogenization, the mixture was centrifuged in a refrigerated centrifuge at 5 °C for 10 min at a speed of 5000 rpm. This step allowed the separation of the solid residues from the liquid phase. After centrifugation, the supernatant was carefully collected and filtered through qualitative filter paper (Whatman®, grade 4) to remove any remaining particulates. The resulting clear extract was then used for subsequent analyses.

This extraction procedure was applied to all variables tested, including fresh and frozen samples of both wild and cultivated plants (CFR, CFZ, WFR, WFZ, CFRs25, CFZs25, CFRs29, CFZs29), to ensure consistency and comparability of results.

Total phenolic content (TPC)

In accordance with Sepahpour et al. (2018), the total polyphenol content was determined using the Folin-Ciocalteu colorimetric method. To prepare the diluted extract, 250 µL of the plant extract were mixed with 750 µL of distilled water, resulting in a 1:4 (*v/v*) dilution. This mixture was then transferred to a 10 mL flask, to which 5 mL of Folin-Ciocalteu reagent (1:10, *v/v*) was added. The solution was left to stand for 5 min to allow the reaction to occur.

Following this initial reaction time, 4 mL of 7.5% sodium carbonate (Na_2CO_3) were added to the mixture. The flask was briefly vortexed to ensure homogeneity and then incubated in the dark for 1 h to complete the reaction. The absorbance of the final solution was measured at 765 nm using a UV–Vis spectrophotometer (UV–Vis k2, PerkinElmer Inc., Waltham, MA, USA).

To account for background absorbance, a blank sample was prepared following the same procedure but using distilled water instead of the extract. The total polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight. The concentration range of gallic acid used as a reference standard was between 3 and 10 ppm.

Total flavonoid content (TFC)

In accordance with Sepahpour et al. (2018), the flavonoid content was determined using a colorimetric method. To prepare the diluted extract, 250 µL of the plant extract were mixed with 250 µL of distilled

water, resulting in a 1:1 *(v/v)* dilution. This mixture was then transferred into a 5 mL flask, to which 2.5 mL of distilled water and 150 µL of 5% sodium nitrite ($NaNO_2$) were added. The solution was allowed to stand for 5 min to facilitate the reaction.

Subsequently, 1 mL of 1 M sodium hydroxide (NaOH) was added to the mixture, and the volume was adjusted to 5 mL with an additional 550 µL of distilled water. The final solution was vortexed briefly to ensure thorough mixing and then incubated for 15 min. A blank sample was prepared in the same way but using the same amount of distilled water instead of the plant extract.

The absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh weight. The concentration range of quercetin used as a reference standard was between 10 and 50 ppm.

Antioxidant activity (AA): ABTS$^+$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and DPPH· (2,2-diphenyl-1-picrylhydrazyl) radicals

Antioxidant Activity (AA) was assessed using ABTS$^+$ and DPPH· assays.

For the determination of ABTS$^+$, the ABTS$^+$ solution was prepared by mixing 88 µL of potassium persulfate ($K_2S_2O_8$) with the appropriate volume of ABTS$^+$ reagent to obtain a final concentration. A volume of 2980 µL of this solution was then placed into a plastic cuvette, and the absorbance was measured at 734 nm. Subsequently, 20 µL of the plant extract were added, and the mixture was incubated in the dark under agitation for 6 min. After the incubation period, the absorbance was measured again. A blank sample was prepared using ethanol to ensure accurate baseline readings. The results of the ABTS$^+$ assay were expressed as a percentage of inhibition.

Similarly, for the determination of DPPH·, a DPPH· solution (1×10^{-4}) was prepared, and 2980 µL of this solution were transferred to a cuvette. The initial absorbance was recorded at 517 nm. Then, 20 µL of the extract were added to the cuvette, and the mixture was incubated in the dark with continuous agitation for 30 min. After this time, the absorbance was measured again. A blank was prepared using methanol. The results were expressed as a percentage of inhibition.

The percentage of inhibition for both ABTS$^+$ and DPPH· radical generation was calculated using the following formula:

$$RadicalScavengingAssay(RSA)\% = \left[\frac{(A0 - A1)}{A0}\right] * 100 \quad (5)$$

where A0 represents the absorbance of the control, while A1 denotes the absorbance of the sample at the designated time points (6 min for ABTS$^+$; 30 min for DPPH·).

Statistical analysis

All experiments were conducted twice with the same methodologies and materials. For morphometric measurements, a Principal Component Analysis (PCA) was carried out accompanied by visual box plot analyses. For the box plot, statistical comparison between groups was performed using the student's t-test. These analyses were performed using the software PAST version 4.15 (Hammer et al. 2001). The analytical data of all were reported as mean ± standard deviation (SD) $n = 2$. Analysis of variance (one-way ANOVA) was conducted by applying the Tukey post hoc test at $p < 0.01$ (SPSS software, version 29.0, Armonk, NY, USA). The following symbols were used to indicate significance: ** $p \leq 0.01$; * $p < 0.05$; n.s. (not significant) $p > 0.05$.

Results and discussion

Morphometric measurements

Morphometric analysis proved to be an effective tool for distinguishing the growth characteristics of *C. aspromontana* in cultivated (CFR) and wild (WFR) conditions. As reported in the literature, morphometric measurements are particularly useful in the Asteraceae family for assessing phenotypic variation and adaptation strategies (Cano et al. 2017).

As illustrated in Fig. 2, a clear differentiation between wild (WFR) and cultivated (CFR) plant samples is evident. The results of our analysis revealed significant differences between cultivated and wild plants, with cultivated specimens

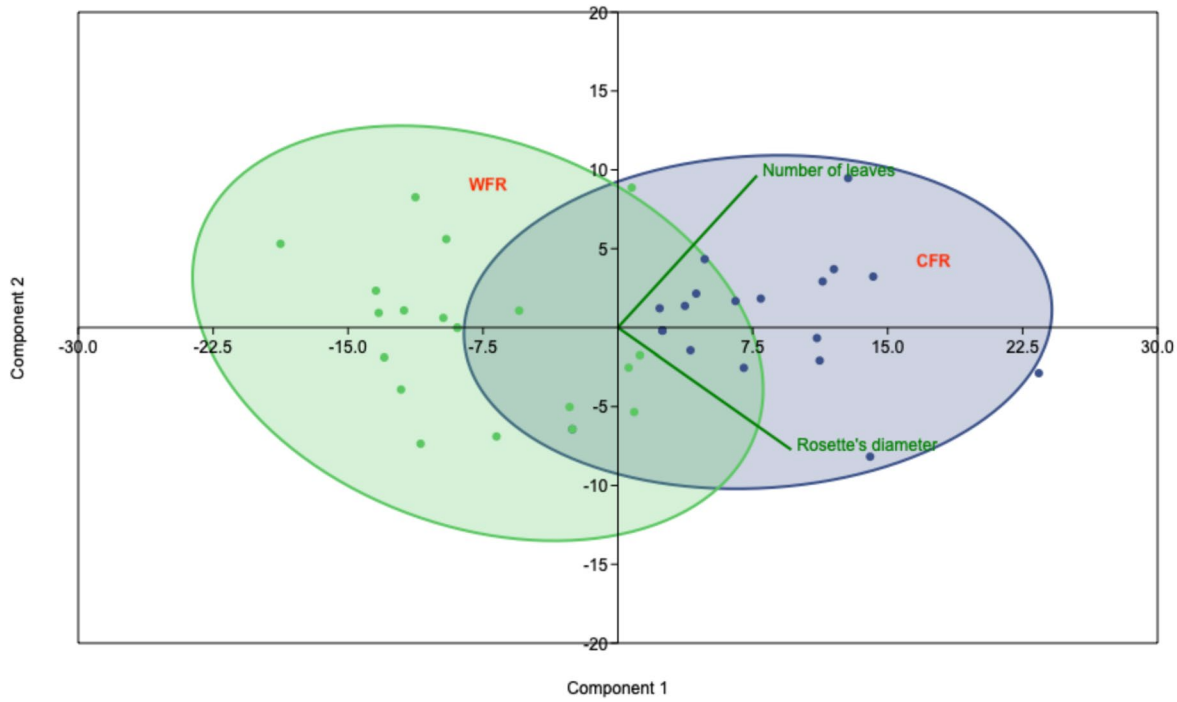

Fig. 2 Principal Component Analysis (PCA) of the wild (WFR) (in green) and cultivated (CFR) (in blue) samples of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) performed on 20 plant samples for each of the two theses by measuring the number of leaves and the diameter of the rosettes

exhibiting a larger rosette diameters and a greater number of leaves. This outcome was expected, as domestication and optimized growing conditions generally enhance vegetative growth. Cultivated plants benefit from nutrient-rich soil and controlled watering, which promote the development of larger rosettes and more abundant foliage.

The distinct separation observed in the Principal Component Analysis (PCA) between wild and cultivated samples confirms this divergence. The analysis revealed that Component 1 (PC1) explained 83.74% of the total variance, while Component 2 (PC2) explained 16.26%. The separation along PC1 was primarily driven by both variables, though slightly more by 'Rosette's diameter' (loading score: 0.780), while PC2 separated 'Number of leaves' (loading: 0.780) from 'Rosette's diameter' (loading: − 0.626). Notably, other investigations on species within the genus *Crepis* have reported similar trends. For example, *Crepis tectorum* L. exhibits pronounced morphometric variability between cultivated and wild populations, reflecting the influence of environmental conditions on plant morphology (Andersson 1993).

The results presented in Table 1 further support this interpretation, showing that

Table 1 Measurements of rosette diameters and number of leaves for wild (WFR) and cultivated (CWR) *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) plants

Sample typology	Rosette diameters (cm)	Number of leaves (no unit)
CFR	38.80 ± 5.08^{a}	22.75 ± 4.96^{a}
WFR	27.10 ± 6.06^{b}	12.45 ± 4.45^{b}
sign	**	**

CFR: Cultivated Fresh; WFR: Wild Fresh. Rosette diameters values are expressed in cm. Data are reported as mean ± standard deviation (SD) ($n=2$). **significance at $p \leq 0.01$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

cultivated plants have significantly larger rosette diameters (38.80 ± 5.08 cm) and higher leaf numbers (22.75 ± 4.96) compared to wild specimens (27.10 ± 6.06 cm and 12.45 ± 4.45, respectively). The statistical analysis indicates that the differences between the two groups are highly significant ($p \leq 0.01$), confirming that cultivation positively affects these morphological traits. Such differences are likely linked to the improved growing conditions provided during cultivation, which include more consistent irrigation and nutrient availability.

Box plot analysis (Fig. 3) provided additional insights into the distribution and variability of the data. For the number of leaves (a), the median value of the CFR group was symmetrically positioned within the box, indicating a balanced distribution. In contrast, the WFR group displayed a downward-shifted median, suggesting an asymmetrical distribution and greater variability. The presence of outliers in the WFR group suggests environmental heterogeneity in the wild populations, whereas the CFR group, with fewer outliers, indicates greater homogeneity likely due to uniform cultivation practices.

Regarding rosette diameters (b), the box plot revealed that the CFR group showed a more compact box and shorter whiskers, indicating less variability compared to the WFR group. This pattern aligns with observations from other Asteraceae species, where domestication and controlled growth conditions lead to reduced phenotypic variation. For example, *Helianthus annuus* L. shows morphological differences between wild and cultivated forms, particularly in cypselae size, underlining the adaptive plasticity of Asteraceae to cultivation practices (Tarighat et al. 2011).

These results suggest that cultivation positively influences plant vigour in *C. aspromontana*, making it a promising candidate for domestication. However, the greater variability observed in wild plants may also reflect genetic diversity and adaptation to harsh environments, as seen in other endemic Asteraceae. Further research comparing different *Crepis* species under cultivation could provide deeper insights into the phenotypic plasticity within this genus (Andersson 1989).

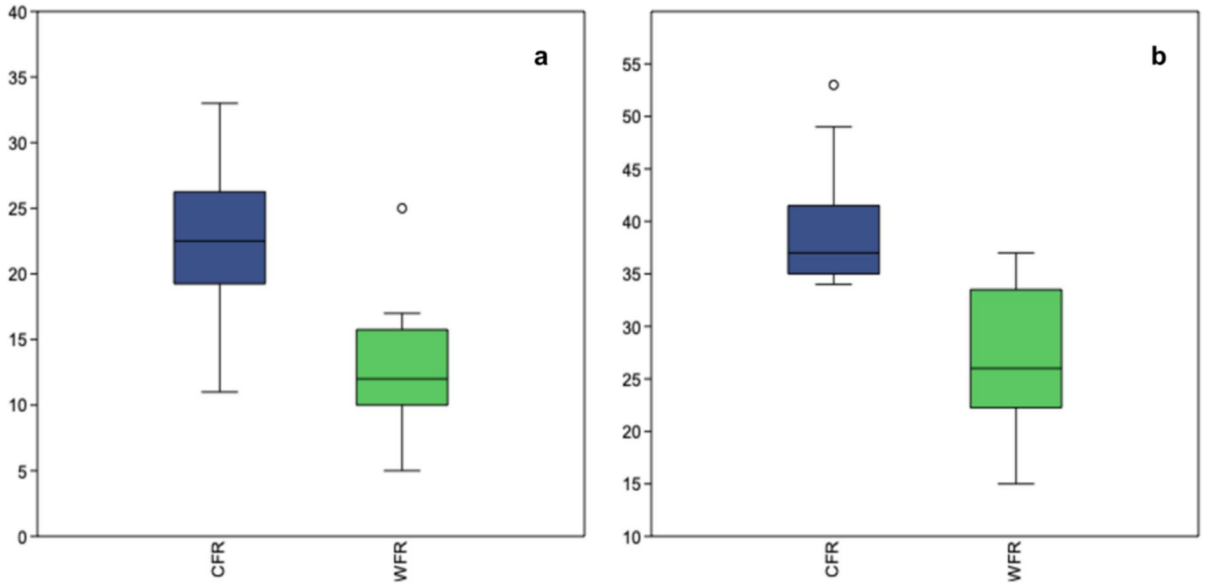

Fig. 3 **a, b** Box plot of the variables "number of leaves" (**a**) and "rosette diameters" (**b**) for the cultivated fresh (CFR) and wild fresh (WFR) groups of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) plants. Outliers were not excluded. Statistical comparison between groups was performed using the student's t-test, and significant differences were observed in both variables ($p < 0.05$). These graphs were performed using the software PAST version 4.15 (Hammer et al. 2001)

Colorimetric analysis

The colorimetric analysis of *C. aspromontana* leaves revealed significant variations in Lightness (L), red-green coordinate (a), and blue-yellow coordinate (b) across different growth conditions and heat stress treatments. As shown in Fig. 4, the top and bottom surfaces of leaves from each matrix (CFR, WFR, CFRs25, and CFRs29) exhibit notable chromatic differences.

These visual differences were quantitatively assessed through the mean chromatic values presented in Table 2, allowing for a comprehensive comparison of the colorimetric parameters (Lightness, red-green coordinate, and blue-yellow coordinate) across different growing conditions and heat stress treatments.

For the upper front of the leaves, the Lightness (L) values were similar between cultivated fresh (CFR) and wild fresh (WFR) leaves, at 44.5 ± 3.5 and 45.1 ± 2.8, respectively, indicating no significant differences. However, the cultivated plants subjected to high temperatures displayed distinct patterns: CFRs25 (cultivated fresh stressed at 25 °C) exhibited the highest lightness (60.9 ± 9.0), significantly differing from all other groups. Cultivated Fresh stressed at 29 °C (CFRs29) presented an intermediate lightness (51.0 ± 4.4), significantly different from both CFR and CFRs25. These results suggest that heat stress, especially at 25 °C, markedly influences the lightness of cultivated leaves, possibly due to changes in pigment synthesis or degradation induced by temperature variation. Similar effects of temperature on leaf chromatic characteristics have been observed in other Asteraceae species and medicinal herbs exposed to

Fig. 4 Colorimetric analysis on *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) to assess the colour differences of the different samples analysed: cultivated fresh (CFR), wild fresh (WFR), cultivated fresh stressed at 25 °C (CFRs25), cultivated fresh stressed at 29 °C (CFRs29)

Table 2 Mean chromatic values (L, a, b) of the upper and lower front of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) leaves under different growing conditions and heat stress

Upper front of leaves				Lower front of leaves			
Sample typology	L	a	B		L	a	b
CFR	44.5 ± 3.5^{c}	-9.5 ± 1.4^{d}	18.5 ± 4.5^{b}	CFR	50.2 ± 3.4^{c}	-8.2 ± 1.1^{c}	17.7 ± 3.7^{b}
WFR	45.1 ± 2.8^{c}	-5.3 ± 1.3^{a}	10.2 ± 3.1^{c}	WFR	49.1 ± 3.1^{c}	-5.9 ± 0.8^{a}	12.7 ± 1.9^{c}
CFRs25	60.9 ± 9.0^{a}	-7.2 ± 2.3^{b}	28.2 ± 5.8^{a}	CFRs25	64.1 ± 8.5^{a}	-5.9 ± 2.6^{a}	22.8 ± 6.5^{a}
CFRs29	51 ± 4.4^{b}	-8.2 ± 1.4^{c}	19.3 ± 5.0^{b}	CFRs29	56.8 ± 3.7^{b}	-7.1 ± 1.1^{b}	17.7 ± 3.1^{b}
Sign	**	**	**	Sign	**	**	**

CFR: Cultivated Fresh, WFR: Wild Fresh, CFRs25: Cultivated Fresh stressed at 25 °C, CFRs29: Cultivated Fresh stressed at 29 °C. L: brightness; a: red/green coordinate; b: yellow/blue coordinate. Data are reported as mean ± standard deviation (SD) ($n = 2$). **significance at $p \leq 0.01$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

stress, such as *Loropetalum chinense* (R.Br.) Oliv. and its variety *L. chinense* var. *rubrum* Yieh, where thermal stress influenced photosystem function and leaf coloration (Cai et al. 2023).

Regarding the red-green coordinate (a), CFR leaves had the most negative value (-9.5 ± 1.4), indicating a stronger tendency towards green, significantly different from the other groups. Wild fresh leaves (WFR) showed the least negative value (-5.3 ± 1.3), leaning more towards red, and were significantly different from the cultivated variant (CFR). Heat-stressed plants (CFRs25 and CFRs29) had intermediate values, yet remained significantly distinct from plants grown at optimal temperatures (CFR). This trend may reflect a stress-induced shift in leaf pigmentation, commonly observed in other Asteraceae under thermal stress. Research has shown that temperature stress can lead to a reduction in chlorophyll content and an increase in carotenoids as a photoprotective mechanism (Wang et al. 2016).

For the blue-yellow coordinate (b), both CFR and CFRs29 leaves displayed similar values (18.5 ± 4.5 and 19.3 ± 5.0, respectively), while WFR leaves exhibited the lowest value (10.2 ± 3.1), indicating a stronger blue tendency. On the other hand, CFRs25 leaves had the highest b value (28.2 ± 5.8), showing a marked shift towards yellow, with significant differences compared to CFR and CFRs29. This change could be related to increased carotenoid content under heat stress, a response aimed at protecting the photosynthetic apparatus from damage (Wang et al. 2016).

The lower front of the leaves displayed a similar pattern. Lightness (L) values for CFR and WFR were 50.2 ± 3.4 and 49.1 ± 3.1, respectively, without significant differences. However, CFRs25 again showed the highest lightness (64.1 ± 8.5), while CFRs29 had an intermediate value (56.8 ± 3.7), significantly different from CFRs25 and CFR. These data indicate that heat stress significantly increases leaf brightness, particularly at 25 °C.

Regarding the red-green coordinate (a), CFR had the most negative value (-8.2 ± 1.1), significantly different from the others. CFRs29 presented intermediate values (-7.1 ± 1.1), significantly different from both CFR and CFRs25. WFR leaves showed a less negative value (-5.9 ± 0.8), indicating a trend towards red, which is typical for leaves grown in natural, non-stress conditions.

For the blue-yellow coordinate (b), WFR leaves had the lowest value (12.7 ± 1.9), indicating a stronger blue tendency, while CFRs25 showed the highest value (22.8 ± 6.5), both significantly different from the other groups. The increased yellow component in stressed leaves might indicate higher carotenoid synthesis, as previously observed in other Asteraceae under environmental stress (Wang et al. 2016).

These results align with previous research on Asteraceae that demonstrated how heat stress can alter pigment composition, particularly affecting chlorophyll degradation and carotenoid synthesis. The observed stress-induced variation in leaf chromaticity supports the hypothesis that environmental factors significantly shape phenotypic traits in endemic Mediterranean species (Wang et al. 2016; Cai et al. 2023). Future research should focus on correlating pigment composition changes with functional traits,

providing deeper insights into the adaptive strategies of *C. aspromontana* under fluctuating environmental conditions.

Chemical parameters: % ash, pH, titratable acidity (TA), total soluble solids (TSS), relative humidity and water activity

The results of the chemical parameter analysis of *C. aspromontana* leaves collected under different conditions are presented. Fresh cultivated (CFR) and wild (WFR) samples, as well at fresh cultivated samples subjected to heat stress at 25 °C (CFRs25) and 29 °C (CFRs29), were analysed. The evaluated parameters include Ash content, pH, Relative Humidity (RH), Titratable Acidity (TA), Total Soluble Solids (TSS), and Water Activity (a_w). These measurements provide a comprehensive overview of the chemical characteristics of the leaves, allowing for a comparison of the impact of different cultivation and treatment conditions (Table 3).

From Table 3, it is evident that the chemical characteristics of *C. aspromontana* leaves are influenced by cultivation conditions and heat stress. The chemical properties of *C. aspromontana* leaves varied significantly across different cultivation conditions and heat stress treatments. Ash content was notably higher in wild fresh (WFR) samples (2.86 ± 0.09) compared to cultivated fresh (CFR) samples (1.78 ± 0.08), suggesting a greater mineral content in wild plants, likely due to the less controlled, natural soil conditions in which they grow (Turan et al. 2003). The increase in Ash content observed in heat-stressed samples (CFRs25 and CFRs29) compared to CFR could indicate a concentration of minerals resulting from moisture loss (Cakmak et al. 2008), aligning with typical Ash content ranges found in commercial vegetables (0.4–2.0%) (Roe et al. 2013.

The pH of the samples also differed, with CFR samples having a slightly higher pH (6.1 ± 0.07) than WFR samples (5.92 ± 0.01), reflecting potential differences in acid–base metabolism between cultivated and wild plants. Interestingly, heat-stressed samples, particularly CFRs29 (6.35 ± 0.01) and CFRs25 (6.27 ± 0.04), exhibited even higher pH values, indicating that heat stress may alter the plants' acid–base balance (Hewitt et al. 2017).

Relative Humidity (RH) was higher in CFR samples (90.89 ± 0.08) than in WFR samples (85.49 ± 0.00), possibly due to intrinsic water content differences between cultivated and wild plants. However, in heat-stressed samples (CFRs25 and CFRs29), Relative Humidity decreased, which is consistent with moisture loss at elevated temperatures (Wahid et al. 2007).

Titratable Acidity (TA) was higher in WFR samples (0.21 ± 0.00) compared to CFR (0.14 ± 0.00), suggesting a difference in organic acid content between wild and cultivated plants. This parameter increased significantly in heat-stressed samples (CFRs25 and CFRs29), indicating that heat stress could lead to alterations in the organic acid composition (Hewitt et al. 2017).

Total Soluble Solids (TSS) showed no significant differences between CFR and WFR samples or among the stressed samples (CFRs25 and CFRs29),

Table 3 Analysis of chemical parameters of different leaf samples of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae)

Samples typology	Ash (%)	pH	Relative humidity (RH) (%)	Titratable acidity (TA) (% of citric acid)	Total soluble solids (TSS) (%)	Water activity (a_W) (no unit)
CFR	1.78 ± 0.08^{c}	6.1 ± 0.07^{b}	90.89 ± 0.08^{a}	0.14 ± 0.00^{c}	0.40 ± 0.00^{a}	0.97 ± 0.00^{a}
WFR	2.86 ± 0.09^{a}	5.92 ± 0.01^{c}	$85.49 \pm 0.00^{b,c}$	$0.21 \pm 0.00^{b,c}$	0.50 ± 0.00^{a}	0.97 ± 0.00^{a}
CFRs25	2.36 ± 0.01^{b}	$6.27 \pm 0.04^{a,b}$	83.31 ± 1.84^{c}	0.40 ± 0.03^{a}	0.40 ± 0.00^{a}	0.94 ± 0.00^{b}
CFRs29	2.50 ± 0.01^{b}	6.35 ± 0.01^{a}	$87.94 \pm 1.28^{a,b}$	0.29 ± 0.03^{b}	0.70 ± 0.00^{a}	0.94 ± 0.00^{b}
sign	**	**	*	**	n.s	**

CFR: Cultivated Fresh, WFR: Wild Fresh, CFRs25: Cultivated Fresh stressed at 25 °C, CFRs29: Cultivated Fresh stressed at 29 °C. Values for Ash, Relative Humidity, Total Soluble Solids are given in percent (%). Water activity (a_w) is a dimensionless parameter (range 0–1). Titratable acidity is expressed as a percentage of citric acid. Mean values are presented with the standard deviation (±) ($n = 2$). **significance at $p \leq 0.01$, *significance at $p < 0.05$, n.s. (not significant) $p > 0.05$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

suggesting that soluble solids remained stable across various conditions. Water Activity (a_W) was similar between CFR and WFR samples (0.97 ± 0.00), implying that the availability of water for biochemical reactions was consistent. In contrast, heat-stressed samples (CFRs25 and CFRs29) showed slightly lower Water Activity (0.94 ± 0.00), which may suggest reduced water availability under stress conditions (Hewitt et al. 2017).

Chlorophylls and carotenoids content

The results in Tables 4 and 5 show significant differences in chlorophyll *a*, chlorophyll *b* and *β*-carotene contents between fresh and frozen matrices, and between cultivated and wild samples of *Crepis aspromontana*.

These measurements provide a comprehensive overview of the variations in the content of photosynthetic pigments, allowing for a comparison of the impact of different cultivation, storage, and heat treatment conditions.

Table 4 shows the contents of chlorophyll *a*, chlorophyll *b* and β-carotene in *Crepis aspromontana* leaves, analysed in both fresh (CFR, WFR) and frozen (CFZ, WFZ) samples.

The results indicate that there are no statistically significant differences between the fresh and frozen samples about chlorophyll *a* and chlorophyll *b* content ($p > 0.05$). In particular, the chlorophyll *a* content is slightly higher in the fresh wild samples (WFR: 1.85 ± 0.56 mg/100 mL) than in the fresh

Table 4 Contents of chlorophyll *a,* chlorophyll *b* and β-carotene in *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) leaves analysed for fresh matrixes (CFR, WFR) and frozen (CFZ, WFZ)

Sample typology	Chlorophyll *a* (mg/100 mL)	Chlorophyll *b* (mg/100 mL)	β-Carotene (mg/100 mL)
CFR	1.20 ± 0.07	3.54 ± 0.36	Not detection
WFR	1.85 ± 0.56	2.61 ± 0.09	Not detection
CFZ	1.80 ± 0.10	3.16 ± 0.06	Not detection
WFZ	1.52 ± 0.21	3.33 ± 0.40	Not detection
sign	n.s	n.s	n.s

CFR: Cultivated Fresh, CFZ: Cultivated Frozen, WFR: Wild Fresh, WFZ: Wild Frozen. All the values are expressed in mg/100 mL. Mean values are presented with the standard deviation (±) ($n = 2$). n.s. (not significant) $p > 0.05$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

Table 5 Contents of chlorophyll *a,* chlorophyll *b* and *β*-carotene in *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) leaves analysed for fresh and frozen cultivated matrixes (CFR, CFZ) and fresh and frozen matrixes stressed at 25 and 20 °C (CFRs25, CFZs25, CFRs29, CFZs29)

Sample typology	Chlorophyll *a* (mg/100 mL)	Chlorophyll *b* (mg/100 mL)	*β*-Carotene (mg/100 mL)
CFR	1.20 ± 0.073^{b}	3.54 ± 0.357^{a}	Not detection
CFZ	1.80 ± 0.102^{a}	$3.16 \pm 0.064^{a,b}$	Not detection
CFRs25	1.10 ± 0.009^{b}	0.43 ± 0.057^{e}	0.27 ± 0.035^{a}
CFZs25	2.04 ± 0.247^{a}	2.19 ± 0.208^{c}	Not detection
CFRs29	1.78 ± 0.215^{a}	$2.54 \pm 0.067^{b,c}$	Not detection
CFZs29	2.11 ± 0.017^{a}	1.47 ± 0.038^{d}	Not detection
sign	**	**	**

CFR: Cultivated Fresh, CFZ Cultivated Frozen, Cultivated Fresh stressed at 25 °C, CFZs25: Cultivated Frozen stressed at 25 °C, CFs29: Cultivated Fresh stressed at 29 °C, CFZs29: Cultivated Frozen stressed at 29 °C. All the values are expressed in mg/100 mL. Mean values are presented with the standard deviation (±) ($n = 2$). **significance at $p \leq 0.01$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

cultivated samples (CFR: 1.20 ± 0.07 mg/100 mL), whereas chlorophyll *b* is higher in the fresh cultivated samples (CFR: 3.54 ± 0.36 mg/100 mL) than in the wild (WFR: 2.61 ± 0.09 mg/100 mL).

These results suggest that cultivation might favour chlorophyll *b* synthesis. This aligns with previous research, such as Mitić et al. (2013), who observed that chlorophyll content in various green leafy vegetables was more abundant than carotenoids, and that differences between species exist depending on cultivation and environmental conditions.

Preservation by freezing appears to preserve photosynthetic pigments, as no significant variations in chlorophyll contents were observed between fresh and frozen samples (CFZ: 1.80 ± 0.10 mg/100 mL for chlorophyll *a*; 3.16 ± 0.06 mg/100 mL for chlorophyll *b*). Similar results have been observed in other research on the effects of freezing on chlorophyll stability in leafy vegetables. Mazzeo et al. (2015) highlighted that freezing can effectively preserve chlorophyll levels, particularly when done shortly after harvest. Additionally, Choi et al. (2024) found that humic substances in soil improved drought and heat stress tolerance in spinach, indirectly supporting the idea that freezing does not significantly alter photosynthetic pigments in certain plant species. Moreover, freezing after harvest has been shown to retain chlorophyll levels in several green leafy vegetables (Yamauchi 2014).

In addition, β-carotene was not detected in any samples, indicating that this carotenoid may be absent or present in undetectable amounts in fresh and frozen leaves of *C. aspromontana*.

Table 5 shows the contents of chlorophyll *a*, chlorophyll *b* and β-carotene in fresh (CFR), frozen (CFZ) and heat-stressed samples at 25 °C and 29 °C, both fresh and frozen (CFRs25, CFZs25, CFRs29, CFZs29).

In this case, the differences between the samples were significant ($p \leq 0.01$) for all the parameters considered. Chlorophyll *a* showed a maximum content in frozen samples stressed at 29 °C (CFZs29: 2.11 ± 0.017 mg/100 mL), while the minimum value was observed in fresh samples stressed at 25 °C (CFRs25: 1.10 ± 0.009 mg/100 mL). These data indicate that heat stress at 25 °C can cause a significant reduction in chlorophyll *a*, as observed by Yamane et al. (1998), who showed a decrease in photosynthetic pigments in response to high temperatures in *Spinacia oleracea* L.

Chlorophyll *b* showed a drastic reduction in CFRs25 samples (0.43 ± 0.057 mg/100 mL), suggesting that heat stress at 25 °C may impair this pigment more than chlorophyll *a*. In contrast, frozen samples stressed at 25 °C (CFZs25: 2.19 ± 0.208 mg/100 mL) maintain a relatively high chlorophyll *b* content, confirming the hypothesis that freezing may offer some protection against thermal degradation of pigments, as reported by Chen et al. (2024) in their study on the effect of temperature on pigment degradation during the freeze–thaw process of celery leaves.

The exclusive detection of *β*-carotene in fresh samples stressed at 25 °C (CFRs25: 0.27 ± 0.035 mg/100 mL) is a notable finding (Table 5). This is not simply a general defense mechanism, but a specific indicator of stress signalling and enzymatic regulation in response to heat. *β*-carotene is a critical non-enzymatic antioxidant, playing a dual role in photoprotection: it participates in the dissipation of excess light energy within the photosystems and directly quenches reactive oxygen species (ROS) to protect membranes from oxidative damage. Its appearance de novo in the CFRs25 sample (and not in the more severe CFRs29) suggests a specific thermal threshold for the enzymatic regulation of its biosynthesis pathway (e.g. the MEP pathway). This aligns with research showing that moderate heat stress can specifically upregulate carotenoid biosynthesis as an adaptive response (Saini et al. 2014; Kohzuma and Miyamoto 2024). These data collectively suggest that heat stress has a more pronounced impact on chlorophyll *b* than chlorophyll *a*, and that freezing, especially at higher temperatures, can preserve chlorophyll *a* levels. The appearance of β-carotene in stressed samples may indicate an activation of biosynthetic pathways to counteract oxidative stress.

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (ABTS.$^+$ and DPPH·)

In this section, the results of the analysis of total polyphenol content (TPC), flavonoid content (TFC), and antioxidant activities measured by $ABTS^+$ and DPPH· assays in *C. aspromontana* leaves collected under different conditions are presented. Fresh cultivated (CFR) and wild (WFR) samples, as well as frozen cultivated and wild samples (CFZ and WFZ),

were analysed. Additionally, fresh cultivated samples subjected to heat stress at 25 °C (CFRs25) and 29 °C (CFRs29), and frozen cultivated samples subjected to heat stress at 25 °C (CFZs25) and 29 °C (CFZs29), were included. These analyses provide a detailed overview of the variations in phenolic compound content and antioxidant capacities, allowing for a comparison of the impact of different cultivation, storage, and heat treatment methods (Table 5).

The results in Table 6 show a clear difference in total polyphenol (TPC) and flavonoid (TFC) contents between fresh and frozen samples of *Crepis aspromontana*, both wild and cultivated. In particular, fresh samples of wild *C. aspromontana* (WFR) presented significantly higher polyphenol (100.68 ± 9.35 mg GAE/100 g) and flavonoid (413.12 ± 24.70 mg QE/100 g) contents than the fresh cultivated samples (CFR), which presented values of 79.26 ± 5.14 mg GAE/100 g for polyphenols and 298.49 ± 24.74 mg QE/100 g for flavonoids. The WFR samples also showed a higher antioxidant capacity, as measured by the $ABTS^+$ (27.25 ± 0.64%) and DPPH· (19.51 ± 2.15%) assays, than the CFR ($ABTS^+$ 21.43 ± 2.29%; DPPH· 11.01 ± 0.88%). This supports the hypothesis that wild plants, exposed to more stressful environmental conditions, may accumulate more bioactive compounds, such as polyphenols, which play a key role in the defence against free radicals (Kumar and Pandey 2013; Agati et al. 2017; Disciglio et al. 2017). This is confirmed by numerous investigations where secondary metabolites are produced following mbiental stresses such as water, nutrient and temperature stress (Zlatev and Lidon 2012; Lipiec et al. 2013).

These findings are in line with research on other *Crepis* species that demonstrated high polyphenolic content and antioxidant potential. For instance, *Crepis crocea* has shown relevant antidiabetic and antioxidant activities due to its acidic-type polysaccharides and phenolic components (Li et al. 2021). Similarly, sesquiterpene lactones with biological activities have been isolated from *C. cameroonica* (Ndom et al. 2006). These examples support the idea that the genus *Crepis* holds promising nutraceutical potential, and that *C. aspromontana* may represent a valuable endemic resource for future exploitation.

In frozen samples (WFZ, CFZ), a decrease in phenolic compounds is observed compared to fresh samples. In particular, wild frozen samples (WFZ) show TPC values of 84.55 ± 1.87 mg GAE/100 g and TFC values of 140.64 ± 4.55 mg QE/100 g, lower than the respective fresh samples. This phenomenon is consistent with previous research indicating some loss of antioxidant activity during freezing Zlatev and Lidon (2012) although phenolic compounds may be partially preserved (Arfaoui 2021). The reduction in wild frozen samples could result from a lowering of flavonoid and polyphenol concentrations during freezing (Lipiec et al. 2013).

The data in Table 7 clearly indicate a profound biochemical response to abiotic stress. The dramatic increase in Total Phenolic Content (TPC) under heat stress is a classic example of plant biochemical adaptation. This is particularly evident in the CFRs29 (438.72 mg GAE/100 g) and CFRs25 (319.59 mg

Table 6 Mean contents of Total Polyphenols (TPC) (mg GAE/100 g), Total Flavonoids (TFC) (mg QE/100 g) and antioxidant activities ($ABTS^+$ and DPPH· radicals) in different samples of fresh and frozen, cultivated and wild *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) leaves and heat stressed at 25 °C and 29 °C

Sample typology	Total Phenolic content (TPC) (mg GAE/100 fw)	Total Flavonoid content (TFC) (mg QE/100 g fw)	$ABTS^+$ (% inhibition)	DPPH· (% inhibition)
CFR	79.26 ± 5.14	298.49 ± 24.74^{b}	$21.43 \pm 2.29^{a,b}$	11.01 ± 0.88^{b}
WFR	100.68 ± 9.35	413.12 ± 24.70^{a}	27.25 ± 0.64^{a}	19.51 ± 2.15^{a}
CFZ	95.31 ± 8.60	$357.34 \pm 25.75^{a,b}$	$22.82 \pm 1.75^{a,b}$	17.67 ± 0.05^{a}
WFZ	84.55 ± 1.87	140.64 ± 4.55^{c}	18.63 ± 2.49^{c}	$14.73 \pm 1.89^{a,b}$
sign	n.s	**	*	*

CFR: Cultivated Fresh, CFZ: Cultivated Frozen, WFR: Wild Fresh, WFZ: Wild Frozen. TPC values are expressed as mg GAE/100 g of fresh weight, TFC values are expressed as mg QE/100 g of fresh weight; $ABTS^+$ and DPPH· values are expressed as % inhibition. Mean values are presented with the standard deviation (±) ($n = 2$). **significance at $p \leq 0.01$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

Table 7 Mean contents of Total Polyphenols (TPC) (mg GAE/100 g), Total Flavonoids (TFC) (mg QE/100 g) and antioxidant activities ($ABTS^+$ and DPPH· radicals) in different samples of fresh and frozen, cultivated and wild *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) leaves and heat stressed at 25 °C and 29 °C

Sample typology	Total phenolic content (TPC) (mg GAE/100 fw)	Total flavonoid content (TFC) (mg QE/100 g fw)	$ABTS^+$ (% inhibition)	DPPH· (% inhibition)
CFR	79.26 ± 5.139^{d}	$298.49 \pm 24.742^{c,d}$	21.43 ± 2.287^{d}	11.01 ± 0.878^{d}
CFZ	95.31 ± 8.597^{d}	357.34 ± 8.597^{c}	$22.82. \pm 1.750^{d}$	17.67 ± 0.047^{c}
CFRs25	319.59 ± 3.570^{b}	588.04 ± 16.483^{a}	68.88 ± 0.033^{a}	71.25 ± 0.572^{a}
CFZs25	132.92 ± 0.515^{c}	$310.41 \pm 1.888^{c,d}$	33.26 ± 1.430^{c}	30.00 ± 1.080^{b}
CFRs29	438.72 ± 3.059^{a}	436.75 ± 7.311^{b}	38.89 ± 0.563^{b}	33.18 ± 0.362^{b}
CFZs29	46.92 ± 0.216^{e}	248.79 ± 4.299^{d}	7.76 ± 0.200^{e}	12.14 ± 1.950^{d}
sign	**	**	**	**

CFR: Cultivated Fresh, CFZ: Cultivated Frozen, CFRs25:Cultivated Fresh stressed at 25 °C, CFZs25: Cultivated Frozen stressed at 25 °C, CFRs29:Cultivated Fresh stressed at 29 °C, CFZs29: Cultivated Frozen stressed at 29 °C. TPC values are expressed as mg GAE/100 g of fresh weight, TFC values are expressed as mg QE/100 g of fresh weight; $ABTS^+$ and DPPH· values are expressed as % inhibition. Mean values are presented with the standard deviation (±) ($n = 2$). **significance at $p \leq 0.01$. The significance level (sign.) is derived from the ANOVA test, indicating the overall statistical significance among the groups. Pairwise comparisons between groups were conducted using Tukey's multiple interval test, and differences are indicated by different letters

GAE/100 g) samples, which showed TPC values 5.5-fold and fourfold higher, respectively, than the non-stressed cultivated control (CFR). This physiological response is likely due to the heat-induced production of reactive oxygen species (ROS), which is known to trigger the oxidative induction of the phenylpropanoid pathway. This pathway is the primary route for the synthesis of most phenolic compounds, which act as powerful antioxidants to mitigate oxidative damage. Our findings strongly support this interpretation and are consistent with previous research on stress-induced phenolic accumulation (Rivero et al. 2001; Kumar and Pandey 2013; Zagoskina et al. 2023). Frozen cultivated samples (CFZ) showed an increase in TFC (357.34 ± 8.597) compared to fresh cultivated leaves (CFR), probably due to damage to cell membranes caused by freezing, which releases previously inaccessible cell contents (Castenmillet et al. 1999).

Furthermore, heat-treated and subsequently frozen samples (CFZs25, CFZs29) retain similar levels of antioxidant activity compared to fresh treated samples, indicating that heat treatment may mitigate the reduction of bioactive compounds caused by freezing (Disciglio et al. 2017). This result could suggest that heat treatment after freezing allows partial regeneration or stabilisation of antioxidant compounds (Levitt 1980; Zagoskina et al. 2023).

In the case of CFZ, for example, there is a significant increase in flavonoids compared to the fresh variant (CFR). This increase could be due to the freezing process, which might induce cell rupture through ice crystal formation, facilitating the release of flavonoid compounds. Furthermore, freezing may stimulate the biosynthesis of these secondary metabolites as part of a protective response to the oxidative stress caused by the freezing process (Arfaoui 2021; Zagoskina et al. 2023).

Conclusion

This study provides a comprehensive analysis of the morphometric and phytochemical characteristics of *Crepis aspromontana*, an endemic species from Calabria with significant ethnobotanical and nutritional potential. The results demonstrated that cultivation practices positively influenced plant vigour, resulting in a greater number of leaves and larger rosettes compared to wild specimens. These traits could increase the commercial appeal of the species, suggesting that domestication may enhance its agronomic value.

Conversely, wild plants exhibited superior phytochemical profiles, with significantly higher concentrations of polyphenols, flavonoids, and antioxidant activity. These findings highlight the rich bioactive composition of wild specimens, reinforcing their potential health benefits and the importance of preserving natural populations Heat stress experiments revealed that cultivated plants subjected to high

temperatures displayed an increase in the synthesis of bioactive compounds, particularly polyphenols and flavonoids, which corresponded to enhanced antioxidant activity. However, heat stress negatively affected photosynthetic efficiency, as evidenced by a reduction in chlorophyll *b* content. In contrast, β-carotene synthesis increased, potentially as a protective response to heat stress. These results align with previous research indicating that heat stress can both challenge photosynthetic function and induce adaptive changes in carotenoid content (Wang et al. 2016; Cai et al. 2023).

Furthermore, the study revealed significant differences in chemical traits between wild and cultivated plants, such as ash content, pH, and titratable acidity. Cultivated specimens generally exhibited lower ash content and pH values compared to their wild counterparts, suggesting that environmental factors and cultivation practices substantially influence the overall quality of *C. aspromontana*.

These findings underscore the importance of integrating domestication strategies to optimize the potential of *C. aspromontana* as a nutrient-rich food source while ensuring the preservation of its biodiversity. The observed differences between wild and cultivated plants also indicate that cultivation may alter not only morphological traits but also the phytochemical composition. Further phytochemical investigations will be necessary to confirm the potential use of *C. aspromontana* in the nutraceutical field and to define its possible application in sustainable local supply chains. Future research should focus on improving the species' adaptability to various environmental stresses to enhance its sustainability and its role in addressing challenges related to food security and climate change resilience.

Author contributions Conceptualization: Miriam Patti, Angelo Maria Giuffrè, Carmelo Maria Musarella, Giovanni Spampinato; Methodology: Miriam Patti, Angelo Maria Giuffrè; Formal analysis and investigation: Miriam Patti, Angelo Maria Giuffrè, Carmelo Maria Musarella, Giovanni Spampinato; Software: Miriam Patti, Angelo Maria Giuffrè; Writing—original draft: Miriam Patti; Writing—review and editing: Miriam Patti, Angelo Maria Giuffrè, Carmelo Maria Musarella, Giovanni Spampinato; Resources: Giovanni Spampinato; Supervision: Giovanni Spampinato; Funding acquisition: Giovanni Spampinato. All authors have read and agreed to the published version of the manuscript.

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Data availability Data will be made available on request to miriam.patti@unirc.it.

Code availability Not applicable.

Declarations

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

Ethics approval and consent to participate We declare that there are no guidelines and/or regional laws prohibiting the harvesting of *Crepis aspromontana* Brullo, Scelsi & Spamp. (Asteraceae) used for the present research. It is included in the Italian Red List and classified according with the IUCN's criteria as Least Concern (LC), indicating that this species is not currently at risk of extinction. Additionally, we confirm that the germplasm collection was conducted without uprooting the plants and from a large population. Lastly, we assert that this activity can be considered as an *ex-situ* conservation tool, having tested the species' domestication for conservation purposes.

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