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Effect of Drying Post-Harvest on the Nutritional Compounds of Edible Flowers

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Abstract: The post-harvest techniques are the most critical point to ensure the quality of edible flowers (EFs) and to keep the bioactive metabolites available for human nutrition. The different species of EFs also represent a problem in improving their consumption with safety. The present study focused on the description of the effects of the commonly used drying treatments in the phytonutritional composition of four species of EFs, *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans*. The bioactive metabolites and antioxidant activity were determined after freeze-drying (FD) and hot-air-drying (HA) treatments in comparison to fresh flowers. All EFs showed different mineral/trace compositions with potassium as the main element and 70–86% water loss. Both post-harvest treatments increased all the metabolites and antioxidant activity in each species. *C. striata* with FD treatment had the highest content of primary and secondary metabolites. *N. strumosa* has the highest ascorbic acid content with the HA treatment. All species had significant antioxidant activity, increasing with FD for *C. striata* while HA is more recommended for the other species. The post-harvest techniques are able to preserve and increase the bioactive metabolites and must be chosen according to each EF species.

Keywords: *Abutilon*; antioxidant activity; *Callianthe*; *Nemesia*; polyphenols; *Salvia*

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1. Introduction

Complete edible flowers (EFs) or their parts, commonly the petals and sepals, have been used since ancient times around the world to add color and flavor to various food preparations. They can be enjoyed fresh, either on their own or incorporated into salads and desserts [1]. In addition to their visual appeal, EFs boast a rich phytonutritional composition that includes minerals, vitamins, proteins, carbohydrates, polyphenols, pigments and a low fat concentration. All of these components contribute to a natural and healthy nutritional profile [2,3]. The different species of EFs have different phytochemical composition, for example, the pansy has greater quantities of water, proteins and dietary fiber in its tissues than the snapdragon [4].

The global market of EFs is promising and attractive with a great potential to increase in terms of horticulture farms, traditional producers and the food industry, as well as to enhance the visual aspects, nutritional aspects and quality of human food consumption [2,3]. All over the world, the EFs that can be purchased in the supermarket are those deriving from annual or perennial herbaceous plants that can easily be grown in pots; however,

there are climbers, shrubs and trees that produce EF, but their cultivation requires larger spaces than EFs produced by herbaceous plants [5]. In fact, the most consumed flowers in Asia are peonies, hibiscus and *Clitoria ternatea*; while in Mexico, the flowers of *Agave salmiana*, *Arbutus xalapensis*, *Myrtillocactus geometrizans*, *Erythrina* spp. and *Yucca filifera* are ingredients of traditional dishes [5]. In Europe, like in Italy, the EFs mainly consumed are violets, begonias, marigolds, nasturtiums, pansies and roses. Furthermore, flowers and inflorescences like artichokes, broccoli, cauliflower and capers are part of the traditional cuisine of many countries; however, they are marketed as vegetables. Actually, it is estimated there are only 180 species of EFs that have been studied and this number is very low [2]. To enhance this market and EF consumption, there are several challenges to overcome; one of them is to increase the number of species used and make their chemical composition very clear to ensure safety and promote diversity in this market [1,6]. Some ornamental plants that produce edible flowers are still little studied and commercialized for food purposes; among these are *Callianthe megapotamica*, *C. striata*, *Nemesia strumosa* and *Salvia elegans*.

Callianthe megapotamica (A. Spreng) Dorr, syn. *Abutilon megapotamicum* (Spreng.) A.St.-Hil. & Naudin (Malvaceae), popularly known as "princess earring", is a woody shrub that occurs in South America such as in Brazil and Colombia [7–9]. Its flowers are solitary, pendulous, lantern-shaped, colorful with a red-to-purple calyx and a yellow corolla with veins not impressed. It produces nectar during all its anthesis and is appreciated in natura due to its sweet flavor [10].

Callianthe striata (Dicks. Ex Lindl.) Donnel ex. *Abutilon striatum* (Malvaceae) is a woody shrub known as "chinese lantern", native to the Brazilian Atlantic Forest. Its flowers are solitary, pendulous, bell-shaped, a chlorophyllous calyx and an orange-to-red corolla with veins very impressed [11–13]. As well as *C. megapotamica*, this species is used as an ornamental plant and the leaves and flowers are consumed in natura, salads or jellies [14,15].

Nemesia strumosa Solander ex. Benth (Plantaginaceae) is a cultivated herbaceous plant from South Africa that has asymmetrical flowers with different colors such as orange, yellow, white, red and others and it is commonly used as an ornamental plant in small gardens [16]. Actually, to the best of our knowledge, there is no information about its chemical composition.

Salvia elegans Vahl (Lamiaceae) is a shrub native to Mexico known as "pineapple sage" due to the particular aroma present in their flowers that is similar to pineapple's aroma [17]. This species is used in folk medicine as an anxiolytic and antidepressant [18,19]. To date, there are only a few studies on the phytonutritional composition of the EFs of *S. elegans* with primary and secondary metabolites as well as descriptions of bioactivity [17,20–22]. Moreover, there are no data about the effects of post-harvest treatments on their metabolites.

These EF species have the potential to be widely produced in horticulture and commercialized, but their chemical composition and their post-harvest techniques are scarce in the literature. The post-harvest techniques are essential to ensure the quality and nutraceutical properties of the EFs as well as to increase their commerce, storage and transport [6,23].

Therefore, the aim of this study was to investigate the chemical composition of *C. megapotamica*, *C. striata*, *N. strumosa* and *S. elegans*, as well as to determine the effects of different post-harvest techniques on their quality and bioactive metabolites' composition.

2. Materials and Methods

2.1. Plant Material and Post-Harvest Treatments

The plants of *Callianthe megapotamica* (A. Spreng.) Dorr (Figure 1a) and *C. striata* were purchased at Vivaio Noaro (Camporosso, Fort Mitchell, Italy); *Nemesia strumosa* Benth. (Figure 1c) was provided by R-Zero Group (Albenga, Italy) and *Salvia elegans* "Scarlet Pineapple" (Figure 1d) is part of the sage collection at CREA—Research Centre for Vegetable and Ornamental Crops (Sanremo, Italy; GPS: 43.816887, 7.758900). *C. megapotamica* and *C. striata* were grown in soil (pH 7.6 and electrical conductivity 0.45 dS/m) and they

were watered twice a week; *N. strumosa* and *S. elegans* were grown in pots (30 cm diameter, 9 l volume) filled with a commercial 7:3 peaty:pumice substrate (Hochmoor–Terflor, Capriolo, Italy) characterized by pH 6.1, electrical conductivity 0.38 dS/m, bulk density 120 kg/m^3 and total porosity 94% *v*/*v*, irrigated with a nutrient solution (Ferti 3, Planta-Düngemittel, Regenstauf, Germany) every week. Plants both in the greenhouse and in the open ground were cultivated using the organic system based on predatory insects, parasites and antagonists of plant pathogens [24] and. *C. megapotamica* and *C. striata* were neither fertilized nor treated with pesticides. Fresh flowers were harvested, weighed (FW), washed and kept in the refrigerator at −20 °C until the data collection; other fresh flowers were weighed (FW) and vacuum freeze-dried (FD) (Labconco, Kansas City, MO, USA) at −50 °C for 48 h (Figure 1e–h) or hot-air-dried (Tecnocalor 2000, Technovetro, Italy) at 60 °C for 72 h until constant weight (HA) (Figure 1i–l).

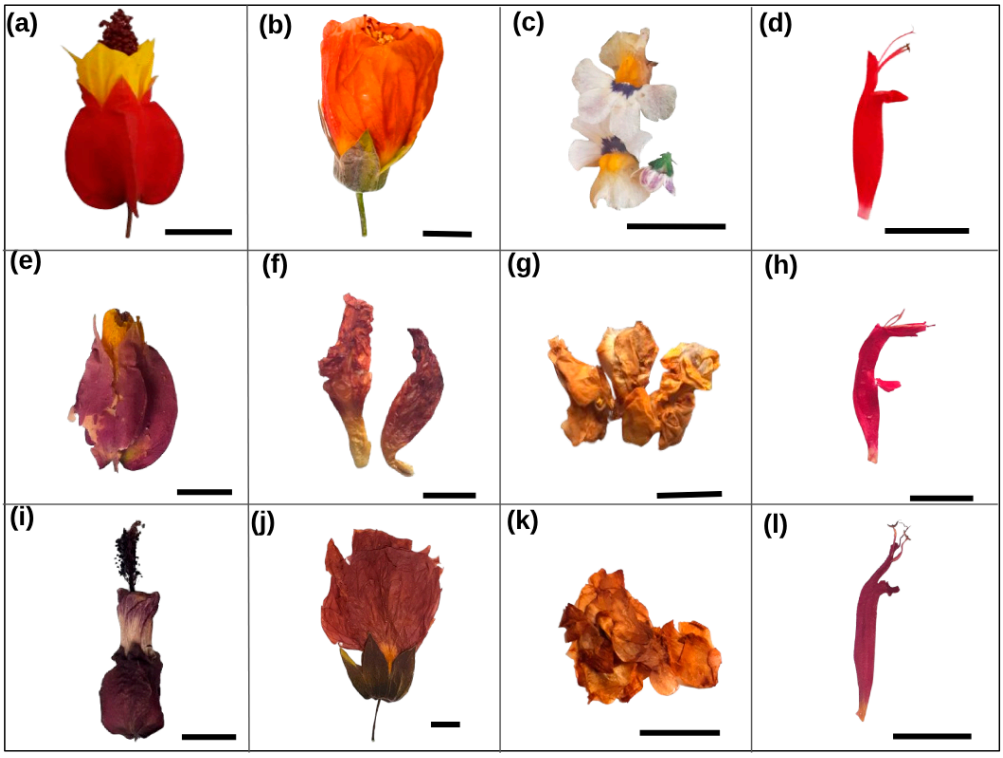


Figure 1. Edible flowers of *Callianthe megapotamica* (**a**,**e**,**i**), *Callianthe striata* (**b**,**f**,**j**); *Nemesia strumosa* (**c**,**g**,**k**) and *Salvia elegans* (**d**,**h**,**l**) in the post-harvest conditions: fresh (**a**–**d**), vacuum freeze-dried (**e**–**h**) and hot-air-dried (**i**–**l**). Bars 1 cm.

2.2. Water Loss Percentage, Crude Protein Percentage and Mineral/Trace Content

For the water loss percentage, the fresh flowers were weighed (FW) and hot-air-dried according to the description above. The water loss percentage was calculated by Equation (1):

$$(Fresh\ weight - Dry\ weight) \times 100\ /\ Fresh\ weight \quad (1)$$

Two hundred milligrams (mg) of dry flowers were powdered and mineralized with a solution of H_2SO_4 and H_2O_2 (15 min at 380 °C) and used to obtain the total organic nitrogen using the Kjeldahl method [25]. The nitrogen percentage results were also used for the calculation of the percentage of crude protein content, obtained by the multiplication of the nitrogen percentage to the coefficient factor 6.25 [26,27]. For the mineral composition, the dried samples were mineralized in a solution of HNO_3:$HClO_4$ (2.5:1; *v*:*v*) for 90 min at 220 °C. The minerals and tracers were analyzed by an atomic absorption spectrometer (Varian 240FS AA, Sydney, Australia). The results were expressed in g mineral per kg and mg trace element per g of DW [28].

2.3. Biochemical Analyses

Flowers (100 mg fresh, or 50 mg dried and freeze-dried) were homogenized with absolute methanol and centrifuged for 10 min at 14,000 rpm to extract the total carotenoid content. The supernatant was measured at 662, 652 and 470 nm and the values were calculated with the formula according to Lichtenthaler [29]. The results were expressed in µg per g.

Two hundred milligrams (mg) of the fresh flowers and 20 mg of the HA and FD flowers were used for the extraction of total polyphenols, flavonoids and anthocyanins, as well as for the radical scavenging activity assays. The flowers were homogenized with methanol 70% in an ice bath and centrifuged for 10 min at 14,000 rpm [30]; the supernatant was recovered and used for the analysis.

For the quantification of the total polyphenols content (TPC), the aliquots were submitted to the Folin–Ciocalteu method [31], measured at 765 nm and expressed as mg of gallic acid equivalent (GAE) per g. The total flavonoid content (TFC) was measured according to Kim et al. [32], measured at 510 nm and expressed in mg of (+)-catechin equivalent (CE) per g.

The anthocyanins were quantified by the differential pH method [33], and the aliquots were diluted in an aqueous buffer at pH 1 or 4.5 before the measurement of the absorbances at 510 and 700 nm. The monomeric anthocyanin content was calculated [34] and the results were expressed in mg of cyanidin-3-O-glucoside (C3G) per g.

The methanolic extracts were used to measure antioxidant activity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity [33] and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) antiradical activity, according to Re et al. [35]. Both assays were expressed in µM of Trolox equivalent antioxidant activity (TAE) per g. The samples were also tested with the Ferric ion Reducing Antioxidant Power (FRAP) assay to confirm the antioxidant activity [36] and were expressed in mM Fe^{2+} per g. The FRAP assay measures ferric-reducing antioxidant power and does not include the activity of thiol groups and proteins.

For the total ascorbic acid measurement, 200 mg of fresh flowers and 20 mg of dried treated flowers were used for the extraction with 6% trichloroacetic acid (TCA) [37]. The samples were submitted to the Kampfenkel method [38] with modifications by Najar et al. [24] for the reduced (AsA), oxidized (DAsA) and total (AsA+DAsA) ascorbate contents. The results were expressed in mg of AsA per 100 g.

An amount of 200 mg of fresh flowers and 20 mg of dried treated flowers were used for the extraction of reducing sugars with 80% ethanol [39]. The samples were submitted to the 3,5-dinitrosalicylic acid (DNS) method [40]. The absorbance was measured at 540 nm and the results were expressed in mg of glucose per g. All measurements were performed with an ultraviolet UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan) with three repetitions for each sample (n = 3).

2.4. Statistical Analysis

For the comparison between the species, as in water loss percentage, crude protein percentage and mineral/trace composition, the data were submitted to variance analysis (ANOVA) with an error probability lower than 0.05 ($p < 0.05$) and the averages were compared by Tukey's test. These analyses were made in the software Past4.03.

The investigated biochemical parameters were also subjected to analysis of variance (ANOVA) to evaluate the presence of statistically significant differences among the samples. Even for this analysis, averages were separated by Tukey's post hoc test, using a $p < 0.05$. Furthermore, the biochemical data were also submitted to multivariate statistical analyses with Principal Component Analysis and Hierarchical Cluster Analysis methods. The PCA was performed on a 9 × 12 correlation data matrix (9 variables × 12 samples = 90 data), selecting the two highest PCs obtained by the linear regressions: the chosen PC1 and PC2 covered 61.4% and 15.1%% of the variance, respectively, for a total variance of 76.5%. The HCA was performed using Ward's method on scaled data, with squared Euclidean

distances as a measure of similarity. The statistical analyses on biochemical data were performed using the JMP Pro 14.0.0 software package (SAS Institute, Cary, NC, USA).

3. Results and Discussion

3.1. Water Loss Percentage, Crude Protein Percentage and Mineral/Trace Content

The water loss percentage was 87% for *C. striata*, *N. strumosa* and *S. elegans*, while for *C. megapotamica*, it was 79% (Figure 2a). Water is the main constituent of EFs and its values vary between the species from 70 to 95% [2]; for example, in *Begonia cucullata*, the water loss varies from 92 to 96% [41], while in *Begonia boliviensis*, the moisture is 86% [28] and in *Rosa micrantha*, it is 72% [42]. The percentages obtained for the four EFs are in agreement with the literature.

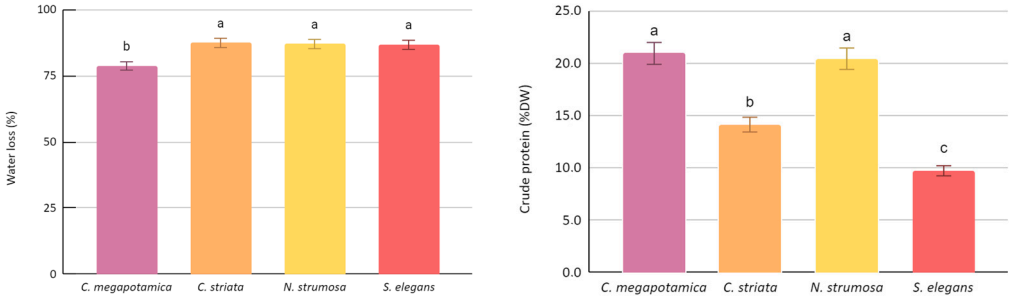


Figure 2. Percentages of water loss (**a**) and crude protein (**b**) in edible flowers of *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans*. Data with the same letter did not differ by Tukey's test with $p < 0.05$. Bars represent mean ± standard error (SE, n = 3).

The crude protein percentage (Figure 2b) was higher in *C. megapotamica* (21%) and *N. strumosa* (20.4%), followed by *C. striata* (14.1%) and *S. elegans* (9.7%). The protein content can greatly vary in the EFs [2]; for some Lamiaceae species, the values vary from 3.2 to 16.2% of DW [43]. Considering other *Salvia* species, the crude protein percentage in *S. elegans* is higher than *Salvia discolor* and *Salvia microphylla*, which have 3.2 and 6.3%, respectively [43]. In this same study, the highest protein content was 16.2% in *Ocimum basilicum* blue spice, with a similar content for *C. striata*. Despite belonging to the same genus, *C. megapotamica* and *C. striata* had a significant difference in the protein content with the highest protein found in *C. megapotamica*. *N. strumosa* and *C. megapotamica* represent the species with the highest protein content in this study and the values are similar to fertilized flowers of *R. micrantha* [42], *Antirrhinum majus*, *Impatiens walleriana* and *Tagetes patula* [27].

The four species analyzed showed similar mineral and trace element composition (Table 1). Calcium (Ca) was the principal mineral found in all species with values varying from 35 to 61 g/KgDW. Potassium (K) is present in all flowers with the highest concentration in *N. strumosa* at 36 g/KgDW, while the other species had 21 g/KgDW. Among the macroelements, sodium (Na) had a lower concentration in the species with values from 0.7 to 1.8 g/KgDW. For magnesium (Mg), *C. megapotamica*, *C. striata* and *N. strumosa* had similar contents varying from 2.4 to 3.3 g/KgDW; in this case, *S. elegans* (1.4 g/KgDW) had the lowest concentration.

Few studies of EFs have performed an analysis of their mineral/trace element composition and its importance to human nutrition [44,45]. To the best of our knowledge, this is the first description of mineral/trace composition for *C. megapotamica*, *C. striata* and *N. strumosa*. All species analyzed showed a particular composition with Ca as a major element followed by K and Mg. The composition and concentration of these elements greatly vary between the species and in the same species according to cultivation and variety [2]. The main minerals in EFs are the macronutrients K, phosphorus (P), Ca and Mg. For the EFs analyzed, Ca was the major element and it is among the four macroelements most found [2]; however, this is in contrast with some studies that described K as the main macronutrient

in EFs [27,44,46]. Ca is important for the human diet because it is a component of the bones and teeth and acts in enzyme cycles such as in promoting locomotion [27]. In humans, K is known as the principal positive ion in cells and nerve and muscle action for locomotion and protein synthesis that contribute to homeostasis [47]. A low concentration of Na was found for these EFs. This fact is interesting because a ratio where K is higher than Na is considered a benefit to cardiovascular disease protection and this proportion was observed in other EFs [2,27].

Table 1. Mineral and trace element composition of edible flowers: *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans*.

Mineral/Trace	*C. megapotamica*	*C. striata*	*N. strumosa*	*S. elegans*
Ca (g/Kg DW)	42.0 ± 28.0 a	61.0 ± 17.0 a	40.0 ± 28.0 a	35.0 ± 13.0 a
K (g/Kg DW)	20.0 ± 1.0 a	19.0 ± 1.0 a	36.0 ± 5.0 a	21.0 ± 1.0 a
Na (g/Kg DW)	0.7 ± 0.7 a	1.0 ± 0.2 a	1.8 ± 1.4 a	0.8 ± 0.4 a
Mg (g/Kg DW)	3.3 ± 0.3 a	2.4 ± 0.8 a	2.9 ± 0.2 a	1.4 ± 0.0 b
Cu (mg/Kg DW)	4.0 ± 4.0 a	5.2 ± 0.8 a	2.4 ± 2.4 a	1.5 ± 0.8 a
Fe (mg/Kg DW)	nd *	5.0 ± 5.0 a	13.0 ± 13.0 a	nd *
Mn (mg/Kg DW)	0.1 ± 0.1 a	13.0 ± 5.0 a	9.4 ± 9.0 a	6.2 ± 3.0 a
Zn (mg/Kg DW)	0.1 ± 0.1 a	6.0 ± 6.0 a	14.0 ± 14.0 a	4.0 ± 2.0 a

Different letters indicate statistically significant differences ($p < 0.05$) when comparing treatments (across the lines of the table). * nd: not detected, DW: dry weight. Data represented the mean ± standard error (n = 3).

For the trace element composition, all EFs showed a different composition, and copper (Cu) was present in all species with values varying from 1.5 to 5.2 mg/KgDW. Iron (Fe) was detected only in *C. striata* and *N. strumosa* (5 and 13 mg/KgDW, respectively). Manganese (Mn) was low in *C. megapotamica* (0.1 mg/KgDW), while it varied in the other EFs from 6.2 to 13 mg/KgDW. Zinc (Zn) varied from 0.1 mg/KgDW in *C. megapotamica* to 14 mg/KgDW in *N. strumosa*; however there were no significant differences among them.

The trace composition varied between the four EFs analyzed and the variation in the same species denied the identification of one main trace element in these species. Beyond the variation in the EFs, Fe was observed as the major trace element in *S. elegans* [21] with 2.6 mg/100 g. In contrast, in the present study, Fe was not detected in this species and its composition is diverse. The trace elements or micronutrients are important in several enzyme activities as cofactors and active sites, in the electron transport chain, in vitamins and in red blood cell formation [27], and EF consumption can contribute to adding these nutrients to the human diet [2].

3.2. Biochemical Analysis

The carotenoid content in the fresh flowers was high in *N. strumosa* with 147.4 µg/g, followed by *C. striata* with 133 µg/g, *C. megapotamica* with 89.3 µg/g and *S. elegans* with 55.3 µg/g (Figure 3a). The post-harvest treatments increased the carotenoid content in all species compared to fresh flowers, due to the water loss. In the freeze-drying (FD) treatment, the highest content was 587 µg/g in *C. striata*, followed by *N. strumosa* > *C. megapotamica* > *S. elegans* with 316, 149 and 129.6 µg/g, respectively. The hot-air-dried (HA) flowers had the highest carotenoid content of all treatments for *N. strumosa* (450.7 µg/g), *C. megapotamica* (292.2 µg/g) and *S. elegans* (158.6 µg/g). However, for *C. striata,* this treatment decreased the carotenoid to 343 µg/g.

The carotenoid content in EFs is responsible for the colors of petals: yellow, orange and, in some cases, red and it varies greatly between the species. The principal carotenoids in EFs are xanthophylls such as lutein and zeaxanthin and carotenes such as lycopene and β-carotene [48]. In the fresh flowers, the highest carotenoid content was in *N. strumosa* with yellow petals (Figure 1c) followed by *C. striata* with orange-red petals (Figure 1b). *S. elegans* had the lowest carotenoid content in all treatments; however, these concentrations were higher than in other studies in the same species with fresh flowers [20]. In other *Salvia* species, the variation in the carotenoids is due to the colors of the different petals, as

observed in dark-purple petals of *S. discolor* (61 µg/g) and white/red petals of *S. microphylla* (4.2 µg/g) [43]. No data about the effects of post-harvest treatments on the carotenoids were found for this species.

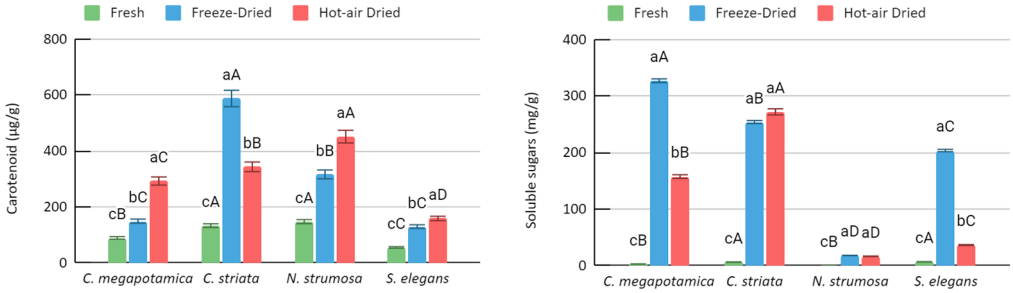


Figure 3. Carotenoid content (**a**) and total soluble sugars (**b**) in edible flowers *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans* in different post-harvest conditions: fresh, vacuum freeze-dried and hot-air-dried. Data with the same capital letters did not differ between species in the same post-harvest condition and lowercase letters between the same species with $p > 0.05\%$. Bars represent mean ± standard error (SE, n = 3).

Both post-harvest treatments increased the carotenoid content in the EFs. The highest content was in *C. striata* after the FD treatment. This treatment also increased the carotenoids in other species such as *Agastache aurantiaca* [49,50] and *Hemerocallis disticha* [50], being the recommended post-harvest treatment for these species. For the other species analyzed, the HA showed the highest carotenoid content. This treatment also increased the carotenoids in *Tagetes erecta* [51] and in tea flowers [52]. The high carotenoid content in the HA treatments may be due to their stable structures that are able to resist the elevated temperatures present in the HA [51]. Therefore, focused on the carotenoids, the HA is more recommended for *C. megapotamica*, *N. strumosa* and *S. elegans*, while FD is more adequate for *C. striata*.

The total soluble sugars content (Figure 3b) was very low in the fresh flowers of *S. elegans* (6.7 mg/g), *C. striata* (6.3 mg/g) and *C. megapotamica* (2.8 mg/g) and null in *N. strumosa*. These contents had increased with the FD treatment. The highest concentration, in this case, was in *C. megapotamica* (326.7 mg/g), followed by *C. striata* (253.8 mg/g), *S. elegans* (203.4 mg/g) and *N. strumosa* (17.9 mg/g). The HA treatment decreased the soluble sugar content to 157.4 mg/g in *C. megapotamica*, 36.4 mg/g in *S. elegans* and 16.5 mg/g in *N. strumosa*. For *C. striata*, this treatment increased the soluble sugars to 343 mg/g.

The soluble sugars are in low concentrations in the fresh flowers and increased with the post-harvest techniques. Carbohydrates are the major macromolecules in plants. Glucose and fructose are the reducing sugars most commonly described in the EFs; they are present in the nectar and give the sweet taste to the flowers [2,53]. Despite the increase in the post-harvest treatments, the soluble sugars in *N. strumosa* were very low compared to the other species and it is interesting because this species could be applied to reduced-sugar diets.

Both post-harvest treatments are able to increase the soluble sugars in EFs. However, the amount and its efficiency varied in each species. For *C. striata*, the HA was more efficient than FD and this result is similar to *A. aurantiaca* [49], where the HA at 50 and 60 °C increased the soluble sugars more than the fresh and FD treatments. Higher temperatures (70 and 80 °C) also increased the soluble sugars in *B. cucullata* [41]. The high temperature present in this treatment may be the principal reason for improving the sugar content due to the inactivation of several enzyme responses by sugar consumption and by the hydrolysis of some sugar molecules.

In contrast, for *S. elegans* and *C. megapotamica*, the highest soluble sugar content was found in the FD flowers and *C. megapotamica* also had a higher concentration of this metabolite in this condition. The FD is considered a more expensive and slow technique compared to other drying methods already used to preserve the EFs; however, this technique also im-

proves and maintains the quality and bioactive metabolites of EFs, and it is recommended for a lot of species [23,54].

The total polyphenols content (TPC) was low in the fresh flowers when compared to the post-harvest treated flowers (Figure 4a). In the fresh flowers, the content was similar between the species with 6.1, 6, 5.3 and 4.9 mgGAE/g for *N. strumosa*, *C. striata*, *S. elegans* and *C. megapotamica*, respectively. The FD treatment increased the polyphenols, with the highest content in *C. striata* (61.2 mgGAE/g) followed by *N. strumosa* < *S. elegans* < *C. megapotamica* (38.9, 26.7 and 15.5 mgGAE/g, respectively). The HA treatment decreased the polyphenols in *C. striata* and *N. strumosa* to 30.6 and 28.4 mgGAE/g, respectively, and increased them in *S. elegans* (41.6 mgGAE/g) and *C. megapotamica* (32.1 mgGAE/g).

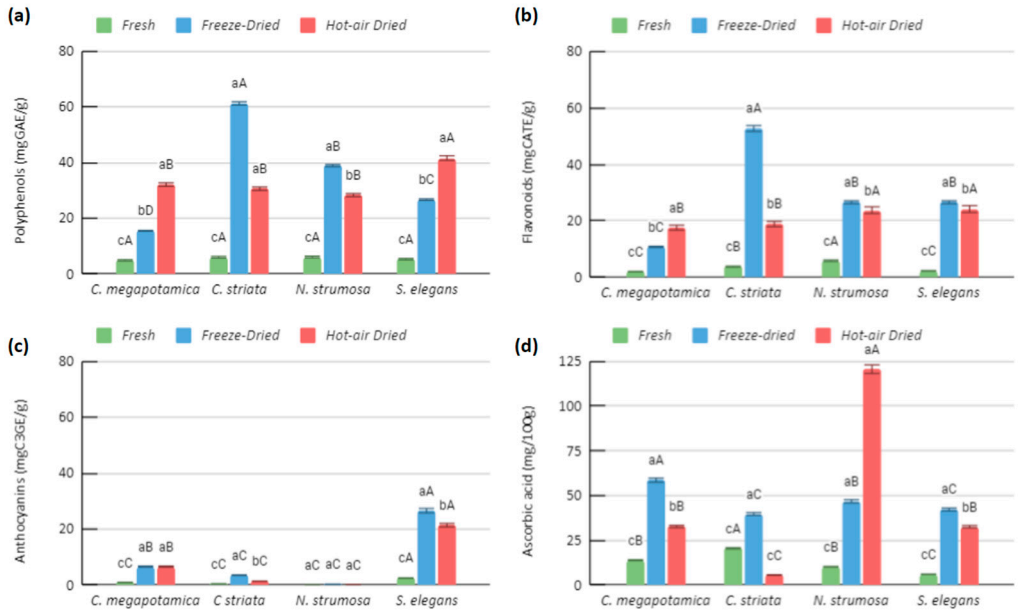


Figure 4. Total polyphenols (**a**), flavonoids (**b**), anthocyanins (**c**) and ascorbic acid (**d**) contents in the edible flowers of *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans* in different post-harvest conditions: fresh, vacuum freeze-dried and hot-air-dried. Data with the same capital letters did not differ between species in the same post-harvest condition and lowercase letters between the same species with $p > 0.05\%$. Bars represent mean ± standard error (SE, n = 3).

The *S. elegans* fresh flowers had lower TPC compared to another study with the same species with 2.321 mgGAE/g [20]. Considering other *Salvia* species, the TPC content is more similar with values from 2.4 to 6.5 mgGAE/g in *S. discolor*, *S. microphylla* [43], *S.* purple queen, *S. x jamensis* J. Compton and *S. farinacea* [55]. This variation could be explained by the growth conditions and different species or varieties.

Both post-harvest techniques increased the TPC content in *S. elegans* with the highest content in the HA condition. This result is very similar to another study where the flowers of *S. elegans* were maintained at 40 °C for 3 days and had 40.8 mgGAE/g [21]. The HA also increased the TPC content in *C. megapotamica* and in four varieties of *Viola x wittrockiana* [56]. This technique is considered more economical and makes it easier to preserve the EFs [23]. The polyphenol increase in the HA is related to the inactivation of the polyphenol oxidase (PPO) that occurs at high temperatures [41]. However, for *C. striata* and *N. strumosa*, the HA decreased the TPC content when compared to FD. In the FD, these last species had an increase in the TPC and *C. striata* had the highest content of all species and treatments. The FD also increased the TPC content in *B. cucullata* and *A. aurantiaca* [41,49].

As well as the polyphenols, the TFC is lower in the fresh flowers (Figure 4b) with the following trend: *N. strumosa* > *C. striata* > *S. elegans* > *C. megapotamica* with 5.9, 3.8, 2.3 and 2 mgCATE/g, respectively. There was an increase in the flavonoids in the FD treatment, with the highest content in *C. striata* with 53.7 mgCATE/g, followed by *N. strumosa* and

S. elegans both with 26 mgCATE/g and *C. megapotamica* with 10.1 mgCATE/g. In the HA, the flavonoids decreased in *C. striata*, *N. strumosa* and *S. elegans* with 18.9, 23.8 and 24.2 mgCATE/g, respectively. For *C. megapotamica,* an increase in the flavonoids was observed in this treatment (17.6 mgCATE/g).

The TFC is similar to the TPC trend with a lower concentration in the fresh flowers followed by an increase in the treated flowers. In the fresh flowers, *N. strumosa* had the highest content among the species analyzed. *S. elegans* and *C. megapotamica* had the lowest TFC. For *S. elegans,* this content was higher compared to other studies with the same species [20] and is very similar to *S. farinacea* fresh flowers [55]. The highest TFC was found in *C. striata* with the FD treatment. The FD was also able to increase this content in *N. strumosa* and *S. elegans*. For the flavonoids, the FD treatment was also more recommended for *B. cucullata* and *A. aurantiaca* with increases in the TFC compared to fresh and HA-treated flowers [41,49].

For the anthocyanins, *S. elegans* had the highest content in all treatments with 2.5 mgC3GE/g in the fresh, 26.6 mgC3GE/g in the FD and 21.4 in the HA-dried flowers (Figure 4c). For *C. megapotamica,* the anthocyanins were 0.9 mgC3GE/g in the fresh flowers and increased to 6.6 mgC3GE/g in both post-harvest treatments. In *C. striata,* the flavonoid content was 0.4 in the fresh flowers, and it increased in the FD (3.5 mgC3GE/g) and the HA-dried flowers (1.4 mgC3GE/g). The lower content was observed in *N. strumosa* with 0.3 mgC3GE/g in FD, 0.1 mgC3GE/g in HA-dried and null in the fresh flowers.

The fresh flowers had low anthocyanin content and it is known that they are responsible for the blue/purple and red colors of the flowers [23]. *N. strumosa* has white/yellow petals and it was expected that the anthocyanins had low or null concentrations. For *C. megapotamica* and *C. striata,* the values were also low even in the post-harvest treatments. These flowers have orange/red petals; however, the red color is present. Other pigments such as carotenoids are present in these species and contribute to their colors. On the other hand, *S. elegans* has vibrant red petals, and its anthocyanin content was higher between the species and increased with the post-harvest treatments. In this case, the FD was more efficient to improve the anthocyanins.

Despite the increase in FD, the anthocyanin content in the HA is also high. This result is similar to *A. aurantiaca* when the FD and HA at 50 and 60 °C increased the anthocyanins compared to fresh flowers and had similar contents between the post-harvest treatments [49]. In contrast, the higher anthocyanin content was in the HA treatment for some species such as *B. cucullata* and *V. wittrockiana* [41,49,56]. According to these authors, the increase in HA could be due to the inactivation of the PPO and the cycle time of drying.

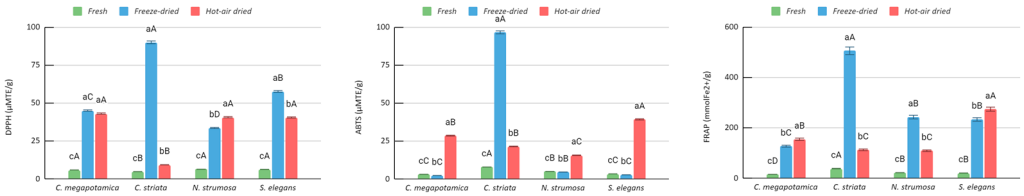
The total ascorbic acid content (AsA+DAsA) was low in the fresh flowers (Figure 4d). In this case, the highest content was in *C. striata* followed by *C. megapotamica* > *N. strumosa* > *S. elegans,* with 20.5, 14, 10.2 and 6 mg/100 g, respectively. It increased in the FD treatment as follows: *C. megapotamica* > *N. strumosa* > *C. striata* > *S. elegans* with 59, 47, 39.7 and 42.2 mg/100 g, respectively. The HA drastically reduced the total ascorbic acid content to 5.6 mg/100 g in *C. striata*, *C. megapotamica* and *S. elegans* (both with 33 mg/100 g) and it increased to 120.6 mg/100 g in *N. strumosa*.

The fresh flowers showed low ascorbic acid content. This vitamin is known as the principal antioxidant in plants, essential for humans, and its content varies greatly between the species. For fresh *S. elegans* flowers, the ascorbic acid content was lower than a previous study with the same species [20]; however, for other *Salvia* species, this content was higher [43,55]. To the best of our knowledge, at the moment, there are no studies about the ascorbic acid content in *C. megapotamica*, *C. striata* and *N. strumosa* as well as the effects of the post-harvest treatments in this vitamin.

Considering the post-harvest treatments, the FD increased the ascorbic acid content in all species, being more recommended for *C. megapotamica*, *C. striata* and *S. elegans*. The HA greatly increased this content in *N. strumosa*; however, for the other species, this treatment decreased the ascorbic acid content, in particular for *C. striata* that had a great decrease in this vitamin. The ascorbic acid content also decreased in the storage of some EFs maintained

under 5 °C for two weeks in *Cucurbita pepo* flowers [57]. According to these authors, the ascorbic acid degradation in these conditions is due to cell damage that leads to oxygen exposition. Apparently, the high temperature can also contribute to cell damage and oxygen exposition. The species and variety have great importance in this process, as in *N. strumosa*, the elevated temperature increased the ascorbic acid content while it decreased for other species. These effects are interesting and need more investigation.

The antioxidant activity by DPPH assay (Figure 5a) was low and similar in the fresh flowers with values around 5–6 µMTE/g in all species. In the FD, the activity increased and the highest value was found in *C striata* followed by *S. elegans* < *C. megapotamica* < *N. strumosa* with 90, 57.7, 45, and 33 µMTE/g, respectively. The HA decreased the antioxidant activity in *C. megapotamica* and *S. elegans* and increased it in *N. strumosa* with all values around 40 µMTE/g. For *C. striata*, this treatment drastically reduces the antioxidant activity to 9.1 µMTE/g.



(**a**) (**b**) (**c**)

Figure 5. Antioxidant activity by DPPH activity (**a**), ABTS (**b**) and FRAP (**c**) assays of edible flowers *Callianthe megapotamica*, *Callianthe striata*, *Nemesia strumosa* and *Salvia elegans* in different post-harvest conditions: fresh, vacuum frozen-dried and hot-air-dried. Data with the same capital letters did not differ between species in the same post-harvest condition and lowercase letters between the same species with $p > 0.05\%$. Bars represent mean ± standard error (SE, n = 3).

The antioxidant activity by the ABTS assay (Figure 5b) revealed a low activity in the fresh and FD flowers of *C. megapotamica*, *N. strumosa* and *S. elegans* with values varying from 2 to 5 µMTE/g. The exception is *C. striata*, which had a value of 7.7 µMTE/g in the fresh flowers and a great increase (96.5 µMTE/g) with FD. The HA increased the antioxidant activity with 39 µMTE/g in *S. elegans*, 28.4 µMTE/g in *C. megapotamica* and 15.5 µMTE/g in *N. strumosa*. For *C. striata*, it was decreased to 21.3 µMTE/g.

In the FRAP assay (Figure 5c), the results' tendency is evidenced. The fresh flowers had lower antioxidant activity compared with the post-harvest treatments, and the highest value was 37 µMTE/g in *C. striata* followed by *N. strumosa* < *S. elegans* < *C. megapotamica* (21, 19 and 14 µMTE/g, respectively). The FD increased the activity in all flowers, with highest value in *C. striata* (505 µMTE/g) followed by *N. strumosa* < *S. elegans* < *C. megapotamica* (242, 232 and 126 µMTE/g, respectively). The HA increased the antioxidant activity in *S. elegans* and *C. megapotamica* (273 and 154 µMTE/g, respectively). For *C. striata* and *N. strumosa*, the HA decreased the antioxidant activity to 112 and 108 µMTE/g, respectively.

The antioxidant activity was monitored by three methods: radical scavenger activity (DPPH assay), the antiradical activity (ABTS assay) and FRAP assays. In all tests, the fresh flowers had low activity with similar values varying from 2 to 7 µMTE/g in the DPPH and ABTS assays or 14 to 37 µMTE/g in the FRAP assay, due to their different detection of the leakage of hydrogen and/or electrons. These results for fresh flowers are similar to other EF species such as *B. cucullata*, *A. aurantiaca* and those in [41,49]. In this condition, the highest activity was found in *C. striata*. Contrastingly, *Abutilon indicum* has a similar antioxidant activity with HA-dried flowers [58]. The *Abutilon* genus was reviewed and species were moved to the new *Callianthe* genus [11]. The *Abutilon* species have some studies focused on the chemical and antioxidant activity of their aerial parts; however, few species were studied and with respect to their flowers, the studies are more scarce [59]. Although *C. striata* and *C. megapotamica* belong to the same genus, the chemical composition and the effects of the post-harvest treatments in their flowers are very different and due to this fact,

studies with other species are recommended. *S. elegans* fresh flowers showed a very similar antioxidant activity to *S. microphylla* and *S. farinacea* [43,55].

Both post-harvest treatments increased the antioxidant activity in the DPPH and FRAP assays, while the ABTS assay for the FD treatment had similar results compared to fresh flowers. For *C. striata*, the highest antioxidant activity was in the FD treatment in all assays; FD also increased this activity in *C. megapotamica* and *S. elegans* in the DPPH assay and in *N. strumosa* in the FRAP assay. On the other hand, HA increases the activity for *C. megapotamica* and *S. elegans* in the ABTS and FRAP assays and in *N. strumosa* in the DPPH and ABTS assays. Considering other studies, the FD increased the antioxidant activity in *B. cucullata* while the HA is more recommended for *V. wittrockiana* at 60 °C and *A. aurantiaca* at 30 °C [41,49]. Therefore, the post-harvest technique recommended for each species varies greatly and must be considered for each case.

The significant antioxidant activity in the post-harvest treatments can be compared to the increase in the bioactive compounds analyzed such as the polyphenols, flavonoids, anthocyanins, ascorbic acid and carotenoids, where their antioxidant power is well known. *C. striata* with FD treatment has the highest antioxidant activity and also has the highest content of carotenoids, polyphenols and flavonoids compared to other species and treatments, and it has more ascorbic acid between the treatments in the same species. This chemical composition is very interesting and can be responsible for this great antioxidant activity. The other species analyzed also have interesting bioactive compounds and antioxidant activity; in particular, *N. strumosa* had the highest ascorbic content in HA-dried flowers. In general, all EFs analyzed showed an increase in the primary and secondary metabolites and in the antioxidant activity in both post-harvest treatments with particularities that must be considered for each species and each metabolite desired.

3.3. Statistical Analyses

Biochemical parameters of the analyzed samples were subjected to multivariate statistical analyses with Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) methods. The dendrogram of the HCA (Figure 6) evidenced a clear partition of the fresh samples that clustered separately from the other in the red macro-cluster. HA and FD samples, instead, were grouped together in the second macro-group, even though a tendency to gather according to the plant species was observed.

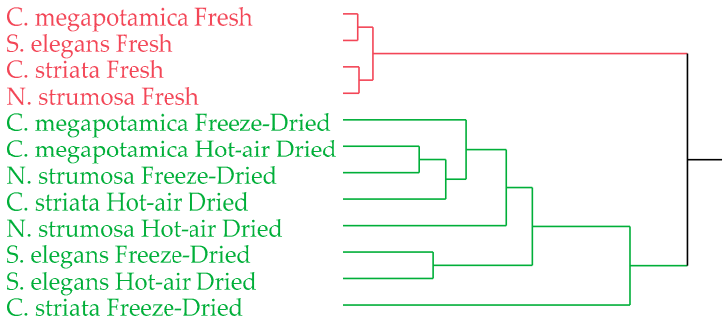


Figure 6. Dendrogram of the HCA performed on the investigated biochemical parameters. Fresh flower samples (in red) cluster separately in a red macro-cluster compared to dried samples (in green).

Similarly, the score plot of the PCA, reported in Figure 7, evidenced a clear separation of fresh samples, plotted in the left quadrant (PC1 < 0), from the treated ones, which instead occupied the right quadrants (PC1 > 0). This sharp detachment was determined by the different contents of the investigated biochemical variables in the fresh plant material compared to the post-harvest plant material. Simultaneously considering the results of the biochemical analyses and the loading plot of the PCA, it was possible to highlight that the obtained separation among them was due to the greatest content of all the investigated parameters in post-harvested material whose vectors were directed toward the rightmost area of the loading plot of the PCA, determined by the conspicuous water loss.

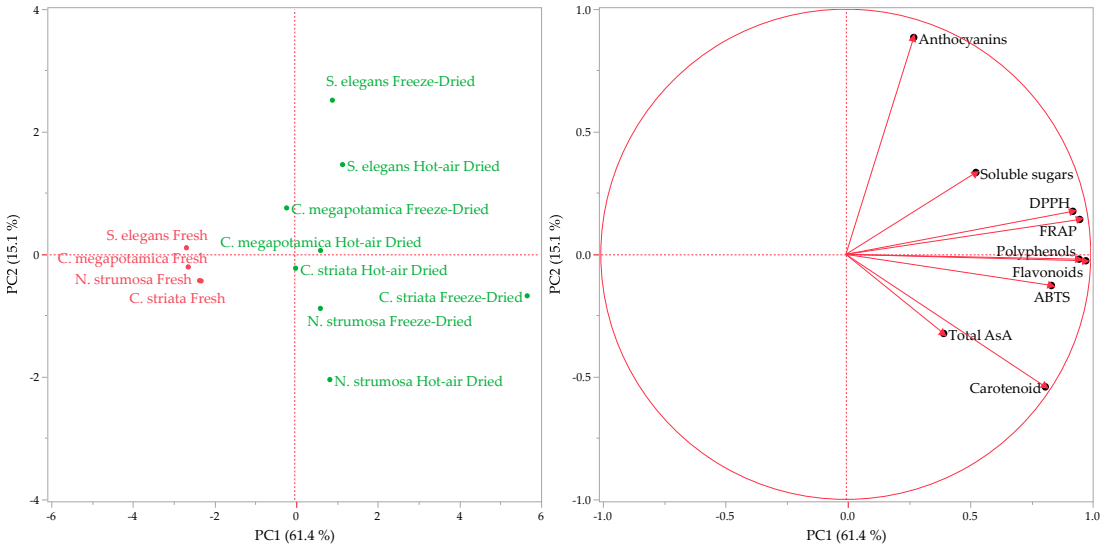


Figure 7. Score and loading plots of the PCA performed on the investigated biochemical parameters. Fresh flower samples (in red) cluster separately in a red macro-cluster compared to dried samples (in green).

Concerning the treated samples, PCA was not able to discern between HA and FD ones. Conversely, the same species subjected to the two different post-harvest treatments showed a tendency to be grouped closely. In particular, the HA and FD samples of *S. elegans* were plotted in the superior part of the upper right quadrant of the score plot thanks to their major content of anthocyanins. Conversely, the great amounts of ascorbic acid and carotenoids were responsible for the positioning of *N. strumosa* in the bottom right quadrant, near to *C. striata* samples also characterized by comparable amounts of carotenoids.

4. Conclusions

This study showed the moisture, protein, mineral and trace element composition, as well as the effects of the post-harvest treatments in four EFs not yet widely exploited as fresh food. The post-harvest treatments increased all bioactive metabolites and the antioxidant activity in the EFs. This fact is very interesting because it allows several opportunities for the EFs' use, such as the possibility of storage during prolonged periods while retaining quality for human consumption and as products of the food and pharmaceutical industries. Fresh or dried flowers can be used by the food industry as ingredients to produce new food products such as sauces, juices and pastes, so it is very important to establish the best protocol for preserving them maintaining the nutritional and functional properties of the flower so that these are transferable to the new food. Therefore, both drying techniques are recommended for these species, even if freeze drying allows for maintaining a greater content of carotenoids, polyphenols, anthocyanins and flavonoids and increased the antioxidant activity in *C. striata*. However, due to different morphological and chemical main compounds, the choice between freeze or hot-air drying must be considered for each species and the metabolites required.

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