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Phytochemical characterization of *Borago officinalis* L. and *Centaurea cyanus* L. during flower development

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Abstract

The present work details the nutritional and chemical compositions of borage and centaurea, at three flowering stages. Water was the main constituent, followed by total dietary fiber. Both flowers showed statistically different ($p < 0.05$) nutritional and chemical profiles, although in both, polyunsaturated fatty acids (PUFAs) (mainly linoleic and α -linolenic acids), free sugars (3.9-28.9 % dw as fructose, glucose, and sucrose), tocopherols (with the major contribution of α -tocopherol from 1.24 to 2.75 mg/100g dw), carotenoids (0.2-181.4 mg/100g dw, mainly as lutein, and organic acids (6.1-14.4 g/100g dw, mainly malic, succinic, and citric acids) were quantified. Concerning flowering, significant differences ($p < 0.05$) were found for some components, particularly carotenoids; however no specific trend was observed in either of the two flower species. Thus, the present study shows that each flower species, as well as their flowering stages, may have different phytochemical and nutritional compositions.

Keywords:

Carotenoids; Edible flowers; Fatty acids; Flower development; Free sugars; Organic acids ;Tocopherols; Vitamin E.

1. Introduction

The commercial value of edible flowers is increasing in the food sector, mostly due to consumer' interest in natural products, new ingredients with potential health benefits, and the aesthetic benefits of their use in food and beverages (colors, textures, and flavor) (Mlcek and Rop, 2011; González-Barrio et al., 2018). Flowers may be consumed as buds and/or the whole flower, or only petals, depending on the flower species. The period of flower development from mature bud to open flower encompasses one of the most active growth periods in the entire developing process of plants (Reid, 2005). During this process, plants undergo different stages, in which a variety of physiological, biochemical and structural processes occur (Collier, 1997; Chen et al., 2012; Çirak et al., 2007). Sometimes the chemical changes that happen during flower development affect their nutritional and beneficial health properties (Shahri et al. 2011; Dar et al. 2014). Thus, consumers are likely to eat flowers of different compositions, according to the part of the flower or development stage. Hence, understanding changes in chemical and nutritional composition during flower development can help to enhance their quality and bioactive potential, and thus, increase edible flowers' commercial value. However, publications on the chemical and nutritional characterization of edible flowers during flower development are limited. A small number of studies have focused primarily on protein, anthocyanins, sugars and enzymes in rose species (*Rosa hybrida*, *R. damascena* Herrm., and *R. bourboniana* Desp.) (Dafny-Yelin et al., 2005; Sood et al., 2006), bioactive compounds in rosemary (*Rosmarinus officinalis* L.) (Del Baño et al., 2003), carbohydrates and anthocyanin pigments in nasturtium flowers (*Tropaeolum*

majus L.) (Silva, 2012), the chemical composition of *Opuntia ficus-indica* (L.) Mill. and *O. stricta* Haw. flowers (Ammar et al., 2014), and physiological and biochemical changes in carnations (*Dianthus chinensis* L.) (Dar et al., 2014).

In particular, centaurea and borage are two important edible flowers that have been sold in different areas, and includes examples, such as Ervas Finas®, BloomBites®, Meadowsweet Flowers®, and Petite Ingredient®. These are used in culinary preparation by both chefs and consumers.

Centaurea (*Centaurea cyanus* L.), also called ‘blue cornflower’ or ‘bachelor’s button’, is a plant native to Europe. Its flowers have no fragrance, but they have a sweet-to-spicy clove-like flavor. Centaurea petals are ideal for mixing with other flowers to make dishes more attractive, for sprinkling over salads (Chaitanya, 2014), or to be used as an ingredient in tea (Chaitanya, 2014). Dried flowers have been proven to contain a long list of medicinal properties, such as being anti-inflammatory, skin cleansing, assisting regulating digestion and kidney, gall bladder, liver and menstrual disorders, and increasing immunity (Chaitanya, 2014; Pirvu et al., 2012).

Borage (*Borago officinalis* L.) is an annual herb, that is native to some parts of the Mediterranean region, and is cultivated for medicinal and culinary uses, but primarily for borage seed oil (Asadi-Samani et al., 2014). Borage flowers are blue and on rare occasions appear white or pink (Asadi-Samani et al., 2014). Petals have a cucumberlike taste and the stamens add a hint of sweetness. The flowers are used in vegetable dishes and fruit salads, to garnish soups or decorate desserts (Husti et al., 2013), as well as in ice cubes (Husti et al., 2013).

To the authors’ best knowledge, no studies on the phytochemical modifications during borage and centaurea flower development have been conducted. Thus, the present study was conducted to determine the evolution and changes that occur in nutritional and

chemical composition (fatty acids, free sugars, vitamin E, carotenoids and organic acids) of these two edible flowers, along with their flower development (tight and mature buds, and fully open flowers). The main objective of doing so is to gather important scientific information for the academic community, and for those with an interest in the subject such as flowers producers, and for the community in general. By valorizing these products, the research can contribute to the popularization of flowers as food or supplements for food, highlight their use to cosmetics and pharmaceutical industries.

2. Material and methods

2.1. Plant material

The flowers of *B. officinalis* and *C. cyanus* were collected from the greenhouse of the School of Agriculture of the Polytechnic Institute of Bragança (Bragança, Portugal). Once harvested, flowers at different stages of development were immediately transported to the laboratory under refrigeration. Flower development was divided into three stages (Figure 1): stage one (tight bud stage) – closed bud flower, showing some petal color at the tip of the bud end; stage two (mature bud stage) – closed bud flower, showing the color of petals in their entire surface; stage three (fully open) – completely opened flower, without symptoms of senescence. Furthermore, petals (true petals, not bracts) were also picked, because they are often the sole parts of flowers used for culinary purposes.

Flowers were evaluated for their moisture content before being freeze-dried. All chemical analyses were performed using the freeze dried samples, with the results presented in dry weight (dw) or converted to fresh weight (fw).

2.2. Moisture, dietary fiber, and nutritional composition

The moisture, ash, fat, carbohydrates, and dietary fiber of each sample were analyzed using AOAC procedures (1995) and expressed as g/100g fresh weight (fw). Moisture content was determined by drying the sample to a constant weight at 105 °C; ash content was measured by calcination at 550 °C for at least 2h, until achieving white ashes. The protein content of samples was estimated using the macro-Kjeldahl method, with a conversion factor of 6.25, as per to Sotelo et al. (2007) and Rop et al. (2012). Lipids were determined by extracting a known mass of powdered sample with petroleum ether, with 0.01% BHT (2,6-di-*tert*-butyl-4-methylphenol) to prevent oxidation, using a Soxhlet apparatus. Dietary fiber was determined by an enzymatic-gravimetric method based on AOAC official method No. 985.29 (AOAC, 2003).

Free sugars were extracted by following the procedure described by Barros et al. (2010). Sugars were analyzed in a Jasco integrated high performance liquid chromatographic system (Tokyo, Japan), equipped with a PU-980 intelligent pump and a refractive index detector (Gilson, USA). Separation was achieved with a SUPELCOGEL Ca column (30 cm × 7.8 mm I.D., Supelco, USA), operating at 80 °C, with ultra-pure water at a flow rate of 0.5 mL/min as eluent. Sugar identification was made by comparing the retention times of sample peaks with standards. Quantification was performed by the external standard method with individual calibration curves for each sugar at concentrations ranging from 0.5 to 10 mg/mL. The results were expressed as g/100 g dw.

2.3. Analysis of lipids' minor components

2.3.1. Lipids compounds extraction

Lipid extraction for compositional analysis was performed according to the method described by Cruz et al. (2013). A 250 mg portion of a lyophilized sample was weighted

in a plastic tube. «Two internal standards solutions, tocol (20 μL ; 1 mg/mL) for vitamin E quantification, and triundecanoin (200 μL ; 1 mg/mL), for total fatty acids quantification, were added. Following on, two antioxidants, BHT (20 μL , 10 mg/mL in methanol) and ascorbic acid (50 mg), were also added. Propan-2-ol (1.6 mL) and cyclohexane (2.0 mL) were mixed and agitated briefly by vortexing and kept overnight under refrigeration (4 $^{\circ}\text{C}$). The non-lipidic fraction was removed by washing with 2.5 mL of a 1% NaCl solution (m/v), followed by agitation by vortexing and centrifugation (3000 rpm, 5 min; Heraeus Sepatech, Germany). The organic phases were dehumidified with anhydrous sodium sulfate and divided into two portions. For fatty acid analysis, the solvent was evaporated under a nitrogen stream (60 $^{\circ}\text{C}$). Hydrolysis was performed with 1.5 mL of KOH (0.5 M in methanol) at 100 $^{\circ}\text{C}$ (10 min) in a dry heating block (Stuart SBH200D/3, Staffordshire, UK). After reaching room temperature, methylation was completed by the addition of 1.5 mL of BF_3 (14% in methanol) and heated for a further 30 min at 100 $^{\circ}\text{C}$. After cooling, 1% NaCl (m/v) (2.5 mL) and *n*-hexane (2 \times 2 mL) were added and the mixture was mixed by vortexing, followed by centrifugation (3000 rpm, 5 min). The supernatant was transferred to a tube with anhydrous sodium sulfate, followed by centrifugation at 5000 rpm for 5 min. A supernatant portion (2 mL) was directly transferred to a clear glass vial (Supelco, Sigma, Bellfonte, USA). The solvent was evaporated under a nitrogen stream (60 $^{\circ}\text{C}$), re-suspended with heptane (1 mL), and the solution mixed in a vortex. This solution was analyzed in a gas chromatograph for fatty acid analysis, as detailed below. The remaining lipid extract (half of the re-suspended extract) was transferred to an amber glass vial. The solvent was evaporated under a gentle nitrogen stream (40 $^{\circ}\text{C}$), resuspended in about 600 μL of *n*-hexane (HPLC grade) and placed in the HPLC autosampler for vitamin E and carotenoids quantification.

2.3.2. Fatty acids

Fatty acids were analyzed according to the method described by Amaral et al. (2003), with slight modifications. The temperatures of the injector and detector were 250 and 270 °C, respectively. Separation was achieved on a 100 -m × 0.25 -mm i.d. Select-FAME column (0.19 -µm film; Agilent, Santa Clara, USA). Helium was used as carrier gas at an internal pressure of 180 kPa. Fatty acids identification (C11:0 C22:6) was accomplished by comparing the relative retention times of FAME peaks with the standards gained from a diverse range of suppliers, from literature data, and confirmed by GC-MS on a Agilent chromatograph 7890A with a 5977B MSD (MS source –230 °C; MS QUAD 150; aux 280 °C; m/z 30-800), using the NIST/EPA/NIH Mass Spectral Library (NIST 14). For quantification purposes, FID peaks were corrected the using response factors obtained with standard FAME solutions. The fatty acids results were calculated on a relative percentage basis.

2.3.3. Vitamin E and carotenoids

Separation was achieved by normal-phase HPLC, based on the method described by Cruz and Casal (2018). The liquid chromatograph consisted of a Jasco integrated system (Easton, USA) equipped with an autosampler (AS-2057 Plus), a PU-980 intelligent pump, and a multi-wavelength DAD (MD-910), recording at 450 nm for carotenoids, connected to a fluorescence detector (FD) (FP-2020 Plus; $\lambda_{\text{excitation}} = 290 \text{ nm}$ and $\lambda_{\text{emission}} = 330 \text{ nm}$) for vitamin E. Chromatographic separation was achieved on a Luna Silica column (100 mm × 3 mm; 3 µm) (Phenomenex, USA), operating at constant room temperature (23 °C), with a gradient of *n*-hexane and 1,4-dioxane at a flow rate of 1.0 mL/min. The compounds were identified by chromatographic comparisons using

authentic standards. Tocopherols and tocotrienols (α -, β -, γ -, and δ -) were acquired from Supelco (USA) and Larodan AB (Sweden). Carotenoids standards (all-*trans*- β -carotene and lutein) were sourced from Sigma Aldrich (USA). Identified tocopherols and carotenoids were quantified by the internal standard method, reported on adw basis.

The total carotenoids contents was estimated according to the method described by Aquino-Bolaños et al. (2013). One gram of frozen-dried powder of each sample was extracted twice using a 20 mL acetone:hexane solution (1:1, v/v). Both extracts were put into a separation funnel, 200 mL distilled water was then added to eliminate acetone. The acetone-free phase was mixed with anhydrous sodium sulfate (5 g) to eliminate any residual water, and the remaining solution filtered and completed to 100 mL with hexane. Carotenoids content was determined by reading the absorbance at 450 nm and comparing the results with a β -carotene calibration curve (0.22 – 8.8 $\mu\text{g/mL}$). Results were expressed as mg β -carotene equivalent/100 g dw.

2.4. Organic acids

The organic acids in the edible flowers were determined by gas chromatography, after methylation, following the procedure described by Sharma et al. (2016) and Kumar et al. (2017), with minor modifications. The analytical column used was a HP-5MS (30 m \times 0.25 mm i.d. \times 0.25 μm thickness ultra-inert capillary column, by Agilent Technologies). Mass spectra were obtained by electron ionization (EI) at 70 eV, in full scan mode, with a spectrum range of ion mass captured between 25 and 400 m/z, and an average of 3.5 scans/s (sample rate of 2). The mass spectra were evaluated using Enhanced ChemStation software (Version F.01.03.2357, Agilent Technologies). Individual standards of citric, levulinic, fumaric, succinic, malic, salicylic, hydroxycinnamic, malonic, oxalic, tartaric and benzoic acids all supplied by Sigma

Aldrich (Germany) were derivatized under sample conditions. Quantification was based on individual calibration curves, using specific m/z for each compound, as detailed in Kumar et al. (2017).

2.5. Statistical analysis

The statistical analysis was performed using SPSS software, Version 18.0 (SPSS Inc., Chicago, USA). The normality of the data and homogeneity of the variances were confirmed by Shapiro-Wilk and Levene's tests, respectively. To verify if significant differences ($p < 0.05$) existed between samples, an analysis of variance (ANOVA) or ANOVA Welch was performed, depending on the existence or non existence of homogeneity of variances, respectively. When significant differences were detected between treatments, Tukey's honest significant difference test (if variances in different groups were identical) or the Games-Howell test (if they were not) were performed.

3. Results and Discussion

3.1. Moisture, dietary fiber, and nutritional composition

The moisture, dietary fiber, and nutritional compositions of borage and centaurea at three flowering stages are detailed in Table 1. Water was the main constituent in both flowers at all stages (73.7-90.5 g/100 g fw), followed by total dietary fiber (3.84–17.2 g/100g fw), while lipids were minor constituents (0.41-1.13 g/100g fw).

When comparing both flowers, borage always presented significantly higher ($p < 0.05$) moisture content (> 85 g/100g fw) than centaurea (> 73 g/100g fw). The same was observed for ash values (1.74-2.05 vs. 1.20-1.56 g/100g fw, respectively). On the other hand, centaurea always presented significantly higher amounts ($p < 0.05$) of total dietary fiber (14.8–17.2 g/100g fw) than borage (3.84–6.74 g/100g fw). In general,

these values were similar to selected vegetables asparagus (moisture 79.34-92.20, proteins 3.62-4.83, fat 0.33-0.90 g/100g fw) (Ferrara et al., 2011), white cabbage (moisture 91.8, proteins 1.4, fat 0.4, dietary fiber 2.4 g/100 g fw) and cucumber (moisture 95.1, proteins 1.4, fat 0.6, dietary fiber 0.7g/100 g fw) (Instituto Nacional de Saúde Doutor Ricardo Jorge, 2010).

Regarding centaurea, our results expressed in dry weight (data not shown) were similar to those reported by Rop et al. (2012) for fully open flowers (protein 6.9 g/100g dw, in both studies), and the results of Pires et al. (2017) for true petals of this species (protein 5.8 vs 8.5 g/100g dw).

During the flowering stage (from tight bud to fully open flower), the nutritional composition trends for both flowers were different. Borage showed an increase in protein, while in centaurea, a decrease in ash and protein content was observed. Peiretti et al. (2004) reported a progressive decrease in crude protein content during the developmental stages of borage, specifically, 149 g/kg dw for late vegetative stage to 138 g/kg dw for late flower stage. In our work, the lipid content remained almost constant, while dietary fiber showed variable behavior, when both flowers were compared. These patterns are in line with literature results for other flowers, as in the case of the two *Opuntia* species at four stages of development reported by Ammar et al. (2014). The flowers of *O. ficus-indica* showed an increase in fat and a decrease in fiber content (three first stages of flowering), while in *O. stricta*, the opposite was detected (Ammar et al., 2014). Thus, each flower, at each flower development stage, showed a unique composition and nutritional value.

For both flowers studied, petals alone presented the highest moisture content when compared with the entire flower, on both flowers. In borage, the separation of petals from the flower represented a highly significant reduction in protein, lipids, and total

dietary fiber, while in centaurea only minor changes were observed. To gain the highest protein content, borage should be consumed as a fully opened flower, while for centaurea the tight bud should be preferred. To obtain a higher fiber content, tight buds should be chosen.

Three free sugars were identified, namely, sucrose, glucose, and fructose. Fructose was the major sugar in both flowers at all flowering stages (1.57-10.4 g/100g dw), except in centaurea petals, in which glucose dominated (7.30 g/100 g dw). Similar results were reported for other flowers by Guimarães et al. (2010) and Pires et al. (2017), who detected fructose as the main sugar in *Rosa micrantha*, dahlia, and centaurea petals. Borage presented higher values for all free sugars, which can likely be associated with its sweet, sensory attribute. Concerning different flowering stages and parts of the flower, petals showed the highest values of free sugars for both flowers, except for sucrose in centaurea. Different functions in flower development have been attributed to sugars, namely as an energy source (Moalem-Beno et al., 1997), osmotic regulators (Bialeski, 1993), and precursors of metabolic processes. During flower development (from tight bud to fully open flower), the sucrose and glucose remained constant in borage, while fructose increased from 5.72 to 6.97 g/100 g dw. In centaurea, sucrose also remained constant, while glucose and fructose increased (from 0.91 to 1.74 and 1.57 to 2.71 g/100g dw, respectively). This may be related to the fact the sugar availability is an important signal during floral induction; in particular sucrose was described as a major photosynthetic product, and acts as a signal to control the timing of flowering (Cho et al. 2018, Tognetti et al. 2013). Moreover, its hydrolytic products (glucose and fructose), as well as their downstream metabolic intermediates, trehalose and trehalose-6-phosphate, also act control as signals (Cho et al. 2018).

3.2. Fatty acids

The fatty acids profiles of borage and centaurea at three flowering stages are shown in Table 2, presented as individual, and grouped as total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), together with the ratios of PUFA/SFA and n-6/n-3. Nineteen fatty acids were identified and quantified. The major fatty acid found in borage at all stages was linoleic acid (C18:2n6) (19.1-23.7%), followed by palmitic acid (C16:0) (17.1-18.0%). These results are similar to Sayanova et al. (1999), who noted that the fatty acids composition of borage flowers was characterized by having the highest content of linoleic (41.4%) and palmitic (31.9%) acids, although our values were lower.

In centaurea, oleic acid (C18:1) was the major fatty acid in tight buds (26.4%), while in mature buds and fully open flowers, this was C16:0 (30.0 and 25.9%, respectively). In petals, α -linolenic acid (C18:3n3) was the prevalent fatty acid (27.7%). However, C16:0, C18:1, and C18:3n3 acids were not significantly different ($p>0.05$) during the flower development of development. Furthermore, γ -linolenic (C18:3n6) and eicosenoic (C20:1) acids were not detected in centaurea, unlike borage. During the three stages of flowering, borage did not reported significant differences ($p>0.05$) in the major fatty acids, with the exception of C18:3n3, which increased from 10.4 to 13.0%. In all stages of development, PUFAs dominated over SFA in borage, followed by MUFA, due to the abundance of C18:2 and C18:3. Similar results were obtained by Peiretti et al. (2004), who reported that during the flower development of borage high percentages of PUFA were detected between 65 to 80%.

Contrastingly, in centaurea the following increasing order was observed: SFA > PUFA > MUFA, due to the contribution of C16:0, although SFA values were not very different from PUFA values. Furthermore, for the human diet, it is

recommended that PUFA/SFA ratios be higher than 0.45, and n-6/n-3 ratios lower than 4.0 (Guil et al., 1996), which was observed in almost all stages of flowering, except in borage petals (PUFA/SFA = 0.40), mature buds in centaurea (PUFA/SFA = 0.20), and centaurea petals (PUFA/SFA = 0.44). Thus, in general terms, both edible flowers at all flowering stages and petals had healthy lipid profiles.

3.3. Vitamin E

Free vitamin E was evaluated in all flowering stages, as detailed in Table 3. Results show that α -tocopherol was the major compound in all samples, followed by γ -tocopherol in borage and β -tocopherol in centaurea. This richness in α -tocopherol is important from a health perspective, since it represents a primary defense against lipid peroxidation (Bartoli et al., 1997), and such richness is similar to that found in other floral species such as calendula (Pires et al., 2017; Miguel et al., 2016), centaurea (Pires et al., 2017), rose species (Pires et al., 2017, Guimarães et al., 2010), capper (Tili et al., 2009), and liliun (Arrom and Munne-Bosch, 2010). In almost all flower development stages, borage showed a higher tocopherols content than centaurea; however, centaurea presented the highest value of α -tocopherol in petals (3.47 mg/100g dw), as well as higher β -tocopherol values than borage (0.41-1.01 vs 0.29-0.33 mg/100g dw). No tocotrienols were detected in centaurea, while borage showed β - and γ -tocotrienols in their composition in reduced amounts. Pires et al. (2017) also described the presence of α - and γ -tocopherols in centaurea flowers. Contrastingly, Lockowandt et al. (2019) did not detect β - or δ - tocopherols in centaurea flowers, although the values for α -tocopherol (1.01 mg/100g dw) and γ -tocopherol (0.29 mg/100 g dw) were similar to ours.

Throughout flower development, the content of total tocopherols decreased in both flowers, between 3.7 to 3.2 mg/100g dw (borage) and 3.0 to 2.4 mg/100 g dw (centaurea). However, borage and centaurea showed significant differences in individual tocopherols. In borage, α - and β -tocopherols decreased (from 2.75 to 2.21 and 0.33 to 0.29 mg/100g dw, respectively) from tight bud to fully open stage, while γ -tocopherol increased (from 0.34 to 0.43 mg/100g dw). In centaurea, the α -tocopherol remained constant, while β - and γ -tocopherols decreased (from 1.01 to 0.66 and 0.32 to 0.28 mg/100g dw, respectively), indicating that different flowers had distinctive trends in vitamin E composition throughout flower development.

When analyzing caper from different Tunisian regions, Tlili et al. (2009), found that buds presented higher α - and γ - tocopherols values than flowers. An increase in α -tocopherol content during the progression of senescence in chrysanthemum petals, followed by a decline in advanced stages (stage four roughly 50% of full browning of the petal and evidence of wilting, and stage five - browning involved 50- 100% of the petal), were been reported by Bartoli et al. (1997). In liliium floral organs, α - and γ -tocopherols also increased alongside the progression of tepal senescence, between stages one (closed flowers, with fully compacted organs and green tepals) and five (flowers with dehydrated tepals, just before abscission) (Arrom and Munné-Bosch, 2010). It must be stated that centaurea petals were richer in α - and γ -tocopherols than the entire flower in each flowering stage. Thus, the consumption of borage and centaurea can contribute to the daily recommend dose of vitamin E.

3.4. Carotenoids

The results for total and individual carotenoids are shown in Table 4. Carotenoids are pigments with provitamin and antioxidant roles. Regarding total carotenoids, borage

always showed significantly higher amounts than centaurea at all stages of flower development (23.5-181.4 vs 0.2-7.8 mg β -carotene/100g dw), except in petals, where centaurea petals had a higher content than those of borage ones (4.8 and 3.3 mg β -carotene/100g dw, respectively). This indicates that borage carotenoids are more prevalent in other flower components than in petals.

Regarding individual carotenoids, lutein was the main carotenoid in both flowers (0.03-43.7 mg β -carotene equivalent/100g dw), as in other edible flowers, namely: chrysanthemum (1.18-30.7 mg lutein/100g dw) (Park et al., 2015), snapdragon (1.41 mg lutein/100g dw) (González-Barrio et al., 2018), garden nasturtium (35.0-45.0 mg lutein/100g fw) (Niizu and Rodriguez-Amaya, 2005), marigold (106.2 mg lutein/100g fw) (Tinoi et al., 2006), and pansies (5.11 mg lutein/100 g dw) (González-Barrio et al., 2018). Borage presented consistently higher lutein contents than centaurea (0.73-43.73 vs 0.03-1.16 mg β -carotene equivalent/100g dw, respectively), except in petals, in which similar results were obtained (0.73 and 0.69 mg β -carotene equivalent/100 g dw, respectively). β -carotene was also found in both flowers, but in much smaller amounts than lutein, ranging between 0.04-8.50 mg β -carotene equivalent/100 g dw for borage and between 0.002-0.091 mg β -carotene equivalent/100g dw in centaurea.

Concerning flower development, different behavior was observed for each of the two flower species. Borage had the highest lutein concentration in the last flowering stage (fully open), with values three times higher than in previous stages, whereas centaurea presented an increase from tight to mature bud, and then a non-significant decrease to the fully open stage, but always in a very low range when compared to borage. Throughout borage flowering (tight bud to fully open stage), β -carotene also increased from 0.45-8.50 mg β -carotene /100g dw, while in centaurea it was almost residual, but remained more constant from mature bud to fully open flower. This different behavior

between the flower species is also described in Ohmiya et al. (2013), who detected that red carnation flower accumulated small amounts of carotenoids at stage one (bud flower), followed by mature flower and decreased at stage four, i.e. – fully open flower; while in green carnations, the amount of carotenoids accumulated in petals during flower development (stage four). Yamamizo et al. (2010) also detected a different behavior for petals of different species of *Ipomoea* during flowering development. In *I. obscura* and *I. nil*, the values of lutein and β -carotene decreased from stage one (12 h before flower opening) to stage four (fully opened flowers), while in *Ipomoea* sp. this content increased. Thus, each plant employs a different strategy for controlling quantitatively and qualitatively controlling carotenoid levels in petals (Ohmiya et al., 2013).

Furthermore, carotenoids extracted from flowers can be used in the food, pharmaceutical, and cosmetics industries. In food industry, carotenoids can be used as a natural colorant, as is already the case; for example, the yellow color derived from the lutein of *Tagetes erecta* L. flowers (Lin et al., 2005). On the other hand, some carotenoids are precursors of vitamins and also present actions that are anti-inflammatory, have antioxidant properties, immunomodulatory, and anti-cancer (Cardoso et al., 2017). As such, the inclusion in pharmaceutical and food products of these bioactive compounds in their concentrated form also represents an approach for promoting health. In fact, some dietary supplements, processed foods, beverages, confectionery, and dairy products already include carotenoids from flowers in their formulations (Berman et al., 2015).

3.5. Organic acids

The organic acids profiles of borage and centaurea during flowering development are presented in Table 5. Eight organic acids were detected, with distinct profiles for both flowers. In borage and centaurea, the total contents of organic acids ranged between 6.1-9.2 and 8.6-14.4 g/100g dw, respectively. The major organic acid in borage was malic acid (2.09 – 4.66 g/100g dw), followed by citric (0.52-1.73 g/100g dw) and levulinic (0.69–1.58 g/100g dw) acids. Contrastingly, in centaurea, the major organic acid was succinic acid (1.89-9.68 g/100g dw), followed by malic (1.47-2.17 g/100g dw) and citric (1.09-2.76 g/100g dw) acids. Petals had the highest total organic acid content, with different patterns in both flower species, but similar to those presented in flower development, with malic acid being prevalent in borage petals (50.6%), and succinic acid in centaurea petals (67.2%). Pires et al. (2017) also reported different organic acids profiles for dahlia, rose, calendula, and centaurea petals, for which the major organic acids were: malic acid (0.74 g/100g dw), quinic and malic acids (1.52 and 1.23 g/100g dw, respectively), succinic acid (1.77 g/100 g dw), and oxalic acid (0.18 g/100g dw), respectively. Furthermore, when analyzing dried centaurea petals (commercial sample) Pires et al. (2017), did not detect any of the current study's major organic acids, reporting only oxalic and shikimic acids. On the other hand, Lockowandt et al. (2019) also detected succinic (3.62 g/100g dw), citric (2.29 g/100g dw), malic (0.36 g/100g dw), and fumaric (0.0021 g/100 dw) acids in fully open centaurea flowers, the first two organic acids being in line with our results.

In both flowers, from tight bud to fully open stage, citric acid decreased (from 1.73-0.92 and 2.76-1.88 g/100g dw in borage and centaurea, respectively). In borage, levulinic and malic acids increased during flower development (0.69-1.17 and 2.09 to 2.88 g/100g dw, respectively). In centaurea, succinic acid increased from tight bud (1.89 g/100g dw) to fully open (3.62 g/100g dw) stages, while malic acid decreased from

2.17-1.84 g/100g dw. Borage petals showed higher values for all organic acids quantified, except citric and salicylic acids. In centaurea petals, only succinic, hydroxycinnamic and malonic acids increased with flower development. Thus, food, cosmetics and pharmaceutical industries can use edible flowers as a new source of different organic acids, depending on the stage of flowering, as well as the part of the flower to be consumed (petals or whole flower).

Furthermore, malic and citric acids, which are important components in centaurea and borage flowers, have been reported to have protective effects on myocardial ischemia/reperfusion injury (Tang et al., 2013) and as having antimicrobial activity against some pathogenic microorganisms (Eswaranandam, 2004). Moreover, some of these organic acids are already used as food additives, such as citric acid, which demonstrated efficacy for pathogen control in fresh and processed meat (Anyasi et al., 2017), or citric, malic, and tartaric acids as antioxidants in the manufacturing of beverages (Scherer et al., 2012).

4. Conclusions

In summary, the present work describes, for the first time, the chemical and nutritional composition of two edible flowers, centaurea and borage, at three flowering stages, and for their petals. Both flowers species have a high-water content (>73%). In general, centaurea presented the highest values for total dietary fiber, while borage showed the highest protein, PUFA and total carotenoid content. During flowering, few changes were observed in the compounds analyzed for both flower species, the major differences detected being between flower and petals. In borage petals, the lowest protein and total carotenoid values were observed.

In conclusion, both edible flower species showed a nutritional composition and compounds with functional properties. Despite the lower amounts of some compounds, the daily consumption of edible flowers may contribute to supplying some macronutrients, vitamins, and organic acids to the organism. The human diet these characteristics may therefore encourage the use of edible flowers (whole flower and petals) for human consumption.

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Figure 1 - Petals and three flowering stages of borage (*Borago officinalis*) and centaurea (*Centaurea cyanus*)

Table 1 – Composition in moisture, nutrients and total dietary fiber of borage and centaurea at three flowering stages plus isolated petals (g/100 g fw)

	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
Moisture	86.9±0.4 ^{a,B}	85.3±1.8 ^{a,B}	86.6±1.8 ^{a,B}	90.5±0.4 ^{b,B}	73.7±2.2 ^{a,A}	76.3±1.2 ^{a,A}	76.7±0.6 ^{a,A}	80.5±0.7 ^{b,A}
Ash	2.02±0.14 ^{c,B}	1.74±0.04 ^{b,B}	2.05±0.11 ^{c,B}	0.88±0.04 ^{a,A}	1.56±0.06 ^{c,A}	1.43±0.11 ^{b,c,A}	1.20±0.11 ^{a,b,A}	1.11±0.04 ^{a,A}
Protein	2.24±0.09 ^{b,A}	2.62±0.10 ^{c,B}	3.04±0.10 ^{d,B}	0.89±0.02 ^{a,A}	2.89±0.55 ^{b,A}	1.78±0.28 ^{a,A}	1.60±0.06 ^{a,A}	1.66±0.24 ^{a,B}
Lipid	0.68±0.16 ^{a,A}	0.56±0.02 ^{a,A}	0.66±0.15 ^{a,A}	0.41±0.08 ^{a,A}	0.89±0.08 ^{a,A}	1.13±0.20 ^{a,B}	0.80±0.03 ^{a,A}	0.86±0.16 ^{a,B}
Soluble sugars								
Sucrose	3.55±0.53 ^{a,B}	4.92±0.67 ^{a,B}	3.86±0.75 ^{a,B}	9.60±1.67 ^{b,B}	1.45±0.15 ^{a,A}	1.30±0.05 ^{a,A}	1.33±0.06 ^{a,A}	1.18±0.45 ^{a,A}
Glucose	5.12±0.44 ^{a,B}	6.94±0.71 ^{b,B}	5.96±0.75 ^{a,b,B}	8.85±0.15 ^{c,B}	0.91±0.10 ^{a,A}	2.10±0.04 ^{b,A}	1.74±0.07 ^{b,A}	7.30±0.46 ^{c,A}
Fructose	5.72±0.36 ^{a,B}	7.86±0.87 ^{b,B}	6.97±0.38 ^{b,B}	10.4±0.30 ^{c,B}	1.57±0.12 ^{a,A}	2.49±0.04 ^{b,A}	2.71±0.10 ^{b,A}	3.43±0.40 ^{b,A}
Total dietary fiber	6.28±0.16 ^{c,A}	6.74±0.05 ^{d,A}	4.74±0.10 ^{b,A}	3.84±0.03 ^{a,A}	17.2±1.4 ^{b,B}	14.8±0.8 ^{a,B}	15.7±0.5 ^{a,b,B}	14.8±0.7 ^{a,B}

Values are expressed as: Mean±Standard deviation. fw- fresh weight. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$).

Table 2 – Fatty acid composition (relative %) of borage and centaurea at three flowering stages.

Fatty acids (%)	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
SFA								
C12:0	0.5±0.2 _{a,b,A}	0.6±0.2 ^b _A	0.5±0.1 ^a _{b,A}	0.3±0.1 _{a,A}	0.7±0.2 ^a _A	0.9±0.1 ^a _B	0.6±0.2 ^a _A	0.8±0.4 ^a _B
C14:0	0.5±0.3 _{a,A}	0.8±0.3 ^a _A	0.7±0.1 ^a _A	2.1±0.1 _{b,B}	0.7±0.3 ^a _A	1.0±0.5 ^a _A	0.7±0.2 ^a _A	0.7±0.3 ^a _A
C15:0	0.2±0.1 _{a,A}	0.2±0.1 ^a _A	0.2±0.1 ^a	0.4±0.1 _{b,B}	0.1±0.1 ^a _A	0.2±0.2 ^a _A	nd	0.2±0.2 ^a _A
C16:0	18.0±0.8 ^a _A	17.8±0.9 _{a,A}	17.1±0.7 _{a,A}	17.2±0.1 ^a _{a,A}	24.8±4.5 _{a,B}	30.0±1.2 _{a,B}	25.9±4.4 _{a,B}	25.2±4.2 _{a,B}
C17:0	0.9±0.5 _{a,B}	1.0±0.1 ^a _A	0.9±0.2 ^a _A	1.3±0.1 _{a,A}	0.4±0.1 ^a _A	1.2±0.3 ^b _{c,A}	0.6±0.3 ^a _{b,A}	1.3±0.6 ^c _B
C18:0	4.9±0.4 _{a,A}	3.8±1.8 ^a _A	4.8±0.4 ^a _A	5.0±0.1 _{a,A}	5.8±1.1 ^a _A	8.6±1.7 ^a _{b,B}	7.2±1.7 ^a _B	10.6±3.2 _{b,B}
C20:0	1.5±0.8 _{a,A}	1.8±0.2 ^a _A	1.8±0.2 ^a _A	1.8±0.1 _{a,A}	2.5±0.8 ^a _A	2.9±0.4 ^a _B	2.5±0.7 ^a _B	2.9±1.1 ^a _B
C22:0	2.4±0.7 _{a,B}	2.6±0.3 ^a _B	2.5±0.4 ^a _A	3.2±0.4 _{a,B}	1.2±0.4 ^a _A	2.0±0.3 ^a _A	1.4±0.5 ^a _B	1.6±0.5 ^a _A
C24:0	2.4±1.3 _{a,A}	2.6±0.2 ^a _B	2.6±0.3 ^a _B	3.9±0.2 _{b,B}	1.3±0.5 ^a _A	2.0±0.3 ^a _A	1.6±0.6 ^a _A	1.3±0.4 ^a _A
Total SFA	31.0	31.0	31.0	35.0	37.0	49.0	40.0	45.0
MUFA								
C16:1	0.1±0.1 _{a,A}	0.3±0.2 ^a	0.2±0.1 ^a _A	0.2±0.1 _a	0.2±0.1 ^a _A	nd	0.3±0.2 ^a _B	nd
C18:1	12.3±1.5 ^a _A	10.3±7.7 _{a,A}	9.9±8.8 ^a _A	6.7±1.2 _{a,A}	26.4±15.3 ^a _B	9.04±3.4 _{5^a,A}	25.9±17.1 ^a _{1^a,A}	19.8±18.5 ^a _{5^a,B}
C20:1	0.4±0.2 _a	0.6±0.4 ^a	0.3±0.0 ^a	0.5±0.1 _a	nd	nd	nd	nd
C22:1	2.5±0.9 _{a,b,B}	2.1±0.5 ^a _B	1.8±0.2 ^a _B	3.2±0.4 _{b,B}	0.7±0.5 ^a _A	0.75±0.6 _{6^a,A}	0.9±0.8 ^a _A	0.5±0.4 ^a _A
C24:1n9	2.6±0.8 _{a,B}	2.2±0.3 ^a	2.0±0.3 ^a	3.5±0.3 _b	0.1±0.1 ^A	nd	nd	nd
Total MUFA	18.0	16.0	14.0	14.0	27.0	10.0	27.0	20.0
PUFA								
C18:2n6	19.1±1.2 ^a _{2^a,B}	21.3±1.5 _{a,b,A}	21.2±2.2 _{a,b,B}	23.7±0.8 ^b _{8^b,B}	15.6±2.4 _{b,A}	20.7±3.0 _{c,A}	12.8±2.9 _{a,b,A}	10.6±0.7 _{a,A}
C18:3n6	7.0±1.4 _a	9.1±1.2 ^b	9.6±1.4 ^b	6.1±0.2 _a	nd	nd	nd	nd
C18:3n3	10.4±1.7 ^b _{7^b,A}	12.5±1.3 _{b,c,A}	13.0±1.5 _{c,A}	8.2±0.7 _{a,A}	19.4±6.7 _{a,B}	20.3±2.2 _{a,B}	19.6±5.6 _{a,B}	27.7±7.8 _{a,B}
C18:4n3	5.9±1.1 _{a,b}	6.2±0.8 ^b _B	6.5±0.8 ^b	4.7±0.3 _{a,B}	nd	0.5±0.6 ^a _A	nd	1.8±0.7 ^b _A
C22:4n6	8.5±1.5 _b	4.2±0.5 ^a	4.5±0.6 ^a _B	8.4±0.3 _{3^b}	nd	nd	0.1±0.1 ^A	nd
Total PUFA	51.0	53.0	55.0	51.0	35.0	42.0	32.0	35.0
PUFA/SFA	0.58	0.52	0.45	0.40	0.73	0.20	0.68	0.44

n6/n3 2.13 1.86 1.83 2.96 0.80 1.00 0.65 0.36

Values are expressed as: Mean±Standard deviation. Nd - not detected. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$)

Table 3 – Vitamin E contents (mg/100 g dw) in borage and centaurea at three flowering stages.

Tocols (mg/100g)	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)
α -tocopherol	2.75±0.16 ^{c,B}	2.24±0.05 ^{a,A}	2.21±0.06 ^{a,B}	2.47±0.11 ^{b,A}	1.32±0.20 ^{a,b,A}	1.78±0.39 ^{b,A}	1.24±0.01 ^{a,A}	3.47±0.29 ^{c,B}
β -tocopherol	0.33±0.01 ^{b,A}	0.30±0.01 ^{a,A}	0.29±0.01 ^{a,A}	0.33±0.01 ^{b,A}	1.01±0.07 ^{c,B}	0.61±0.06 ^{b,B}	0.66±0.07 ^{b,B}	0.41±0.02 ^{a,B}
γ -tocopherol	0.34±0.01 ^{a,B}	0.43±0.01 ^{c,B}	0.43±0.01 ^{c,B}	0.38±0.02 ^{b,A}	0.32±0.01 ^{b,A}	0.34±0.04 ^{b,A}	0.28±0.01 ^{a,A}	0.39±0.01 ^{c,A}
δ -tocopherol	0.24±0.01 ^{a,A}	0.25±0.01 ^b	0.24±0.01 ^{a,A}	0.24±0.01 ^{a,b,A}	0.35±0.01 ^{b,B}	nd	0.26±0.01 ^{a,A}	0.34±0.03 ^{b,B}
Total tocopherol	3.7	3.2	3.2	3.4	3.0	2.7	2.4	4.6
β -tocotrienol	0.23±0.01 ^b	0.28±0.01 ^c	0.28±0.01 ^c	0.22±0.01 ^a	nd	nd	nd	nd
γ -tocotrienol	0.23±0.01 ^b	0.20±0.01 ^a	0.19±0.01 ^a	0.25±0.01 ^c	nd	nd	nd	nd
Total tocotrienol	0.5	0.5	0.5	0.5	nd	nd	nd	nd

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$).

Table 4 - Total and individual carotenoids in borage and centaurea at three flowering stages and petals.

Flowers	Flowering stages	Total carotenoids (mg β -carotene/100 g dw)	β -carotene (mg β -carotene equivalent/100 g dw)	Lutein
Borage	Tight bud	23.5±4.2 ^{b,B}	0.45±0.03 ^{b,B}	13.04±0.61 ^{b,B}
	Mature bud	23.8±0.1 ^{b,B}	1.04±0.07 ^{c,B}	14.95±0.32 ^{c,B}
	Fully open	181.4±13.9 ^{c,B}	8.50±0.61 ^{d,B}	43.73±2.24 ^{d,B}
	(Petals)	3.3±0.5 ^{a,A}	0.04±0.01 ^{a,A}	0.73±0.15 ^{a,A}
Centaurea	Tight bud	0.2±0.2 ^{a,A}	0.002±0.001 ^{a,A}	0.03±0.01 ^{a,A}
	Mature bud	7.8±0.3 ^{c,A}	0.041±0.005 ^{b,A}	1.16±0.06 ^{c,A}
	Fully open	5.8±1.0 ^{b,A}	0.036±0.003 ^{b,A}	1.08±0.03 ^{c,A}
	(Petals)	4.8±0.2 ^{b,B}	0.091±0.039 ^{c,B}	0.69±0.21 ^{b,A}

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$)

Table 5 –Organic acids composition in borage and centaurea at three flowering stages (g/100 g dw)

Organic acids	Borage				Centaurea			
	Tight bud	Mature bud	Fully open	(Petals)	Tight bud	Mature bud	Fully open	(Petals)

Citric acid	1.73±0.05 ^{d,A}	0.99±0.01 ^{c,A}	0.92±0.02 ^{b,A}	0.52±0.02 ^{a,A}	2.76±0.04 ^{d,B}	2.20±0.12 ^{c,B}	1.88±0.08 ^{b,B}	1.09±0.06 ^{a,B}
Levulinic acid	0.69±0.03 ^{a,A}	1.20±0.07 ^{b,B}	1.17±0.07 ^{b,B}	1.58±0.15 ^{c,B}	0.77±0.03 ^{c,B}	0.70±0.01 ^{b,A}	0.86±0.06 ^{d,A}	0.53±0.03 ^{a,A}
Fumaric acid	0.75±0.06 ^{a,B}	0.91±0.07 ^{b,B}	0.87±0.01 ^{b,B}	1.30±0.08 ^{c,B}	0.018±0.001 ^{b,A}	0.017±0.001 ^{b,A}	0.017±0.001 ^{b,A}	0.013±0.001 ^{a,A}
Succinic acid	0.56±0.01 ^{b,A}	0.51±0.01 ^{a,A}	0.49±0.01 ^{a,A}	0.55±0.03 ^{b,A}	1.89±0.09 ^{a,B}	2.94±0.16 ^{b,B}	3.62±0.15 ^{c,B}	9.68±0.21 ^{d,B}
Malic acid	2.09±0.05 ^{a,A}	2.94±0.10 ^{b,B}	2.88±0.03 ^{b,B}	4.66±0.29 ^{c,B}	2.17±0.03 ^{c,B}	2.09±0.23 ^{c,A}	1.84±0.09 ^{b,A}	1.47±0.09 ^{a,A}
Salicylic acid	0.010±0.001 ^{a,A}	0.012±0.001 ^{c,A}	0.011±0.001 ^{b,A}	0.010±0.001 ^{a,A}	0.019±0.001 ^{b,B}	0.019±0.001 ^{b,B}	0.017±0.001 ^{a,B}	0.019±0.001 ^{b,B}
Hydroxycinnamic acid	0.24±0.01 ^{a,B}	0.22±0.01 ^{a,A}	0.22±0.01 ^{a,A}	0.49±0.06 ^{b,A}	0.21±0.02 ^{a,A}	0.27±0.03 ^{b,B}	0.35±0.02 ^{c,B}	0.49±0.05 ^{d,A}
Malonic acid	0.055±0.006 ^{a,A}	0.057±0.002 ^{a,A}	0.053±0.002 ^{a,A}	0.064±0.003 ^{b,A}	0.77±0.04 ^{a,B}	0.73±0.03 ^{a,B}	0.70±0.07 ^{a,B}	1.13±0.02 ^{b,B}
Total	6.1	6.8	6.6	9.2	8.6	9.0	9.3	14.4

Values are expressed as: Mean±Standard deviation. Lowercase letters compared different flowering stages in the same flower species. Uppercase letters compared both flowers at the same flowering stage. Values with the same letter are not statistically different ($p>0.05$)

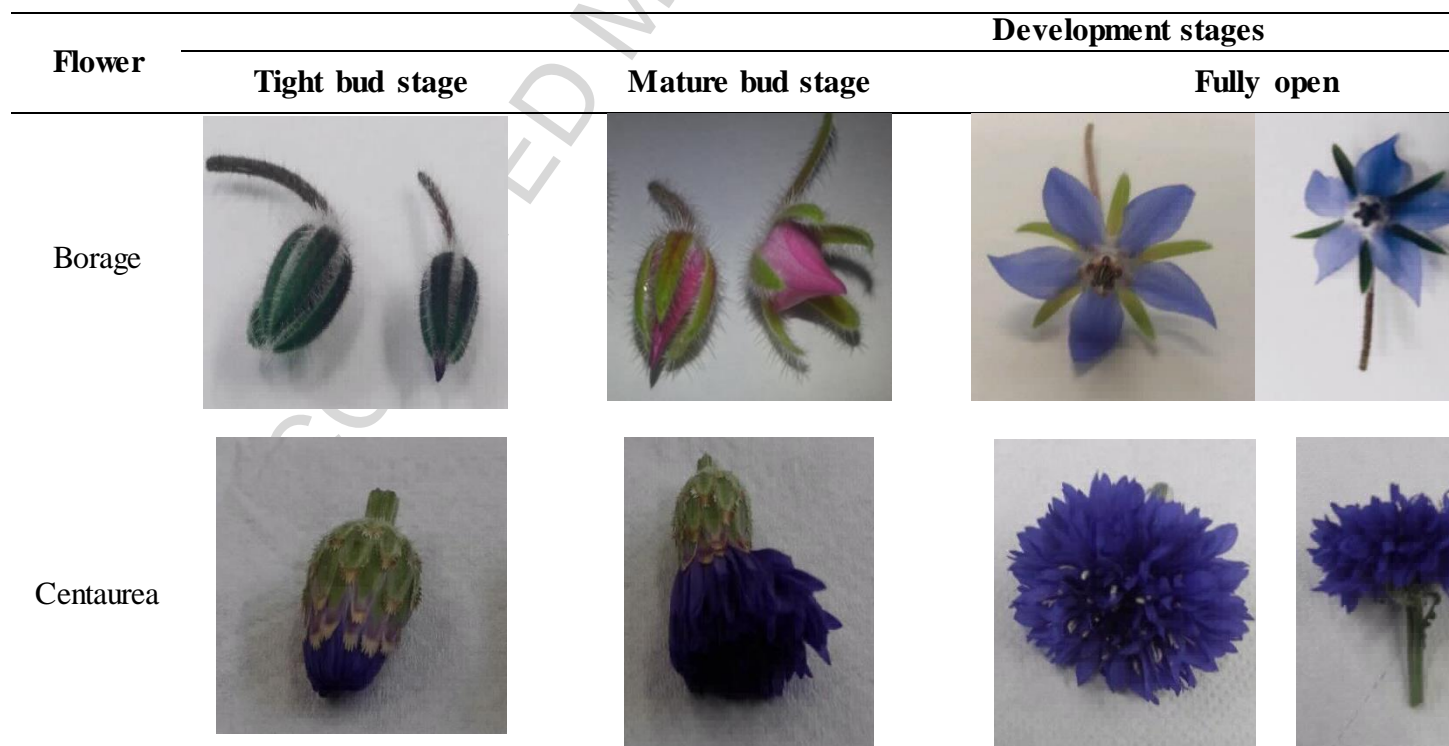
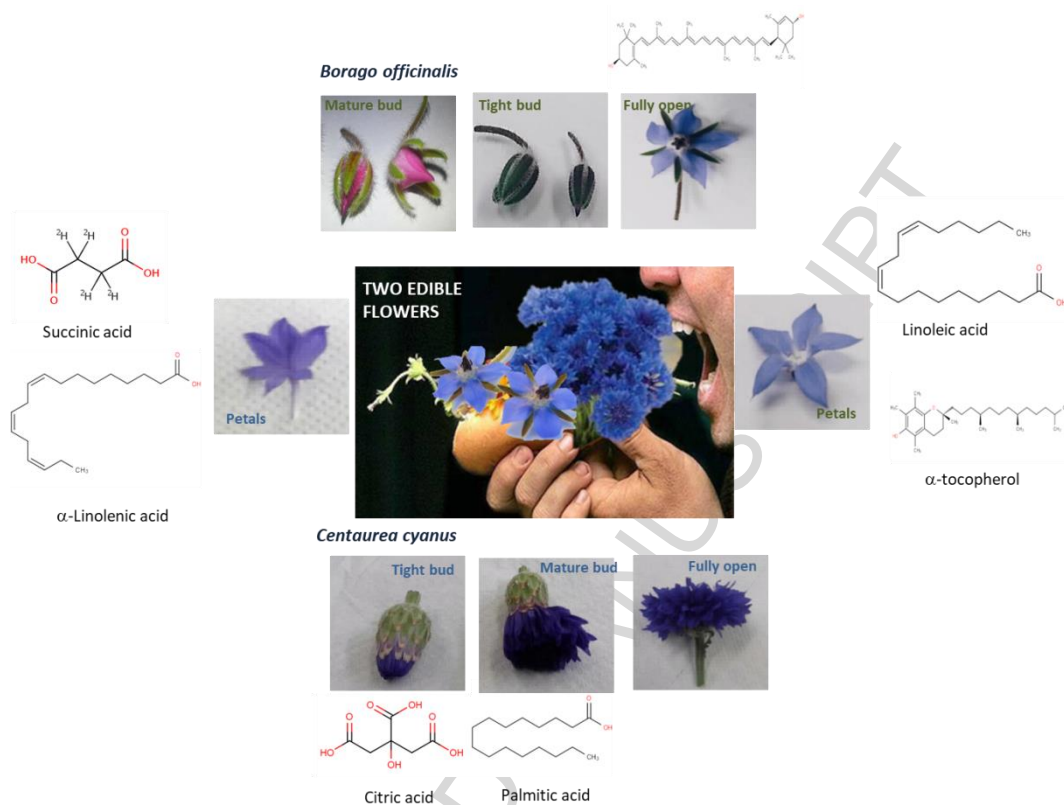


Figure 1

Graphical abstract:



Highlights

- Borago and centaurea showed different nutritional and chemical compositions.
- Centaurea presented the highest values of energy and total dietary fiber.
- Borago showed the highest PUFA and total carotenoids contents.
- Differences in phytochemical profiles were detected along flowering.
- Borago true petals presented the lowest values of protein and total carotenoids.