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Borage, camellia, centaurea and pansies: Nutritional, fatty acids, free sugars, vitamin E, carotenoids and organic acids characterization

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ABSTRACT

The present study aimed to evaluate the nutritional and bioactive potential of four edible flowers (borage, centaurea, camellia, and pansies). Significant differences were observed among the four. Water was the main constituent (> 76%, fresh weight - fw). Linoleic and palmitic acids were the major fatty acids found in borage and red and yellow pansies, while in camellia it was the arachidic acid. In white pansies, behenic and arachidic acids were predominant. Concerning vitamin E, α-tocopherol was the major vitamer. Carotenoids values varied between 5.8 and 181.4 mg β-carotene/100 g dry weight (dw) in centaurea and borage, respectively, being particularly rich in lutein. Malic acid was the major organic acid, except in centaurea, where succinic acid was predominant. Fructose, glucose and sucrose were detected in all flowers. These results can contribute to the knowledge of these edible flowers and consequently increase their popularity among consumers and in the food industry.

1. Introduction

Edible flowers have recently become a new crop, usually grown in conjunction with cut flowers, herbs, and lettuces, to complement producers' income and create opportunities for added-value products. Edible flower production is already common in the Far East, as the consumption of flowers has been practiced around the world since the earliest. Nevertheless, the practice of using edible flowers is still relatively new in modern cuisines, being used either for decorative (adding new colors) or sensory (new textures and flavors) purposes, or for their potential health benefits. However, consumers around the world are becoming health conscious and attracted by the idea of trying new foodstuffs; therefore, the search for new food sources and supplements has increased. In this respect, edible flowers can become a possibility, since the use of flowers as food can be said to be "good for health" (Rodrigues et al., 2017). Thus, it is necessary to help producers by giving them information about the composition of the flowers, to develop strategies to introduce them to the consumers' table.

Globally, the nutritional composition of edible flowers is not different from that of other plant organs (Mlcek & Rop, 2011). Nevertheless, recent studies have been conducted on edible flowers to focus on the analysis of bioactive compounds (carotenoids, flavonoids and anthocyanins) and their antioxidant activity (Chen, Chen, Xiao, & Fu, 2018; Loizzo et al., 2016; Lu, Li, & Yin, 2016; Navarro-González, González-Barrio, García-Valverde, Bautista-Ortín, & Periago, 2015; Petrova, Petkova, & Ivanov, 2016; Rachkeeree et al., 2018). Such studies are justified by the impact of these phytochemicals' on human health and their properties to prevent some diseases. In particular, pansies, centaurea, borage, and camellia are flowers usually included by gourmet chefs in their dishes and sold at specialized sale points for edible flowers around the world, such as Ervas Finas®, BloomBites®, Meadowsweet Flowers®, and Petite Ingredient®. Specifically, pansies present a wide range of petal colours and have a perfumed and sweet taste, which are characteristics valued by chefs and consumers alike (Kelley, Behe, Biernbaum, & Poff, 2004). Also, borage is crispy and has the flavour of cucumber, and centaurea is clove-like, which make them suitable for certain dishes. Furthermore, although camellia can be used as a garnish, it can also be dried and then cooked, as in the case of Asian cuisine. Edible flowers can be consumed on their own fresh, or as a functional food ingredient or an important component of cosmetics and pharmaceutical products. Given these attributes, these flowers are already quite commonly used. However, few studies have focused on their nutritional and bioactive properties.

Concerning the four flowers studied (borage, camellia, centaurea

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and pansies), some studies have already been done on the nutritional and mineral compositions of centaurea and pansies by Rop, Mlcek, Jurikova, Neugebauerova, and Vabkova (2012) and Vieira (2013). Other studies have focused on the antioxidant properties, namely of borage (Aliakbarlu & Tajik, 2012), pansies (Gamsjaeger, Baranska, Schulz, Heiselmayer, & Musso, 2011; González-Barrio, Periago, Luna-Recio, Garcia-Alonso, & Navarro-González, 2018; Skowyra, Calvo, Gallego, Azman, & Almajano, 2014) and centaurea (Lockowandt et al., 2019), as well as phenolic compounds of camellia (Nakajima, Itokawa, & Ikuta, 1984). However, further studies are needed on carotenoids, fatty acids, vitamin E, free sugars, and organic acids to encourage their consumption and use. Since only a small amount of species of edible flowers has been studied, more works are required to explore their potential as food or as natural additives (resulting from the presence of bioactive compounds). Based on this premise, the aim of this study was to determine the nutritional composition and identify some bioactive compounds in four species of edible flowers that are commonly used by chefs, namely: borage (*Borago officinalis* L.), camellia (*Camellia japonica* L.), centaurea (*Centaurea cyanus* L.) and pansies (*Viola* × *wittrockiana* Gams.*)* (white, yellow and red). Furthermore, the present work also investigated if flowers with different colors of the same species (*Viola* × *wittrockiana*) have different nutritional composition and bioactive profile. The novelty of the present study was to characterize more edible flowers species and to find potential compounds that could be used as a new and prospective source for the food industry, as well as to underline the possible nutritional differences between pansies with different colors.

2. Material and methods

2.1. Standards and reagents

All reagents were of analytical, chromatographic or spectroscopic grade. HPLC grade *n*-hexane was purchased from Merck (Darmstadt, Germany) and 1,4-dioxane boron trifluoride in methanol (14%), butylated hydroxytoluene (BHT), and ascorbic acid were obtained from Sigma (Madrid, Spain). Methanol and KOH were acquired from Panreac (Barcelona, Spain). The remaining reagents were supplied by Merck (Darmstadt, Germany) or Sigma–Aldrich (St. Louis, USA). Concerning standards of tocopherols (α, β, γ, and δ) and tocotrienols (α, β, γ, and δ), these were purchased from Calbiochem (La Jolla, USA) and Sigma–Aldrich (St. Louis, USA). The internal standard for vitamin E quantification was tocol (2-methyl-2-(4,8,12-trimethyltridecyl) chroman-6-ol) and was obtained from Matreya LLC (State College, USA). A 1 μg/mL solution was prepared in *n*-hexane and kept at −20 °C. Triundecanoin was used as the internal standard for fat estimation, based on the total fatty acid amounts, and was purchased from Sigma–Aldrich (St. Louis, USA). A 10 mg/mL solution of triundecanoin was prepared in *n*-hexane. A certified fatty acids methyl ester (FAME) reference standard mixture (37 fatty acids from C4 to C24) from Supelco (Bellefonte, USA) was used for the calibration of the flame ionization detector (FID) signals. Concerning carotenoid, organic acid and sugar standards, all were obtained from Sigma–Aldrich (St. Louis, USA).

2.2. Samples

Blue borage (*Borago officinalis* L.), camellia (*Camellia japonica* L.), blue centaurea (*Centaurea cyanus* L.) and pansies (*Viola* × *wittrockiana* Gams.) (white, yellow and red pansies) were collected at full flowering stage (Fig. 1), from the greenhouse of the School of Agriculture, Polytechnic Institute of Bragança (Portugal). The air temperature and relative humidity in the greenhouse were not controlled, although the flowers were grown under organic production mode and the same environmental and agronomic conditions. For further analysis, 250 g of each species of flower were harvested. After harvest, the fresh flowers were immediately transported to the laboratory under refrigerated conditions. For the analysis of pansies, centaurea and borage, the whole flower was considered since in these species all parts are typically consumed. In camellia, only the petals were studied because the pollen was removed due to possible allergies as well as sepals. The 250 g of each species were divided into three independent samples, and the analyses performed as described in the following sections.

2.3. Nutritional composition

The nutritional composition (moisture, ash, fat and dietary fiber) of each flower species was determined by following the AOAC procedures AOAC (1995) and expressed as g/100 g 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 a minimum of 2 h, until obtaining white ashes. The protein content of the samples was estimated by the macro-Kjeldahl method, with a conversion factor of 6.25, following the methodologies described by Sotelo, López-García, and Basurto-Peña (2007) and Rop et al. (2012). Total fat content was determined by extracting a mass of powdered sample with petroleum ether with butylated hydroxytoluene (BHT), using a Soxhlet apparatus. Dietary fiber was determined by an enzymatic-gravimetric method based on the AOAC official method No. 985.29 (AOAC, 2003).

2.4. Extraction of lipid components

A 250 mg freeze-dried sample was weighed and two internal standard solutions were added: tocol (20 μL; 1 mg/mL) for vitamin E quantification and triundecanoin (200 μL; 1 mg/mL) for total fatty acids quantification, followed by two antioxidants: BHT (20 μL, 10 mg/mL in methanol) and ascorbic acid (50 mg). Propan-2-ol (1.6 mL) and cyclohexane (2.0 mL) were added for lipid extraction. The lipid extraction conditions applied were those reported by Cruz et al. (2013) (Fig. 1S).

2.5. Fatty acids

The fatty acid profiles were determined with a Chrompack CP 9001 chromatograph (Chrompack, Middelburg, Netherlands) equipped with a split-splitless injector, a Chrompack CP-9050 autosampler and a flame ionization detector (FID). Helium was used as carrier gas at an internal pressure of 180 kPa. The temperatures of the detector and injector were 250 °C and 270 °C, respectively. Separation was achieved on a 100-m × 0.25-mm ID Select-FAME column (0.19-μm film; Agilent, Santa Clara, USA). The oven temperature was programmed at 180 °C for a period of 35 min and then programmed to increase to 250 °C at a rate of 3 °C/min. The total analysis time was 60 min. The split ratio was 1:50, and the injected volume was 1.2 μL. Fatty acids identification (from C11:0 to C22:6) was accomplished by comparing the relative retention times of FAME peaks with standards from diversified suppliers, taken from literature data, and confirmed by GC-MS on an 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, the FID peaks were corrected using response factors obtained with standard FAME solutions. The fatty acids results were calculated on a relative percentage basis.

2.6. Free sugars

Free sugars were determined by following the procedure mentioned by Barros, Oliveira, Carvalho, and Ferreira (2010), with minor modifications. One gram of dried sample powder was extracted with 40 mL of 80% aqueous ethanol (v/v) at 80 °C for 30 min. The resulting suspension was filtered with a vacuum pump (KNF LABOPORT, Darmstadt, Germany). The supernatant was concentrated at 45 °C in a rotary

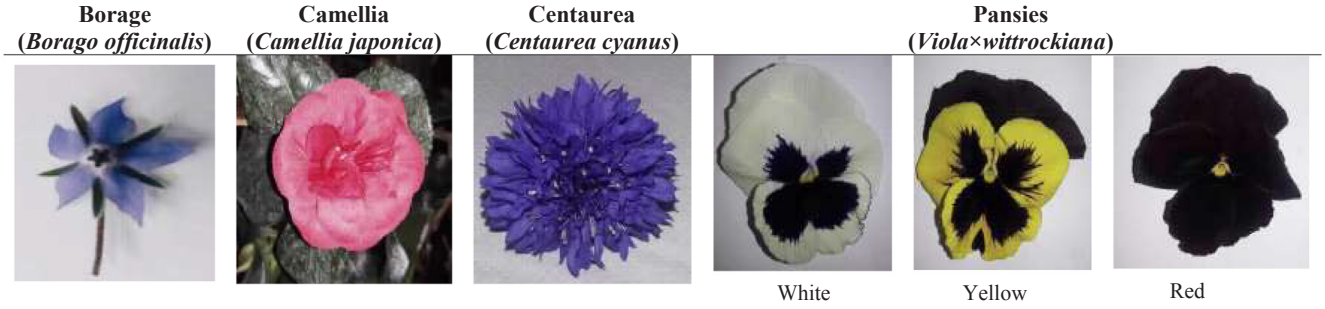

Fig. 1. Edible flowers studied in the present work: borage, camellia, centaurea and pansies (white, yellow, red).

evaporator (Stuart, RE300DB, Stone, UK) and defatted three times in succession with 10 mL of ethyl ether. After concentration at 40 °C, the solid residues were dissolved in water to a final volume of 3 mL and filtered through 0.2 μm nylon filters from Whatman.

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 ID, 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 on g/100 g dw.

2.7. Vitamin E and carotenoids

Vitamin E and carotenoids compositions were determined, according to Cruz and Casal (2018) (Fig. 1S). The separation was achieved by normal-phase HPLC. 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 diode array detector (DAD) (MD-910, recorded at 450 nm), connected to a fluorescence detector (FD) (FP-2020 Plus; $\lambda_{excitation}$ = 290 nm and $\lambda_{emission}$ = 330 nm). The chromatographic separation was achieved on a Luna Silica column (100 mm × 3 mm; 3 μm) (Phenomenex, Torrance, 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 with authentic standards. Tocopherols and tocotrienols (α-, β-, γ-, and δ-) were acquired from Supelco (Bellefonte, USA) and Larodan AB (Solna, Sweden). Carotenoids standards (all-*trans*-β-carotene and lutein) were from Sigma-Aldrich (St. Louis, USA). Identified tocopherols and carotenoids were quantified by the internal standard method, being reported on a dw basis.

Simultaneously, the total carotenoid contents were determined by spectrophotometry, according to the method used by Aquino-Bolaños, Urrutia-Hernández, Del Castillo-Lozano, Chavéz-Servia, and Verdalet-Guzmán (2019). One gram of frozen-dried powder of each sample was extracted twice with a 20 mL acetone:hexane solution (1:1, v/v). Both extracts were put into a separation funnel, 200 mL of distilled water being added to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to remove any residual water, the remaining solution was 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 μg/mL). Results were expressed in mg β-carotene/100 g dw.

2.8. Organic acids

The organic acids in edible flowers were estimated by gas chromatography, after methylation, following the methods reported by Sharma et al. (2016) and Kumar, Sharma, Bhardwaj, and Thukral (2017), using a column HP-5MS (30 m × 0.25 mm ID × 0.25 μm thickness ultra-inert capillary column, Agilent Technologies) instead of the DB-5 ms column mentioned by the authors. Individual standards of citric, levulinic, fumaric, succinic, malic, salicylic, hydroxycinnamic, malonic, oxalic, tartaric, and benzoic acids, were obtained from Sigma-Aldrich (St. Louis, USA), and 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).

2.9. Statistical analysis

The statistical analysis was performed on SPSS software, Version No. 18.0 (SPSS Inc., Chicago, USA). The normality of the data was verified by the Shapiro-Wilk test. Analysis of variance (ANOVA) or ANOVA Welch were carried out to determine if there were significant differences ($p < 0.05$) between samples, depending on the existence or not of homogeneity of variances. Additionally, if significant differences were detected between species, a post hoc analysis was performed, namely: Tukey's honestly significant difference test (if variances in the different groups were identical) or Games-Howell test (if they were not). The homogeneity of the variances was tested by Levene's test.

Table 1
Nutritional composition (g/100 g fw) of borage, camellia, centaurea and pansies.

Components	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola* × *wittrockiana*)		
				White	Yellow	Red
Moisture	86.6 ± 1.8^{b}	87.7 ± 1.3^{b,c}	76.7 ± 0.6^{a}	91.3 ± 2.1^{c}	86.5 ± 0.7^{b}	85.1 ± 0.3^{b}
Ash	2.05 ± 0.11^{c}	0.37 ± 0.01^{a}	1.20 ± 0.11^{b}	0.92 ± 0.08^{b}	1.10 ± 0.19^{b}	0.94 ± 0.13^{b}
Protein	3.04 ± 0.10^{d}	0.76 ± 0.20^{a}	1.60 ± 0.06^{b}	2.03 ± 0.06^{c}	2.06 ± 0.03^{c}	1.36 ± 0.03^{b}
Lipid	0.66 ± 0.15^{a,b}	0.31 ± 0.07^{a}	0.80 ± 0.03^{b}	0.45 ± 0.01^{a,b}	1.31 ± 0.09^{c}	0.67 ± 0.03^{a,b}
Total dietary fiber	4.74 ± 0.10^{b,c}	6.71 ± 1.76^{c}	15.7 ± 0.5^{d}	1.50 ± 0.09^{a}	4.32 ± 0.02^{b}	3.79 ± 0.23^{b}

Mean ± Standard deviation. fw – fresh weight. Values with the same letter in the same row are not statistically different (p > 0.05).

3. Results and discussion

3.1. Nutritional composition

The nutritional composition of the four edible flowers is shown in Table 1. In general, the four flowers differed significantly in their nutritional composition. Water was the main constituent (76.7–91.3 g/100 g fw). Total dietary fiber and proteins ranged between 1.50 and 15.7, and 0.76 to 3.04 g/100 g fw, respectively. Fat was the least abundant macronutrient, ranging between 0.31 and 1.31 g/100 g fw in camellia and yellow pansies, respectively.

Borage had the highest mineral (2.05 g/100 g fw) and protein contents (3.04 g/100 g fw). In contrast, camellia presented the lowest ash (0.37 g/100 g fw), protein (0.76 g/100 g fw) and fat (0.31 g/100 g fw) contents (Table 1). Centaurea also presented one of the lowest protein contents (1.60 g/100 g fw, respectively); however, it had the highest content of total dietary fiber (15.7 g/100 g fw), and approximately nine-fold greater than white pansies, which reported the lowest amount (1.50 g/100 g fw). Concerning the total dietary fiber content obtained for borage and camellia, these were similar to those reported for oat flakes (6.7 g/100 g fw), showing the importance of this component in some edible flowers. Concerning centaurea, the results were different to those described by Pires, Dias, Barros, and Ferreira (2017), who reported lower values of fat; however, this macronutrient was only present in small quantities. Furthermore, Rop et al. (2012) also reported a lower value than ours for protein (0.673 g/100 g fw) for centaurea flowers. Grzeszczuk, Stefaniak, and Pachlowska (2016) described similar moisture (86 g/100 g fw), ash (2.23 g/100 g fw) and protein (2.19 g/100 g fw) contents to ours for borage flowers.

Between pansies of different colors, significant differences were detected ($p < 0.05$). White pansies showed significantly higher values of moisture (91.3 g/100 g fw) than other flowers, while yellow pansies presented the highest fat content (1.31 g/100 g fw). White pansies presented the lowest dietary fiber content. For pansies, Rop et al. (2012) detected similar values compared to ours for moisture (90%), but lower values for protein (0.670 g/100 g fw). Furthermore, González-Barrio et al. (2018) also studied pansies and obtained similar values for moisture (86.32 g/100 g fw), protein (2.11 g/100 g fw), fat (0.44 g/100 g fw), ash (1.11 g/100 g fw), and fiber (5.09 g/100 g fw).

3.2. Fatty acids

The results obtained for the individual fatty acids of the studied edible flowers are shown in Table 2. Each flower showed a different fatty acids profile. Twenty-one fatty acids were identified and quantified, and borage was the flower with the highest number of fatty acids detected. Linoleic acid (C18:2n6) and palmitic acid (C16:0) were the major fatty acids found in borage, and in red and yellow pansies, respectively. The linoleic acid ranged from 3.3 to 27.2% in camellia and yellow pansies, respectively; palmitic acid varied between 1.5 and 25.9% in white pansies and centaurea, respectively. Ramandi, Najafi, Raofie, and Ghasemi (2011) reported a similar profile in borage, where the major fatty acids were palmitic (39.4%), linoleic (26.4%), and oleic (21.1%) acids. However, these authors detected higher percentages than those of the present study. Centaurea and red pansies also showed high percentages of oleic acid (C18:1) (25.9 and 14.0%, respectively). The results found in the present work for centaurea flowers were not in accordance with Pires et al. (2018), who reported that the main fatty acids were eicosapentaenoic acid (C20:5n3; 26.9%) and linolenic acid (C18:3n3; 18.8%), while in the present study they were palmitic acid (C16:0; 25.9%) and oleic acid (C18:1; 25.9%). Camellia presented high percentages of arachidic acid (C20:0, 42.5%), the main fatty acid, followed by palmitoleic acid (C16:1, 23.7%). At the same time, in white pansies it was the behenic acid (C22:0, 24.6%), closely followed by the arachidic acid (C20:0, 24.3%). Pansies with different colors showed a different profile of fatty acids, the yellow pansies being the one with the highest number of different fatty acids.

Globally, polyunsaturated fatty acids (PUFA) were predominant in borage and yellow pansies, while the remaining flowers showed higher concentrations of saturated fatty acids (SFA). Monounsaturated fatty acids (MUFA) were detected in minor amounts in all flowers, ranging from 6 to 27%.

Concerning PUFA/SFA ratios, all flowers were above 0.45, the minimum value recommended by the British Health Department (HMSO, 1994). Furthermore, in the flowers where n-6 and n-3 fatty acids were detected, the ratio of both was calculated, being lower than 4.0, which is recommended for the human diet (Guil, Torija, Gimeénez, & Rodriguez, 1996). High PUFA/SFA and low n-6/n-3 ratios are associated with health benefits, such as a decrease of the “bad cholesterol” in mice blood (Liu et al., 2016), the control of metabolic disorders symptoms, including obesity, insulin resistance, inflammation, and lipid profiles (Liu et al., 2013), and the reduction in the risk of cancer, cardiovascular and inflammatory diseases.

3.3. Tocopherols

The content and composition of tocopherols vary significantly among plant tissues, with higher levels in seeds compared to photosynthetic tissues (Saini and Keum, 2016). Tocopherols are bioactive constituents in human diet. They are well known for their antioxidant properties and potential health benefits, such as anti-inflammatory (Mocchegiani et al., 2014), hypolipidemic (Minhajuddin, Beg, & Iqbal, 2005), and antiatherogenic (Kirmizis & Chatzidimitriou, 2009) effects. However, recent dietary studies show that the recommended daily allowance in terms of tocopherols is often not met. So, improving their quantity in food has become imperative (Péter et al., 2015). Vitamin E composition of the four edible flower species is shown in Table 3. Four tocopherols (α-, β-, γ-, and δ-tocopherol) and two tocotrienols (β- and γ-tocotrienol) were identified and quantified in almost all flowers (Table 3 and Fig. 2S-A). However, significant differences between them were observed ($p < 0.05$). In general, the major component in all samples was α-tocopherol, ranging between 0.67 and 22.21 mg/100 g dw for red and yellow pansies, respectively. This vitamer has an important role in lipid peroxidation inhibition (Ouchikh et al., 2011). γ-Tocopherol was the second most abundant tocopherol detected, except in centaurea, which was β-tocopherol. Regarding total tocopherols, yellow pansies presented the highest content (24.89 mg/100 g dw), mainly due to the presence of α-tocopherol. Pires et al. (2017) only detected α- and γ- tocopherols in centaurea petals, while in our study β- and δ- tocopherols were also identified. Furthermore, values two-fold lower than ours were determined for α-tocopherol (0.55 mg/100 g dw), while a similar value of γ-tocopherol (0.29 mg/100 g dw) was reported (Pires et al., 2017). Regarding tocotrienols, they were not detected in centaurea and red pansies. Borage and camellia flowers showed higher amounts of β-tocotrienol, followed by γ-tocotrienol. Despite not being considered as a great source of tocopherols, due to their low fat content, edible flowers can contribute to the supply of vitamin E to the human organism. Nevertheless, the daily recommended dose for tocopherols for adults is 300 mg/day (EFSA, 2008), meaning that a large quantity of edible flowers would have to be daily ingested.

3.4. Carotenoids

Carotenoids are lipophilic pigments that accumulate in flowers, being responsible for color, which attract pollinators. Flower petals have a wide range of carotenoids levels, depending on the plant species or cultivar (Ohmiya, 2013). The profile and total carotenoids content are shown in Table 4. The studied edible flowers presented significantly different ($p < 0.05$) carotenoid contents, ranging between 5.8 and 181.4 mg β-carotene equivalent/100 g dw in centaurea and borage, respectively. Regarding pansies with different colors, the values of total carotenoids varied between 21.6 and 109.2 mg β-carotene/100 g dw.

Table 2
Fatty acids composition (relative %) in borage, camellia, centaurea and pansies.

Fatty acids	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola* × *wittrockiana*)		
				White	Yellow	Red
SFA						
C12:0	0.46 ± 0.07^{a}	0.14 ± 0.12^{a}	0.62 ± 0.17^{a}	0.56 ± 0.06^{a}	7.65 ± 0.33^{c}	6.24 ± 0.89^{b}
C14:0	0.67 ± 0.14^{a}	0.43 ± 0.09^{a}	0.71 ± 0.16^{a}	2.10 ± 0.82^{a}	10.47 ± 0.44^{b}	19.30 ± 2.16^{c}
C15:0	0.25 ± 0.04^{b}	nd	nd	0.05 ± 0.08^{a}	0.13 ± 0.01^{a}	0.22 ± 0.08^{b}
C16:0	17.11 ± 0.66^{b}	nd	25.88 ± 4.39^{d}	1.51 ± 0.13^{a}	20.39 ± 0.68^{b,c}	23.12 ± 1.38^{c,d}
C17:0	0.87 ± 0.15^{c}	0.10 ± 0.11^{a}	0.59 ± 0.34^{b,c}	0.30 ± 0.30^{a,b}	0.54 ± 0.08^{b,c}	0.76 ± 0.24^{c}
C18:0	4.80 ± 0.36^{c}	0.86 ± 0.24^{a}	7.16 ± 1.74^{d}	0.86 ± 0.15^{a}	2.97 ± 0.16^{b}	4.35 ± 0.32^{c}
C20:0	1.80 ± 0.23^{a}	42.54 ± 2.56^{c}	2.50 ± 0.72^{a}	24.31 ± 0.81^{b}	0.55 ± 0.02^{a}	0.65 ± 0.04^{a}
C22:0	2.49 ± 0.38^{d}	0.11 ± 0.18^{a}	1.45 ± 0.49^{c}	24.57 ± 0.81e	0.93 ± 0.08^{b,c}	0.64 ± 0.14^{a,b}
C24:0	2.58 ± 0.34^{c}	0.38 ± 0.30^{a}	1.55 ± 0.64^{b}	0.23 ± 0.28^{a}	1.24 ± 0.39^{b}	1.71 ± 0.54^{b}
Total SFA	31	45	40	54	45	57
MUFA						
C16:1	0.19 ± 0.07^{a}	23.67 ± 0.96^{c}	0.32 ± 0.19^{a}	19.17 ± 0.60^{b}	0.32 ± 0.09^{a}	0.37 ± 0.23^{a}
C18:1	9.90 ± 8.77^{a}	nd	25.87 ± 17.06^{b}	nd	5.17 ± 4.11^{a}	14.02 ± 7.01^{a,b}
C20:1	0.32 ± 0.02^{c}	0.45 ± 0.04^{d}	nd	0.46 ± 0.05^{d}	0.06 ± 0.03^{a,b}	0.12 ± 0.11^{b}
C22:1	1.79 ± 0.19^{c}	1.25 ± 0.14^{b,c}	0.88 ± 0.81^{a,b}	nd	0.27 ± 0.21^{a}	0.38 ± 0.32^{a}
C24:1n9	1.95 ± 0.26^{b}	0.10 ± 0.13^{a}	nd	0.09 ± 0.22^{a}	nd	0.22 ± 0.26^{a}
Total MUFA	14	25	27	20	6	15
PUFA						
C18:2n6	21.23 ± 2.22^{c}	3.34 ± 0.63^{a}	12.77 ± 2.91^{b}	4.52 ± 0.60^{a}	27.17 ± 1.77^{d}	20.68 ± 1.87^{c}
C18:3n6	9.64 ± 1.37^{b}	7.41 ± 2.88^{b}	nd	3.10 ± 0.48^{a}	nd	nd
C18:3n3	12.89 ± 1.48^{b}	nd	19.64 ± 5.61^{c}	nd	21.15 ± 1.43^{c}	5.92 ± 0.87^{a}
C18:4n3	6.50 ± 0.79^{b}	14.48 ± 1.37^{c}	nd	16.78 ± 0.78^{d}	0.87 ± 0.11^{a}	1.27 ± 0.22^{a}
C20:2n6	nd	0.11 ± 0.18^{b}	nd	nd	0.08 ± 0.01^{a}	nd
C22:3	nd	1.14 ± 0.08^{b}	nd	0.62 ± 0.05^{a}	nd	nd
C22:4n6	4.54 ± 0.64^{c}	1.89 ± 0.37^{b}	0.07 ± 0.07^{a}	0.75 ± 0.66^{a}	0.05 ± 0.05^{a}	nd
Total PUFA	55	30	32	26	49	28
PUFA/SFA	1.77	0.68	0.82	1.10	0.47	0.49
n-6/n-3	1.8	0.8	0.7	0.5	1.2	2.9

Mean ± Standard deviation. nd - not detect. Values with the same letter in the same row are not statistically different ($p > 0.05$).

So, pansies of different colors showed great differences in carotenoids. Lower values of total carotenoids were reported by González-Barrio et al. (2018), namely 146 μg/g dw, probably because they used a mixture of colors. Two pigments were identified and quantified in all samples, namely lutein and β-carotene (Table 4). Except for camellia, lutein was always at higher concentrations than β-carotene, ranging between 0.42 and 43.73 mg β-carotene equivalent/100 g dw (camellia and borage, respectively). Lutein is a macular pigment, which is not synthesized by humans and thus must be obtained from food. Furthermore, some studies reported that high dietary intake and higher serum levels of lutein are associated with a lower risk of age-related macular degeneration (Scripsema, Hu, & Rosen, 2015). According to literature, other edible flowers also showed lutein as the main xanthophyll, such as chrysanthemum (1.18–30.7 mg/100 g dw) (Park et al., 2015), snapdragon (1.41 mg/100 g dw) (González-Barrio et al., 2018), garden nasturtium (35.0–45.0 mg/100 g) (Niizu & Rodriguez-Amaya, 2005), marigold (106.2 mg/100 g fw) (Tinoi, Rakariyatham, & Deming, 2006), and crem flowers (24.3 mg/100 g dw) (Bona et al., 2017). Our results are within the values reported by González-Barrio et al. (2018) regarding the carotenoid content in pansies, who detected lutein as the main carotenoid (5.11 mg/100 g dw), followed by β-carotene (4.15 mg/100 g dw). Although white and yellow pansies had a higher proportion of lutein compared to red ones, the latter have 1.9 and 5.0 times more carotenoids than the yellow and white pansies, respectively. However, other carotenoids not detected in the present study have been mentioned by other authors, such as González-Barrio et al. (2018), who reported violaxanthin, antheraxanthin and zeaxanthin, and Gamsjaeger et al. (2011), who mentioned xanthophylls in pansies flowers. Our results were in line with those reported for *Tagetes erecta* L. and *Calendula officinalis* L. flowers, where lutein content increased from the yellow to the dark orange flowers (Gregory, Chen, & Philip, 1986; Pintea, Bele, Andrei, & Socaciu, 2003). Pansies and

Table 3
Vitamin E in borage, camellia, centaurea and pansies (mg/100 g dw).

Tocols	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola* × *wittrockiana*)		
				White	Yellow	Red
α-tocopherol	2.21 ± 0.06^{b}	9.27 ± 0.42^{c}	1.24 ± 0.01^{a}	8.64 ± 0.66^{c}	22.21 ± 0.35^{d}	0.67 ± 0.07^{a}
β-tocopherol	0.29 ± 0.01^{b}	0.27 ± 0.01^{b}	0.66 ± 0.07^{c}	0.66 ± 0.02^{c}	0.64 ± 0.02^{c}	0.17 ± 0.01^{a}
γ-tocopherol	0.43 ± 0.01^{b}	1.39 ± 0.21^{c}	0.28 ± 0.01^{a,b}	1.41 ± 0.13^{c}	1.58 ± 0.05^{c}	0.22 ± 0.01^{a}
δ-tocopherol	0.24 ± 0.01^{a}	nd	0.26 ± 0.01^{a}	0.57 ± 0.03^{c}	0.46 ± 0.01^{b}	nd
Total tocopherols	3.17	10.92	2.43	11.28	24.89	1.07
β-tocotrienol	0.28 ± 0.01^{b}	0.23 ± 0.01^{a}	nd	0.36 ± 0.01^{d}	0.33 ± 0.01^{c}	nd
γ-tocotrienol	0.19 ± 0.01^{a}	0.21 ± 0.01^{b}	nd	nd	nd	nd
Total tocotrienols	0.48	0.44	—	0.36	0.33	–

nd - not detected; Mean ± Standard deviation. Values with the same letter in the same row are not statistically different ($p > 0.05$).

Table 4
Total carotenoids (mg β-carotene/100 g dw) and individual carotenoids (mg β-carotene equivalent/100 g dw) in borage, camellia, centaurea and pansies.

	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola* × *wittrockiana*)		
				White	Yellow	Red
Total carotenoids	181.4 ± 13.9^{e}	24.7 ± 4.1^{b}	5.8 ± 1.0^{a}	21.6 ± 1.0^{b}	58.0 ± 3.6^{c}	109.2 ± 2.2^{d}
β-carotene	8.50 ± 0.61^{d}	0.59 ± 0.49^{a,b}	0.04 ± 0.01^{a}	1.11 ± 0.04^{b}	2.12 ± 0.19^{c}	0.18 ± 0.01^{a}
Lutein	43.73 ± 2.24^{d}	0.42 ± 0.33^{a}	1.08 ± 0.03^{a}	3.65 ± 0.23^{b}	9.99 ± 1.96^{c}	1.18 ± 0.07^{a}

Mean ± Standard deviation. Values with the same letter in the same column are not statistically different ($p > 0.05$)

centaurea presented lutein values similar to those found in fresh carrot (1.4–7.0 mg lutein/100 g dw) (Heinonen, 1990), and borage having slightly higher values than fresh grapes (34.0 mg lutein/100 g dw) (Pinho, Ferreira, Pinto, Benitez, & Hogg, 2001). In general terms, these edible flowers might be a valuable source of lutein.

As expected, red and yellow pansies showed the highest values of total carotenoids (109.2 and 58.0 mg β-carotene/100 g dw, respectively). This observation is related to the fact that these pigments are responsible for the red, yellow and orange colors in plants. In contrast, purple and blue colors are ascribed to other pigments, such as anthocyanins. Thus, centaurea (5.8 mg β-carotene/100 g dw) and camellia (24.7 mg β-carotene/100 g dw) showed the lowest values of total carotenoids, due to the blue and rose color of their petals.

3.5. Organic acids

Organic acids are involved in various fundamental pathways in plant metabolism and catabolism as intermediate or end products. Furthermore, organic acids (usually citric and malic acids) influence the flavor, color and aroma of vegetables and fruits (Vaughan & Geissler, 1997).

The organic acids profile of the four edible flowers is detailed in Table 5. Statistical differences ($p < 0.05$) were observed between samples. Eight organic acids were identified in almost all flowers species. Malic acid was the major organic acid found in the studied edible flowers, except in centaurea. High quantities of succinic (3.62 g/100 g dw), malic (1.84 g/100 g dw) and citric (1.88 g/100 g dw) acids in centaurea flowers were not reported by Pires et al. (2017). This result may be due to the type of sample analyzed. Pires et al. (2017) worked only on petals and not the whole flower as in the present study. Furthermore, Lockowandt et al. (2019) detected lower contents of total organic acids in centaurea flowers (6.63 g/100 g dw), as well as malic (0.36 g/100 g dw), succinic (2.55 g/100 g dw), and fumaric (0.0021 g/100 g dw) acids compared to our results. The second major organic acid detected in borage, camellia, yellow and white pansies was levulinic acid (0.63–4.12 g/100 g dw). At the same time, for centaurea it was citric acid (1.88 g/100 g dw) and for red pansies it was hydroxycinnamic acid (0.82 g/100 g dw). Thus, edible flowers presented distinct organic acids profiles. Comparing flowers, centaurea and white pansies (9.28 and 10.20 g/100 g dw, respectively) showed the highest amounts of organic acids. In contrast, camellia, followed by red pansies, presented the lowest contents (4.15 and 5.61 g/100 g dw, respectively).

Furthermore, the major organic acids found in the four species - malic, citric and succinic acids -, have been linked to important roles in human health. Malic acid is known for its protective effects on myocardial ischemia/reperfusion injury (Tang et al., 2013) and antimicrobial activity against some pathogenic microorganisms (Eswaranandam, Hettiarachchy, & Johnson, 2004); citric acid is a crystal thickener in bones (Hu, Rawal, & Schmidt-Rohr, 2010); and succinic acid is known to help in the treatment of diabetes (Pari & Saravanan, 2007).

3.6. Free sugars

Free sugars composition of the four edible flower species is presented in Table 5. In the edible flowers studied, three free sugars were identified, namely: sucrose, glucose and fructose, varying between 0.49 and 3.86 g/100 g dw, 1.74–13.1 g/100 g dw, and 2.71–16.6 g/100 g dw, respectively. Borage was the flower that presented the highest sucrose content, while camellia was the one that reported the highest glucose and fructose concentrations (Fig. 2S-C). On the other hand, yellow pansies showed the lowest concentrations of sucrose and glucose. Regarding pansies of different colors, white pansies presented higher values in all detected free sugars than the other two colors (red and yellow). Our results are in line with Pires et al. (2017), who also detected these three sugars in centaurea petals; however, the values reported for fructose (0.65 g/100 g dw), sucrose (0.38 g/100 g dw) and glucose (0.47 g/100 g dw) were lower than ours. The difference in

Table 5
Organic acids and sugars compositions in borage, camellia, centaurea and pansies (g/100 g dw).

Organic acids	Borage (*Borago officinalis*)	Camellia (*Camellia japonica*)	Centaurea (*Centaurea cyanus*)	Pansies (*Viola* × *wittrockiana*)		
				White	Yellow	Red
Citric acid	0.93 ± 0.02^{d}	0.41 ± 0.01^{b}	1.88 ± 0.08^{e}	0.22 ± 0.02^{a}	0.52 ± 0.01^{c}	0.38 ± 0.06^{b}
Levulinic acid	1.17 ± 0.07^{c}	0.63 ± 0.05^{a}	0.86 ± 0.06^{b}	4.12 ± 0.06^{d}	0.91 ± 0.04^{b}	0.59 ± 0.03^{a}
Fumaric acid	0.87 ± 0.01^{d}	0.008 ± 0.001^{a}	0.02 ± 0.01^{b}	0.03 ± 0.01^{c}	0.03 ± 0.01^{c}	0.011 ± 0.001^{a,b}
Succinic acid	0.49 ± 0.01^{c}	0.14 ± 0.001^{a,b}	3.62 ± 0.15^{d}	0.12 ± 0.01^{a,b}	0.23 ± 0.01^{b}	0.11 ± 0.003^{a}
Malic acid	2.88 ± 0.03^{b}	2.82 ± 0.04^{b}	1.84 ± 0.09^{a}	4.92 ± 0.03^{e}	4.44 ± 0.10^{d}	3.59 ± 0.05^{c}
Salicylic acid	0.01 ± 0.01^{a}	0.013 ± 0.001^{a}	0.02 ± 0.01^{a}	0.10 ± 0.008^{b}	0.11 ± 0.01^{b,c}	0.11 ± 0.01^{c}
Hydroxycinnamic acid	0.22 ± 0.01^{a,b}	0.13 ± 0.01^{a}	0.35 ± 0.3^{b}	0.26 ± 0.03^{a,b}	0.59 ± 0.02^{c}	0.82 ± 0.18^{d}
Malonic acid	0.05 ± 0.01^{a}	nd	0.70 ± 0.07^{d}	0.43 ± 0.10^{c}	0.35 ± 0.02^{b}	nd
Total organic acids	6.63	4.15	9.28	10.20	7.16	5.61
Sucrose	3.86 ± 0.75^{d}	2.35 ± 0.09^{c,d}	1.33 ± 0.06^{a,b}	2.26 ± 0.34^{c,d}	0.49 ± 0.09^{a}	1.91 ± 0.50^{b,c}
Glucose	5.96 ± 0.75^{c}	13.1 ± 0.76^{d}	1.74 ± 0.81^{a}	11.8 ± 1.45^{d}	3.74 ± 0.27^{a,b}	4.32 ± 0.04^{b}
Fructose	6.97 ± 0.38^{b}	16.6 ± 0.81^{c}	2.71 ± 0.10^{a}	13.8 ± 1.86^{c}	4.30 ± 0.13^{a,b}	4.13 ± 0.96^{a,b}
Total Sugars	16.8	32.1	20.6	27.9	8.53	10.4

Mean ± Standard deviation. Values with the same letter in the same row are not statistically different ($p > 0.05$).

values may be because in our study, the whole flower was analyzed, whereas, in the study mentioned above, only petals were evaluated. Although there are few works on the sugar profile of edible flowers, other studies have also shown that different flower species have different sugar compositions. For example, fructose was the main sugar in *Rosa canina* L. petals and *C. officinalis* (Barros, Carvalho, & Ferreira, 2011; Miguel et al., 2016, respectively), while sucrose was the major sugar in *Taraxacum* sect. *Ruderalia* (Dias et al., 2014).

3.7. Potential applications

The present study has demonstrated that the chemical composition of edible flowers is species-dependent, and the presence of these compounds may help producers to promote their consumption and sale. Furthermore, some of these compounds can be extracted from flowers and used as food ingredients or supplements in the food, cosmetics and pharmaceutical industries. In particular, pansies (red and yellow) showed the highest amounts of total carotenoids that may be feasible to be used as food colorants (Delgado-Vargas & Paredes-López, 2002). Furthermore, borage and yellow pansies showed high contents of lutein, which might be used as a food additive. Borage, camellia and pansies have high amounts of malic acid, which could be used as a natural antimicrobial agent to be added as an active ingredient in drugs or food, being an excellent alternative to synthetic chemicals (Saeed, Afzaal, Tufail, & Ahmad, 2018). Furthermore, some tocopherols have been identified in the flowers studied, in particular, α-tocopherol, which is valued both nutritionally for its vitamin E and for its bioactive properties of being an antioxidant. Thus, tocopherols from edible flowers may be a viable alternative as a natural antioxidant in place of synthetic antioxidants in the food, pharmaceuticals and cosmetics industries (Kusumawati & Indrayanto, 2013). Furthermore, tocopherols can be extracted and incorporated into new biopolymer-based edible films, especially for preserving fresh produce (Barbosa-Pereira et al., 2013). Besides, palmitic and linoleic acids, the main fatty acids in the four flowers species studied, can be added to coatings used by the food industry, as well as to cosmetics and some industrial polymers (Rajput, Hundiwale, Mahulikar, & Gite, 2014). Centaurea is rich in fiber that can have several applications, such as dietary fibers, and in biodegradable films, natural fiber composites, biopolymers, and pharmaceuticals (Ramawat & Ahuja, 2016). However, in the present study, other phytochemicals, such as flavonoids, anthocyanins, and tannins, also present in edible flowers (Loizzo et al., 2016; Rachkeeree et al., 2018; Navarro-González et al., 2015), were not evaluated. However, in the future, these compounds must be explored due to their strong antioxidant properties that counteract reactive oxygen species (ROS) and are known to reduce the risk of diseases. Some works detected flavonols (quercetin, kaempferol) and phenolic compounds (gallic acid, protocatechuic acid) in camellia flowers (Nakajima et al., 1984); flavonols (quercetin and isorhamnetin glycosides), flavones (apigenin glycosides), and anthocyanins (cyanidin and delphinidin glycosides) in pansies flowers; as well as, anthocyanins (cyanidin glycoside), flavonoids, flavonols and flavones in centaurea (Mishio, Takeda, & Iwashina, 2015). So, the findings of the present study and the information already available in the literature support that the consumption of these edible flowers can be a source of bioactive compounds in the diet and dietary supplements, functional ingredients, and additives.

4. Conclusion

The present study provides valuable information on the nutritional composition of four edible flower species, as well as on some bioactive compounds. Borage has the highest protein content, while centaurea has remarkable fiber content. Concerning fatty acids and vitamin E, PUFA were predominant in borage and yellow pansies. At the same time, the remaining species showed higher percentages of SFA, and all flowers complemented with significant amounts of α-tocopherol. All edible flowers presented sucrose, glucose and fructose. Except camellia, lutein content was always higher than β-carotene, with red pansies and borage reporting the highest content of total carotenoids. This result represents a very important attribute, particularly due to the protective effects of lutein in eye health. Regarding organic acids, all flowers presented high contents of malic acid, except centaurea (with succinic acid as the main organic acid). In conclusion, edible flowers are a valuable source of nutrients so that they may be usefully explored for applications in the food industry and gastronomy. Their diverse chemical profiles could also be explored for the development of flower-based plant products, particularly in the food and nutraceutical industrial sectors.

CRediT authorship contribution statement

Luana Fernandes: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft. **Elsa Ramalhosa:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision, Project administration. **José Alberto Pereira:** Data curation, Writing - review & editing, Supervision. **Jorge Alexandre Saraiva:** Conceptualization, Supervision, Project administration. **Susana Casal:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109070.

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