Nutritional and Nutraceutical Composition of Pansies (*Viola* × *wittrockiana*) During Flowering

Luana Fernandes, Elsa Ramalhosa, Paula Baptista, José A. Pereira, Jorge A. Saraiva, and Susana I.P. Casal

Abstract: Edible flowers consumption and use are an increasing food trend worldwide, although information concerning their nutritional composition and nutraceutical value is still scarce. Thus, the aim of this study was to contribute to the popularization of pansies (*Viola* × *wittrockiana),* through the analysis of the nutritional and nutraceutical features of pansies with different colors (white, yellow, and red) and flowering stages. Both flower type and flowering stage influenced the flower composition. When completely open, white and yellow pansies had the highest contents of protein (>2.00 g/100 g fresh weight), while red pansies had the highest content of carbohydrates (8.0 g/100 g fresh weight). Regarding the fatty acid profiles, linoleic acid was always predominant (ranging between 18.7 and 51.0 g/100 g fatty acids), followed by the palmitic and linolenic acids. During flowering, there was an increase in protein, fat, and linolenic acid contents in white and yellow pansies, whereas in red pansies the values did not change. Red pansies were characterized by the highest contents of total carotenoids (873 to 1300 μg β-carotene/g dry weight) and monomeric anthocyanins (303 to 402 μg Cy-3 glu/g dry weight); however, white and yellow pansies showed an increase in the values of total reducing capacity (total phenols), hydrolysable tannins, flavonoids, monomeric anthocyanins, and antioxidant activity from the bud to completely open flower stage. Our results underline the nutritional differences between pansies with different colors at distinct stages of development and their potential health benefits, suggesting that they can be used as ingredient to improve the nutritional properties of foods.

Keywords: Antioxidant activity, bioactive compounds, fatty acids, flowering stages, nutritional composition, pansies

Practical application: The market of edible flowers is increasing, although little information in nutritional view is available. So, the present study was conducted to contribute to the popularization of edible flowers as a new and prospective source for the food industry, as well as a promising product for human nutrition. The results of the present study underline the nutritional differences between pansies with different colors at distinct stages of development and their potential health benefits, suggesting that they can be used as ingredient to improve the nutritional properties of foods.

Introduction

The consumption and use of edible flowers have increased in recent years. Supermarkets are beginning to sell flowers and gourmet chefs to use them on their dishes, drinks, and desserts. Furthermore, the number of scientific papers regarding this topic has increased compared to the past (Loizzo et al., 2016; Rop, Mlcek, Jurikova, Neugebauerova, & Vabkova, 2012). Even though edible flowers can be considered food sources, they have not been sufficiently exploited from the nutritional and health points of view.

Pansies (*Viola* × *wittrockiana*) from Violaceae family, represents one of the most popular edible flowers. Petals come in a myriad of rainbow pastel colors, often with two or three colors on the same flower (Lim, 2014). The whole flower and buds are edible, although they can have a mild, fresh flavor, or a more prominent wintergreen taste, depending on the part of flower (the whole flower tastes stronger than the petals alone). They are added to salads or used as a garnish and to embellish desserts (frosted cakes, sorbets, and iced drinks), as well as, crystallized and eaten as a sweet treat. However, in past, pansies have also been used as multipurpose medicinal agents, with some laxative, depurative, expectorant, emetic, alterative, anti-inflammatory, diuretic, sedative, antioxidant, and antiseptic properties (Tang et al., 2010). Some of those biological activities can be attributed to their phenolic compounds and carotenoids (Gamsjaeger, Baranska, Schulz, Heiselmayere, & Musso, 2011; Rop et al., 2012; Skowyra, Calvo, Gallego, Azman, & Almajano, 2014; Vukics et al., 2008). Until now, few studies on the physicochemical characterization of pansies have been reported, with some data on antioxidant activity (Carazo, López, Almajano, Rodrigo, & Huguet, 2005; Vukics et al., 2008), mineral composition (Rop et al., 2012), and carotenoids and flavonoids (Gamsjaeger et al., 2011). According to our knowledge, only one work has investigated compositional differences associated with petal colors (red, yellow, and violet) (Skowyra et al., 2014). However, no one has characterized pansies at their different flowering stages, as already studied for safflower (*Carthamus tinctorius* L.) (Salem, Msaada, Hamdaoui, Limam, & Marzouk, 2011), *Acacia cyclops* (Kotze, Jürgens, Johnson, & Hoffmann, 2010), and rose species (Dafny-Yelin et al., 2005;

JFDS-2018-1785 Submitted 11/8/2018, Accepted 1/30/2019. *Authors Fernandes and Saraiva are with Organic Chemistry, Natural Products and Agrifood (QOPNA), Chemistry Dept., Univ. of Aveiro, Campus Universitário de Santiago, 3810-193, Aveiro, Portugal. Authors Fernandes, Ramalhosa, Baptista, and Pereira are with Mountain Research Centre (CIMO), School of Agriculture, Polytechnic Inst. of Bragança, Campus de Santa Apolónia, 5300-253, Bragança, Portugal. Authors Fernandes and Casal are with LAQV@REQUIMTE/Laboratory of Bromatology and Hydrology, Faculty of Pharmacy, Porto Univ., Rua Jorge Viterbo Ferreira, 228, 4050–313, Porto, Portugal. Direct inquiries to authors, Elsa Ramalhosa, (E-mail: elsa@ipb.pt).*

doi: 10.1111/1750-3841.14482

Journal of Food Science A Publication of the Institute of Food Technologists

Schmitzer, Veberic, Osterc, & Stampar, 2010; Sood, Vyas, & Nagar, 2006), focusing on volatiles, phenolics, antioxidant activity, and protein changes during flowering.

This study was conducted to contribute to the popularization of edible flowers as a new and prospective source for the food industry, as well as a promising product for human nutrition. So, the aim of this research was to increase the knowledge in the microscopic structure of petals and in their physicochemical and biological properties (dimensions, water activity (a_w), pH, flowers' color and weight, nutritional composition, individual fatty acids, carotenoids, flavonoids, hydrolysable tannins, monomeric anthocyanins, total reducing capacity, and DPPH radical scavenging activity and reducing power) of pansies with different colors (white, yellow, and red) at different flowering stages (bud, half open, and flower completely open). Thus, this work provides information on pansies of different colors and at distinct flowering stages that affect their nutritional traits. The latter are relevant for the consumer and different applications for the food or supplements industries.

Material and Methods

Samples

Pansies (*Viola* × *wittrockiana*) of three different colors (white, yellow, and red) were obtained from the greenhouse of the School of Agriculture, Polytechnic Inst. of Bragança (Portugal). Flowers were harvested at three flowering stages (bud, half-opened flower, and completely open flower), and plants of each color were randomly selected (Figure 1). For each color and stage of development, 500 g of stemless flowers were collected, from approximately 50 different plants. After harvest, the flowers were transported to the laboratory. Some analyses were performed on fresh flowers, while others were performed later in flowers preserved by lyophilization (Scanvac, Coolsafe, Lynge, Denmark), ground to homogenous powder, and kept at room temperature protected from light.

Microscopic analysis of pansies petals

Three petals of each color of completely open flowers were analyzed regarding their microscopic structure. Thin sections of petals were then cut into random transversal and longitudinal sections, mounted in water and observed on a light microscope (Leitz laborlux 12, Chippenham, UK) equipped with a camera (Nikon SMZ-U, Tallahassee, Florida). The structure of petal epidermis was analyzed in different points to assure repeatability.

Physicochemical analysis

Weight, dimensions, color, pH, and a_w. Ten samples of each flowering stage and color were weighed on a digital balance (Kern, Balingen, Germany). Axial dimensions, length, and width were measured with a digital caliper (0 to 150 mm; Powerfix, Leeds, UK).

pH was measured according to the method described by Aquino-Bolaños, Urrutia-Hernández, Del Castillo-Lozan, Chavéz-Servia, and Verdalet-Guzmán (2013) and AOAC method 920.149, with some modifications. Briefly, 1 g of each sample was mixed with 50 mL of distilled water, boiled for 1 hr, filtered, and the pH measured with a potentiometer (Hanna HI8417, Amorim, Portugal).

Water activity (a_w) was determined in a portable water activity meter (Novasina, LabSwift-aw, Lachen, Switzerland).

Nutritional composition

The nutritional composition (moisture, ash, fat, carbohydrates, and dietary fiber) of each sample were analyzed following the AOAC procedures (1990), and expressed in g/100 g fresh weight (fw). Moisture content was determined by drying the sample to constant weight at 105 °C; ash content was measured by calcination at 550 °C during at least 2 hr, until achieving white ashes. Protein content of the samples was estimated by the macro-Kjeldahl method, with a conversion factor of 6.25, according to Sotelo, López-García, and Basurto-Peña (2007) and Rop et al. (2012). Lipids were determined by extracting a known weight of powdered sample with petroleum ether with 0.01% BHT (2,6-di-*tert*-butyl-4-methylphenol) to prevent oxidation, using a Soxhlet apparatus. These samples were preserved for the fatty acid analysis as detailed below. Dietary fiber was determined by an enzymatic-gravimetric method based on AOAC official method No. 985.29 (AOAC, 2003). Carbohydrates were calculated by mass difference. Energy was calculated according to Eq. (1):

$$\text{Energy}\left(\frac{\text{kcal}}{100\,\text{g fw}}\right) = \left[(4 \times (\text{protein} + \text{carbohydrates})) + (9 \times \text{lipids}) + (2 \times \text{dietary fiber})\right]\left(\frac{\text{g}}{100\,\text{g fw}}\right) \quad (1)$$

Nutraceutical composition

Fatty acids. As reported in the nutritional composition section, the lipid fraction had BHT and was stored at –20 °C for fatty acid analysis. Fatty acid methyl esters were obtained by cold hydrolysis with methanolic potassium hydroxide 2M, according to ISO 12966-2 (2011). Fatty acids were determined by GC (Chrompack, CP-9001 model, Netherlands) with flame ionization detection (GC–FID). Fatty acid separation was carried out on a Select FAME (100 m × 0.25 mm × 0.25 μm) (Agilent, USA) column. Helium was used as carrier gas at a pressure of 190 kPa. The temperatures of the injector and detector were 250 and 260 °C, respectively. The collection and processing of the data were performed by the CP Maitre Chromatography Data System program, Version 2.5 (Chrompack International B.V., Middelburg, Netherlands). The identification of the chromatographic peaks was confirmed by GC–MS using a similar column on an Agilent chromatograph 7890A, with an MSD 5977B detector (MS source 230 °C; MS Quadropole 150 °C; auxiliary temperature 280 °C; and detection in the full scan mode with a m/z of 30 to 800), using analytical standards and the NIST 14 Mass Spectral Library.

Carotenoids. Carotenoid contents were determined according to the method used by Fernandes et al. (2018). One gram of freeze-dried powdered sample was extracted twice with 20 mL acetone:hexane solution (1:1, v/v). Both extracts were combined in a separation funnel and 200 mL of distilled water was added to eliminate acetone. The acetone-free phase was mixed with 5 g anhydrous sodium sulphate to eliminate any residual water, the remaining solution filtered and completed to 100 mL with hexane. Total carotenoid content was determined by reading the absorbance at 450 nm and comparing the results to a β-carotene calibration curve (0.22 to 8.8 μg/mL). Results were expressed in μg β-carotene equivalent/100 g dw.

Monomeric anthocyanins, total flavonoids, hydrolysable tannins, and total reducing capacity. The extraction performed was based on the method described by Li et al. (2014) with slight modifications. Freeze-dried powders (1 g) of each

Figure 1–Flowering stages (bud, half open flower and completely open flower) of *Viola* × *wittrockiana* with three colours (white, yellow, and red).

sample were extracted with 50 mL of water:acetone (6:4, v/v) at 37 °C for 30 min, under agitation (IKA, RCT Model B, Staufen, Germany) at 1000 rpm. The water:acetone extracts were filtered and placed in a rotary evaporator (Stuart, RE300DB, Stone, UK) to remove acetone. Then, all extracts were frozen and stored in a freeze drier (Coolsafe, Lynge, Denmark) for 2 days. The extracts obtained were redissolved with the same solvent (water:acetone) to a concentration of 50 mg extract/mL and covered with aluminum foil under freezing until further analysis.

The total monomeric anthocyanins, total flavonoids and hydrolysable tannins contents, as well as the total reducing capacity (TRC) of the edible flowers extracts were quantified following the methodologies used by Fernandes et al. (2018). All measurements were performed in triplicate. The results for monomeric anthocyanins were expressed in μg cyanidin-3-glucoside/g dry weight (μg Cy 3-glu/g dw), flavonoids in mg of quercetin equivalent/g dry weight (mg QE/g dw), hydrolysable tannins in mg of tannic acid equivalent/g dry weight (mg TAE/g dw), and TRC in mg gallic acid equivalent/g dry weight (mg GAE/g dw).

Antioxidant activity.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The DPPH radical scavenging activities of the extracts were determined by the procedure described by Fernandes et al. (2018). Pansies extract diluted solutions (300 μL; the extract concentration range varied between 0.09 and 1.75 mg extract/mL solution; the dilutions were performed with extraction solution) were added to 2.7 mL of the DPPH methanolic solution (6.09×10^{-5} mol/L). After 1 hr in the dark, at room temperature, absorbance was read at 517 nm. Antioxidant activity was expressed by the percentage of scavenging effect, according to Eq. (2):

$$\text{DPPH radical scavenging effect (\%)} = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100 \quad (2)$$

A_{DPPH} is the absorbance of the DPPH solution and A_{Sample} is the absorbance in the presence of the sample. The extract concentration providing 50% of DPPH radical scavenging effect (EC_{50}) was calculated from the graph of the DPPH radical scavenging effect percentage versus extract concentration.

Reducing power. The reducing power of each extract was determined by the procedure described by Fernandes et al. (2018). To 1.0 mL of pansies extract solutions, at different concentrations, were added 2.5 mL of phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ 1% (m/v). After shaking, the mixtures were incubated at 50 °C for 20 min after which 2.5 mL of 10% trichloroacetic acid (m/v) was added with further stirring. A volume of 2.5 mL of the mixture was transferred to another test tube, to which 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ 0.1% (m/v) were added. The absorbance values were read at 700 nm. From the graph $Abs_{700\ nm}$ versus concentration, the EC_{50} values

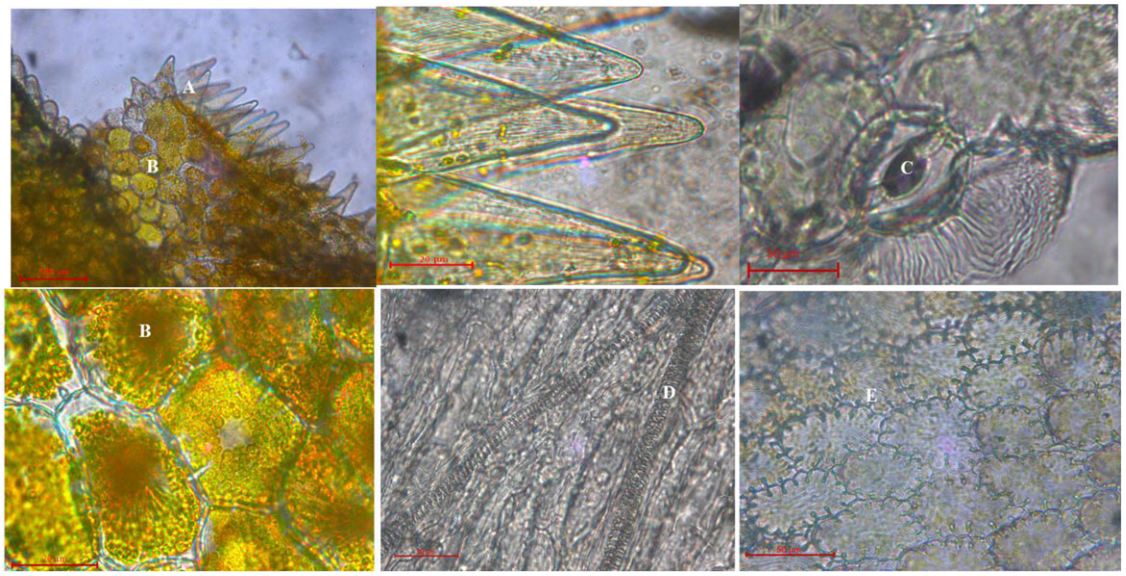

Figure 2–Structure of yellow pansies petals: longitudinal section of the petal with the conical papillae in the adaxial epidermis (A) and mesophyll (B); (C) fragment of the epidermis with a stomata; (D) branched vascular bundles in the petal mesophyll; (E) epidermis cells with folded walls.

were determined corresponding to the extract concentration that gave an absorbance of 0.5.

Statistical analysis

The SPSS Statistical software, v. 18.0 (SPSS Inc., Chicago, IL), was used for the statistical treatment of the data. The normality of the data was verified by 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, respectively. Additionally, if significant differences were detected between treatments, a post hoc analysis was performed, namely, the 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.

Results and Discussion

Microscopic analysis of pansies petals

Petals of yellow *Viola* × *wittrockiana* completely open flowers were examined in relation to their structure (Figure 2). Conical papillae in the adaxial epidermis of all petals (A) and mesophyll (B) were found. By observing in more detail this structure, it was detected that the walls of the papillae cells were thin, covered by a layer of cuticle forming parallel striate. The mesophyll was composed by irregular branched cells, with large intercellular air spaces between them. Furthermore, in mesophyll stomata (C), vascular bundles (D), and epidermis cells with folded walls (E) were found. No structural differences were observed between the three colors. Our results were similar to those described by Weryszko-Chmielewska and Sulborska (2012).

Physical analysis

Weights, lengths, widths, pH, and water activity (a_w) values for pansies with three colors during flowering are presented in Table 1. As expected, the weight, width, and length at the first stage of flowering (bud) showed the lowest values and reached their maximum values when the flowers were completely open. The increase in dimensions was most notable from the half-open flower to completely open flower stage and less prominent from the bud to half-open flower stage. No significant differences in dimensions and weight at distinct flower stages were detected among the cultivars analyzed in the present study. Damalas, Koutroubas, and Fotiadis (2014) reported lower length (18.8 mm) and width (15.2 mm) for *Viola arvensis* flowers; however, this is another species of viola. Regarding pH and a_w, few significant differences between flowering stages as well as between pansies of different colors were detected, but without any pattern.

Nutritional composition

The nutritional composition of pansies of different colors at distinct flowering stages is presented in Table 2. Water was the main constituent in pansies, ranging between 85.0 and 91.3 g/100 g fw, as has been described by other authors for edible flowers (Rop et al., 2012). Carbohydrates were the second most abundant macronutrients in all samples (3.94 to 8.78 g/100 g fw), followed by total dietary fiber (1.50 to 4.66 g/100 g fw), proteins (0.95 to 2.06 g /100 g fw), and ash (0.92 to 1.16 g/100 g fw). Fat was the less abundant macronutrient, ranging between 0.37 and 1.31 g/100 g fw, in white bud and the completely open yellow flower, respectively. The caloric energy varied between 31 and 52 kcal/100 g fw, for completely open white flower and bud and completely open red flower, respectively. Our range of results converted to dry weight (carbohydrates 42.0 to 55.6 g/100 g dw; fiber 17.2 to 43.2 g/100 g dw; protein 9.15 to 23.17 g/100 g dw; fat 4.48 to 5.21 g/100 g dw; ash 6.3 to 10.5 g/100 g dw) is in accordance with the ones described by González-Barrio, Periago, Luna-Recio, Javier, and Navarro-González (2018), who reported similar values of macronutrients (carbohydrates 47.7 g/100 g dw; protein 15.4 g/100 g dw; fat 3.22 g/100 g dw; ash 8.11 g/100 g dw) for pansy flowers, as well as by Rop et al. (2012), who detected 6.7 g/100 g dw of protein. Vieira (2013) reported higher protein (16.8 g/100 g dw) and carbohydrates (64.5 g/100 g dw) contents, and lower fiber (9.3 g/100 g dw) values for pansies.

Table 1–Physico-chemical characterization of *Viola* × *wittrockiana* of three different colors at distinct flowering stages*.

Parameters	Color	Flowering stage		
		Bud	Half open	Completely open
Weight (g)	White	$0.15 \pm 0.05^{a,A,B}$	$0.20 \pm 0.05^{a,A}$	$0.62 \pm 0.12^{b,A}$
	Yellow	$0.18 \pm 0.04^{a,B}$	$0.20 \pm 0.07^{a,A}$	$0.63 \pm 0.15^{b,A}$
	Red	$0.12 \pm 0.03^{a,A}$	$0.20 \pm 0.04^{a,A}$	$0.68 \pm 0.18^{b,A}$
Width (mm)	White	$7.94 \pm 2.01^{a,A}$	$18.33 \pm 5.01^{b,B}$	$53.12 \pm 5.64^{c,B}$
	Yellow	$7.86 \pm 1.57^{a,A}$	$14.97 \pm 3.30^{b,A,B}$	$45.98 \pm 11.42^{c,A}$
	Red	$7.79 \pm 1.37^{a,A}$	$13.84 \pm 3.08^{b,A}$	$49.84 \pm 6.30^{c,A,B}$
Length (mm)	White	$15.86 \pm 3.61^{a,A}$	$19.28 \pm 3.79^{a,A}$	$58.38 \pm 6.18^{b,B}$
	Yellow	$21.02 \pm 2.17^{a,B}$	$20.46 \pm 4.43^{a,A}$	$48.24 \pm 5.45^{b,A}$
	Red	$18.39 \pm 1.52^{a,A,B}$	$22.00 \pm 3.22^{a,A}$	$53.69 \pm 5.48^{b,A,B}$
pH	White	$6.15 \pm 0.07^{b,A,B}$	$6.10 \pm 0.08^{a,b,A}$	$6.03 \pm 0.09^{a,A}$
	Yellow	$6.18 \pm 0.10^{a,B}$	$6.15 \pm 0.05^{a,A}$	$6.21 \pm 0.07^{a,B}$
	Red	$6.08 \pm 0.06^{a,A}$	$6.32 \pm 0.13^{b,B}$	$6.01 \pm 0.08^{a,A}$
a_w	White	$0.985 \pm 0.001^{a,B}$	$0.985 \pm 0.001^{a,A}$	$0.982 \pm 0.001^{a,B}$
	Yellow	$0.984 \pm 0.001^{b,A,B}$	$0.983 \pm 0.001^{a,b,A}$	$0.978 \pm 0.001^{a,A}$
	Red	$0.982 \pm 0.001^{a,A}$	$0.984 \pm 0.001^{b,A}$	$0.984 \pm 0.001^{a,b,B}$

*Values are expressed as mean ± standard deviation. Lowercase letters present the differences among different flower stages of a single color (P-value < 0.05). Uppercase letters present the differences among different colors at each flower stage (P-value < 0.05). a_w, water activity.

Table 2–Nutritional composition of *Viola* × *wittrockiana* of three different colors at distinct flowering stages.

Color	Flowering stage	Moisture (g/100 g fw)	Protein (g/100 g fw)	Fat (g/100 g fw)	Carbohydrates* (g/100 g fw)	Total dietary fiber (g/100 g fw)	Ash (g/100 g fw)	Energy (kcal/100 g fw)
White	Bud	$85.6 \pm 0.3^{a,A}$	$1.05 \pm 0.01^{a,A}$	$0.37 \pm 0.02^{a,A}$	$7.7 \pm 0.1^{a,A}$	$4.5 \pm 0.1^{b,A}$	$1.03 \pm 0.07^{a,A}$	$47 \pm 1^{a,A}$
	Half open	$89.5 \pm 7.0^{a,A}$	$1.10 \pm 0.13^{a,A}$	$0.36 \pm 0.09^{a,A}$	$4.2 \pm 1.3^{a,A}$	$4.5 \pm 0.7^{b,A}$	$1.00 \pm 0.03^{a,A}$	$31 \pm 1^{a,A}$
	Completely open	$91.3 \pm 2.1^{a,B}$	$2.03 \pm 0.06^{b,B}$	$0.45 \pm 0.01^{a,A}$	$3.9 \pm 1.9^{a,A}$	$1.5 \pm 0.1^{a,A}$	$0.92 \pm 0.08^{a,A}$	$31 \pm 8^{a,A}$
Yellow	Bud	$85.6 \pm 0.3^{a,A}$	$0.95 \pm 0.14^{a,A}$	$0.54 \pm 0.04^{a,B}$	$7.1 \pm 0.1^{a,A}$	$4.6 \pm 0.1^{b,A}$	$1.16 \pm 0.09^{a,A}$	$46 \pm 2^{a,A}$
	Half open	$85.3 \pm 1.5^{a,A}$	$0.96 \pm 0.01^{a,A}$	$0.47 \pm 0.04^{a,A}$	$8.8 \pm 1.6^{a,A}$	$4.0 \pm 0.1^{a,A}$	$1.16 \pm 0.08^{a,A}$	$51 \pm 6^{a,A}$
	Completely open	$86.5 \pm 0.7^{a,A,B}$	$2.06 \pm 0.03^{b,B}$	$1.31 \pm 0.09^{b,B}$	$4.8 \pm 0.7^{a,A}$	$4.3 \pm 0.1^{b,C}$	$1.10 \pm 0.19^{a,A}$	$48 \pm 4^{a,A}$
Red	Bud	$85.0 \pm 1.7^{a,A}$	$1.46 \pm 0.05^{b,B}$	$0.75 \pm 0.09^{a,C}$	$7.7 \pm 1.9^{a,A}$	$4.4 \pm 0.1^{b,A}$	$1.08 \pm 0.14^{a,A}$	$52 \pm 8^{a,A}$
	Half open	$85.3 \pm 1.8^{a,A}$	$1.19 \pm 0.06^{a,A}$	$0.80 \pm 0.04^{a,B}$	$6.6 \pm 2.3^{a,A}$	$4.7 \pm 0.1^{b,A}$	$1.02 \pm 0.12^{a,A}$	$48 \pm 9^{a,A}$
	Completely open	$85.1 \pm 0.3^{a,A}$	$1.36 \pm 0.03^{b,A}$	$0.67 \pm 0.03^{a,A}$	$8.0 \pm 0.4^{a,B}$	$3.8 \pm 0.2^{a,B}$	$0.94 \pm 0.13^{a,A}$	$52 \pm 1^{a,A}$

Values are expressed as: mean ± standard deviation. fw, fresh weight.
Lowercase letters present the differences among different flower stages of a single color (P-value < 0.05). Uppercase letters present the differences among different colors at each flower stage (P-value < 0.05).
*Dietary fiber is not included.

Concerning flowering stages, no consistent patterns were observed between pansies with different colors. An increase in protein and fat contents was observed in white and yellow pansies on the last stage of flowering (completely open flower) while dietary fiber decreased, while in red pansies an increase in carbohydrates content was detected. Barros, Carvalho, and Ferreira (2011) also failed to detect a pattern of macronutrient turnover during the development of *Crataegus monogyna* flower. In fact, they stated that moisture and protein contents decreased, while ash, carbohydrates, and fat increased when comparing buds with flowers. A similar pattern for protein was found in roses, namely, from the transition of stage 1 (small bud with petals still covered by sepals) to stage 6 (fully open flower at anthesis) that was accompanied by a decrease in protein content of petals (203.2 to 88.6 g/100 g dw) (Dafny-Yelin et al., 2005). So, considerable variations in the nutritional composition may be observed during the development of different flowers, including pansies. For nutritional purposes, generally the completely open flowers presented the highest protein content, but the lowest total dietary fiber. Nevertheless, all flowers at different flowering stages showed low energy contents, without significant differences between them, being suitable for low calorie diets.

Nutraceutical composition

Fatty acids. The fatty acid composition of pansies at different flowering stages and colors is presented in Table 3. Eighteen fatty acids were identified in pansies. The predominant fatty acids were linoleic acid (C18:2n6), followed by palmitic acid (C16:0) and linolenic acid (C18:3n3). Similar results were reported by other authors (Guimarães, Barros, Carvalho, & Ferreira, 2010; Pires, Dias, Barros, & Ferreira, 2017). The highest relative amounts of linoleic acid (C18:2n6) were detected in red buds (51.0%), of linolenic acid (C18:3n3) in completely open yellow flowers (23.0%) and of palmitic acid (C16:0) in completely open red flowers (17.4%). The first two fatty acids are essential fatty acids, as they cannot be synthesized by the human organism due to the lack of desaturase enzymes required for their production. Moreover, both were mentioned to reduce some heart disease risk factors, as triglycerides blood pressure and cholesterol profile (Miyoshi et al., 2014; Miura et al., 2008; Ramel, Martinez, Kiely, Bandarra, & Thorsdottir, 2010; Shidfar, Keshavarz, Hosseyni, Ameri, & Yarahmadi, 2008; Singer et al., 1990). Furthermore, myristic (C14:0) acid was also detected in high contents in red and yellow pansies, as well as heptadecanoic acid (C17:0) in white pansies. When considering the overall fatty acid profile for pansies, it was found that this flower

Table 3–Fatty acids composition (g fatty acids/100 g fatty acids) of the oils extracted from *Viola* × *wittrockiana* of different colors at distinct flowering stages.

	White			Yellow			Red		
Fatty acid	**Bud**	**Half open**	**Completely open**	**Bud**	**Half open**	**Completely open**	**Bud**	**Half open**	**Completely open**
SFA									
C12:0	2.0 ± 2.5^{a,A}	1.1 ± 0.3^{a,A}	1.1 ± 0.3^{a,A}	3.2 ± 0.7^{a,A}	4.2 ± 0.9^{a,B}	16.1 ± 5.4^{b,B}	2.2 ± 0.3^{a,A}	4.9 ± 3.2^{a,B}	23.4 ± 0.9^{b,C}
C14:0	2.2 ± 1.4^{a,A}	6.9 ± 4.0^{b,A}	5.0 ± 0.9^{a,b,A}	13.8 ± 6.4^{a,B}	14.8 ± 5.8^{a,B}	11.8 ± 5.6^{a,B}	5.0 ± 0.7^{a,A}	8.6 ± 1.5^{b,A,B}	17.8 ± 0.4^{c,C}
C15:0	0.4 ± 0.4^{a,A}	0.2 ± 0.1^{a,A}	0.2 ± 0.3^{a,A}	0.3 ± 0.2^{a,A}	0.5 ± 0.4^{a,A}	0.2 ± 0.2^{a,A}	nd	0.4 ± 0.4^{a,A}	0.3 ± 0.1^{a,A}
C16:0	15.0 ± 2.1^{a,A}	15.4 ± 1.9^{a,A}	16.6 ± 3.7^{a,A}	16.6 ± 0.3^{a,A}	17.0 ± 2.3^{a,A}	16.5 ± 1.0^{a,A}	14.8 ± 1.1^{a,A}	14.7 ± 2.1^{a,A}	17.4 ± 0.6^{b,A}
C17:0	12.3 ± 9.4^{a,B}	8.9 ± 2.9^{a,A,B}	14.9 ± 3.1^{a,B}	2.6 ± 0.7^{c,A}	1.4 ± 0.2^{b,A}	0.5 ± 0.2^{a,A}	1.2 ± 0.3^{a,A}	12.0 ± 9.8^{b,B}	1.0 ± 0.6^{a,A}
C18:0	3.0 ±0.4^{a,b,A}	4.5 ± 1.7^{b,A}	2.4 ± 1.2^{a,A}	3.2 ± 1.6^{a,A}	4.2 ± 0.8^{a,A}	2.9 ± 0.5^{a,A}	3.0 ± 0.2^{a,A}	3.8 ± 0.5^{b,A}	3.6 ± 0.2^{b,B}
C20:0	0.6 ± 0.6^{a,A}	1.2 ± 0.4^{b,A}	nd	1.0 ± 0.5^{a,b,A}	1.3 ± 0.2^{b,A}	0.5 ± 0.4^{a,A}	1.0 ± 0.2^{a,A}	0.6 ± 0.7^{a,A}	0.4 ± 0.2^{a,A}
C22:0	1.6 ± 0.4^{b,A}	2.1 ± 0.8^{b,A}	0.6 ± 0.5^{a,A}	1.8 ± 0.5^{b,A}	2.0 ± 0.5^{b,A}	1.0 ± 0.2^{a,A}	1.3 ± 0.2^{b,A}	1.2 ± 0.7^{a,b,A}	0.7 ± 0.1^{a,A}
C24:0	2.7 ± 3.6^{a,A}	2.3 ± 1.2^{a,A}	1.5 ± 0.6^{a,A}	2.1 ± 1.2^{a,A}	2.0 ± 1.1^{a,A}	1.4 ± 1.2^{a,A}	1.4 ± 0.4^{a,b,A}	2.4 ± 1.2^{b,A}	1.0 ± 0.1^{a,A}
MUFA									
C16:1n7	nd	0.6 ± 0.3^{A}	nd	0.3 ± 0.2^{a,A}	0.2 ± 0.2^{a,A}	0.3 ± 0.2^{a}	0.3 ± 0.1^{A}	nd	nd
C18:1n9	6.8 ± 1.6^{a,b,A}	7.8 ± 1.5^{b,B}	5.3 ± 1.4^{a,B}	10.8 ± 3.4^{b,B}	11.5 ± 2.5^{b,C}	3.6 ± 0.7^{a,A}	6.2 ± 2.0^{b,A}	3.6 ± 0.6^{a,A}	4.4 ± 0.5^{a,b,A,B}
PUFA									
C18:2n6	37.4 ± 5.8^{a,B}	33.6 ± 1.5^{a,A}	33.2 ± 3.8^{a,B}	29.1 ± 1.6^{a,A}	25.6 ± 11.3^{a,A}	21.9 ± 1.9^{a,A,B}	51.0 ± 3.9^{c,C}	32.8 ± 5.6^{b,A}	18.7 ± 0.7^{a,A}
C18:3n3	14.6 ± 1.7^{a,A}	14.3 ± 0.5^{a,A}	19.3 ± 3.6^{b,B}	13.8 ± 3.0^{a,A}	15.4 ± 2.2^{a,A}	23.0 ± 1.5^{b,C}	12.7 ± 3.1^{a,A}	14.1 ± 3.0^{a,A}	11.0 ± 0.3^{a,A}
C18:4n3	0.2 ± 0.4^{A}	nd	nd	1.1 ± 1.2^{a,A}	nd	0.4 ± 0.3^{a,A}	nd	0.8 ± 1.0^{a}	0.4 ± 0.3^{a,A}
SFA	39.9	42.7	42.2	44.5	47.3	50.8	29.9	48.6	65.5
MUFA	7.4	9.1	5.3	11.5	11.7	3.9	6.4	3.6	4.4
PUFA	52.7	48.2	52.5	44.0	41.0	45.3	63.7	47.7	30.0
PUFA/SFA	1.32	1.13	1.24	0.99	0.87	0.89	2.13	0.98	0.46
n6/n3	2.6	2.3	1.7	2.1	1.7	0.9	4.0	2.3	1.7

nd, not detected; mean ± standard deviation ($n = 3$).
Lowercase letters present the differences among different flower stages of a single color (P-value < 0.05). Uppercase letters present the differences among different colors at each flower stage (P-value < 0.05).

Table 4–Nutraceutical composition of dried *Viola* × *wittrockiana* of different colors at distinct flowering stages.

Color	Flowering stage	TRC (mg GAE/g dw)	Total carotenoids (μg β-carotene/g dw)	Hydrolyzable tannins (mg TAE/g dw)	Flavonoids (mg QE/g dw)	Monomeric anthocyanins (μg Cy-3 glu/g dw)	DPPH EC_{50} (mg/ml)	Reducing Power EC_{50} (mg/ml)
White	Bud	$7.3 \pm 0.6^{a,B}$	$404 \pm 30^{c,A}$	$21.5 \pm 1.4^{a,A}$	$40.7 \pm 1.6^{a,A}$	$6.3 \pm 0.3^{a,A}$	$0.43 \pm 0.01^{b,B}$	$0.92 \pm 0.02^{c,B}$
	Half open	$13.0 \pm 0.6^{b,B}$	$278 \pm 8^{b,A}$	$35.5 \pm 2.5^{b,B}$	$77.7 \pm 1.0^{b,C}$	$11.1 \pm 0.7^{b,A}$	$0.38 \pm 0.02^{a,B}$	$0.84 \pm 0.01^{b,B}$
	Completely open	$21.8 \pm 1.1^{c,C}$	$132 \pm 6^{a,A}$	$55.7 \pm 5.0^{c,C}$	$124.5 \pm 4.0^{c,C}$	$35.9 \pm 2.9^{c,A}$	$0.38 \pm 0.01^{a,C}$	$0.67 \pm 0.01^{a,C}$
Yellow	Bud	$5.3 \pm 0.8^{a,A}$	$1073 \pm 28^{c,B}$	$26.5 \pm 1.2^{a,A,B}$	$47.3 \pm 1.0^{b,B}$	$1.5 \pm 0.1^{a,A}$	$0.48 \pm 0.01^{c,C}$	$1.71 \pm 0.02^{c,C}$
	Half open	$7.6 \pm 0.5^{b,A}$	$804 \pm 30^{b,B}$	$23.7 \pm 2.7^{a,A}$	$42.2 \pm 0.6^{a,A}$	$3.1 \pm 0.6^{b,A}$	$0.30 \pm 0.01^{b,A}$	$0.99 \pm 0.01^{b,C}$
	Completely open	$13.3 \pm 1.1^{c,B}$	$576 \pm 36^{a,B}$	$42.8 \pm 4.0^{b,B}$	$82.5 \pm 0.7^{c,B}$	$22.9 \pm 0.8^{c,A}$	$0.20 \pm 0.01^{a,A}$	$0.58 \pm 0.01^{a,A}$
Red	Bud	$13.8 \pm 1.2^{b,C}$	$1133 \pm 36^{b,C}$	$30.1 \pm 4.7^{b,B}$	$68.4 \pm 5.0^{b,C}$	$303 \pm 24^{a,B}$	$0.17 \pm 0.01^{a,A}$	$0.48 \pm 0.01^{a,A}$
	Half open	$12.5 \pm 0.5^{a,b,B}$	$873 \pm 12^{a,C}$	$20.4 \pm 0.5^{a,A}$	$51.5 \pm 1.1^{a,B}$	$353 \pm 44^{a,b,B}$	$0.34 \pm 0.02^{c,A}$	$0.73 \pm 0.01^{c,A}$
	Completely open	$11.6 \pm 1.1^{a,A}$	$1300 \pm 26^{c,B}$	$19.2 \pm 1.6^{a,A}$	$47.9 \pm 4.0^{a,A}$	$402 \pm 42^{b,B}$	$0.26 \pm 0.01^{b,B}$	$0.64 \pm 0.01^{b,B}$

Values are expressed as: mean ± standard deviation. TRC, total reducing capacity. DPPH, 2,2-diphenyl-1-picrylhydrazyl. GAE, gallic acid equivalent. TAE, tannic acid equivalent. QE, quercetin equivalent. Cy-3 glu, cyanidin-3-glucoside. EC_{50}, extract concentration providing 50% of DPPH radical scavenging effect and the extract concentration that gave an absorbance of 0.5 in the reducing power assay. dw, dry weight. Lowercase letters present the differences among different flower stages of a single color (P-value < 0.05). Uppercase letters present the differences among different colors at each flower stage (P-value < 0.05).

showed higher values of PUFA and SFA than MUFA. In general, all pansies at the three flowering stages showed PUFA/SFA ratios higher than 0.45 (ranging from 0.46 to 2.13) and n-6/n-3 ratios lower than 4.0 (varying between 0.9 and 4.0), which are recommended for the human diet (Department of Health, 1994), helping to ensure that flowers are considered a healthy food.

Concerning flowering stages, significant differences were found (Table 3). In general, the main fatty acids detected in pansies showed different patterns during flowering. Linolenic acid (C18:3n3) increased for 32.4% and 66.4% in white and yellow pansies, respectively. Contrary, in red pansies palmitic acid (C16:0) increased from 14.8% to 17.4% and linoleic acid decreased from 51.0% to 18.7%. Similar patterns were observed by other authors in other flower species, particularly, between bud and flower stages of *Crataegus monogyna*, an increase in linolenic acid (26.8% to 29.5%), and a decrease in linoleic acid (15.6 to 14.2%) were observed (Barros et al., 2011). Moreover, in two species of *Opuntia* flowers, from vegetative to full flowering stages, an increase in palmitic (from 38.2% to 43.0% for *Opuntia ficus-indica*; 48.9% to 59.5% for *Opuntia stricta*) and linolenic acids (from 3.7% to 6.2% for *Opuntia ficus-indica*) were reported (Ammar, Ennouri, Bali, & Attia, 2014). In general terms, MUFA percentage decreased from bud to completely open flower in all color pansies (28.4%, 66.1%, and 31.2% in white, yellow, and red pansies, respectively); however, in red pansies SFA increased significantly from 29.9% to 65.5% and PUFA decreased from 63.7% to 30.0%.

Total reducing capacity, total carotenoids, hydrolysable tannins, total flavonoids, and monomeric anthocyanins. Total reducing capacity, total carotenoids, hydrolysable tannins, total flavonoids, and monomeric anthocyanins contents of pansies of three different colors and at distinct flowering stages are presented in Table 4. Quantitative differences in these compounds during flowering and between pansies of different colors have been observed. Regarding the color of the flower, the major differences in values were detected in carotenoids and monomeric anthocyanins, with red pansies showing the highest values for both types of compounds (873 to 1300 μg β-carotene/g dw and 303 to 402 μg Cy-3 glu/g dw, respectively). Regarding total carotenoids, the yellow pansies showed always higher values than white pansies. According to Park et al. (2015), most yellow accents in flowers result from the presence of carotenoids (especially, xanthophylls), whereas anthocyanins are responsible for the most red-, blue-, and purple-colored petals. However, these authors found a "Kastelli" cultivar of chrysanthemum with red colored-petals and high concentrations of carotenoids, probably because this cultivar might accumulate reddish carotenoids that are absent in yellow petals. Regarding anthocyanins, Skowyra et al. (2014) reported similar results to ours, mentioning that violanin was the major anthocyanin in the three different coloured pansies (red, yellow, and violet), highlighting the higher content in red (11.40 mg/g freeze-dried weight) in comparison to yellow (4.69 mg/g freeze-dried weight) petals.

In white, yellow, and red pansies, other bioactive compounds showed similar values, namely the TRC, ranging from 5.3 to 21.8 mg GAE/g dw (yellow bud and completely open white flower, respectively); hydrolysable tannins between 19.2 and 55.7 mg TAE/g dw (completely open red and white flowers, respectively); and flavonoids between 40.7 and 124.5 mg QE/g dw (white bud and completely open white flower, respectively).

Concerning flowering stages, significant differences in pansies of different colors were observed. White and yellow pansies showed much similar behavior than red ones, increasing the values of TRC, hydrolysable tannins, and flavonoids from bud to completely open flower stage. On contrary, in red pansies, opposite patterns were observed. The increase stated in TRC values might be related to the accumulation of phenolic compounds during the full-flowering stage that may also be related to ecological functions, such as the intensification of antifungal defenses and the attraction of pollinators (Langenheim, 1994). Our results are in line with those of Bagdonaite, Martonfi, Repcak, and Labokas (2012), who reported that the bioactive compounds of *Hypericum perforatum* flowers revealed significant differences between two developmental stages (budding and full-flowering stages), being the growth and development of the reproductive parts followed by an increase in the contents of bioactive compounds. Red pansies showed the highest values of TRC (13.8 mg GAE/g dw), hydrolysable tannins (30.1 mg TAE/g dw), and flavonoids (68.4 mg QE/g dw) in the bud stage, slight decreasing until the completely open flower stage. Regarding carotenoids and monomeric anthocyanins, the highest contents (1300 μg β-carotene/g dw and 402 μg Cy-3 glu/g dw, respectively) were obtained in completely open flowers. These different patterns

and contents of bioactive compounds during the development of flowers of the same specie with different colors were also found in other flower species as *Carthamus tinctorius* (Salem et al., 2011). For example, in orange flowers of *Carthamus tinctorius* the phenolic compounds increased during flowering, while in yellow and red flowers, they decreased (Salem et al., 2011). However, in all pansies during flowering, the content of total monomeric anthocyanins increased from the bud to completely open flower stage (6.3 to 35.9, 1.5 to 22.9, and 303 to 402 μg Cy-3 glu/g dw in white, yellow, and red pansies, respectively). Similar results were found in petals of eight cultivars of *Rosa* × *hybrida* (Schmitzer et al., 2010). On contrary, Sood et al. (2006) reported an increase in total anthocyanins content in flowers of *Rose damascene* and *Rose bourboniana* at the first stages of flowering, followed by a decrease in the half and fully opened flowers. So, the contents of anthocyanins do not follow a consistent pattern (Sakata & Uemoto, 1976; Sood et al., 2006).

Antioxidant activity

The antioxidant activity of pansies was determined in flowers with different colors and at three flowering stages, using the DPPH radical scavenging activity and reducing power assays (Table 4). Buds of red pansies were characterized by the lowest EC_{50} values (0.17 mg/mL for DPPH and 0.48 mg/mL for reducing power), indicative of the highest antioxidant activity. This might be related to the fact that the buds of red pansies had a high content of phenols (predicted by TRC assay), major compounds responsible for the antioxidant activity of plant materials (Zhao, Zhang, & Yang, 2014). Yellow and white pansies showed an increase in the antioxidant activity pattern from the bud to completely open stage (lower EC_{50} values), accompanied by an increase in total phenols content. Comparing pansies of different colors, no patterns were observed. In completely open flowers, yellow pansies showed the highest antioxidant activity, followed by red and white. Different results have been reported by Skowyra et al. (2014), who detected higher values of antioxidant activity for red pansies than yellow ones, independently of the solution used in the extraction.

Conclusion

In conclusion, the results obtained indicate the value of pansies as edible flowers as well as provide more information about nutritional and nutraceutical compositions of pansies with different colors during flowering. This can be useful for consumers, chef's cuisine, producers, and food industry to enhance the production and commercialization of edible flowers as new products.

The present work demonstrated that there were significant changes in the nutritional and nutraceutical compositions of pansies with different colors during flowering. In white, yellow, and red pansies, water was the main macronutrient, followed by carbohydrates, proteins, and ash, being appropriate to low calorie diets. During flowering, an increase in protein contents in white and yellow pansies was observed, whereas in red pansies the values remained constant. PUFA and SFA predominated, mainly due to the contribution of linoleic, linolenic, and palmitic acids. The highest total carotenoids and monomeric anthocyanins contents were observed in red pansies. The TRC, hydrolysable tannins, flavonoids, and monomeric anthocyanins increased from the bud to completely open flower stage in white and yellow pansies. So, this study helped to increase the knowledge in pansies flowering behavior, although more studies are necessary to improve the information on other edible flowers already consumed.

Acknowledgments

The authors acknowledge the Portuguese Foundation for Science and Technology (FCT, Portugal) for the financial support provided by the research grant SFRH/BD/95853/2013 to Luana Fernandes and FCT/MEC for the financial support to QOPNA research Unit (FCT UID/ QUI/00062/2013) and LAQV research Unit (UID/QUI/50006/2013-POCI/01/0145/FEDER/007265) through national funds and co-financed by the FEDER, within the PT2020 Partnership Agreement. Furthermore, the authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013).

Author Contributions

L.F., S.C., and E.R. conceptualized the article; L.F., S.C., E.R., J.P., J.S., and P.B. provided the methodology; L.F. performed the investigation; L.F., S.C., P.B., and E.R. performed data curation; L.F. wrote and prepared the original draft; S.C., E.R., J.S., P.B., and J.P. reviewed and edited the article; S.C., E.R., J.S., and J.P. supervised the study; S.C., E.R., J.S., and J.P. were associated with project administration.

References

Ammar, I., Ennouri, M., Bali, O., & Attia, H. (2014). Characterization of two prickly pear species flowers growing in Tunisia at four flowering stages. *LWT - Food Science and Technology*, *59*, 448–454.

AOAC (1990). *Official methods of analysis of AOAC International* (15th ed.). Washington, DC: AOAC International.

AOAC (2003). Total dietary fiber in foods, enzymatic–gravimetric method (985.29). *Official methods of analysis of AOAC International* (17th ed.). Gaithersburg, MD: AOAC International.

Aquino-Bolaños, E. N., Urrutia-Hernández, T. A., Del Castillo-Lozan, M. L., Chavéz-Servia, J. L., & Verdalet-Guzmán, I. (2013). Physicochemical parameters and antioxidant compounds in edible squash (*Cucurbita pepo*) flower stored under controlled atmospheres. *Journal of Food Quality*, *36*, 302–308.

Bagdonaite, E., Martonfi, P., Repcak, M., & Labokas, J. (2012). Variation in concentrations of major bioactive compounds in *Hypericum perforatum* L. from Lithuania. *Industrial Crops and Products*, *35*, 302–308.

Barros, L., Carvalho, A. M., & Ferreira I. C. F. R. (2011). Comparing the composition and bioactivity of *Crataegus monogyna* flowers and fruits used in folk medicine. *Phytochemical Analysis*, *22*, 181–188.

Carazo, N., López, D., Almajano, M. P., Rodrigo, M. C., & Huguet, A. (2005). Actividad antioxidante de los pétalos de flores comestibles. EUITAB-ESAB. 1–5 (Retrieved from https://upcommons.upc.edu/bitstream/handle/2117/18886/Actividad%20antioxidante%20de%20los%20p%C3%A9talos%20de%20flores%20comestibles%20204-232.pdf)

Dafny-Yelin, M., Guterman, I., Menda, N., Ovadis, M., Shalit, M., Pichersky, E., . . . Vainstein, A. (2005). Flower proteome: Changes in protein spectrum during the advanced stages of rose petal development. *Planta*, *222*, 37–46.

Damalas, C. A., Koutroubas, S. D., & Fotiadis S. (2014). Occurrence of European field pansy (*Viola arvensis*) in Orestiada, Greece. *Hellenic Plant Protection Journal*, *7*, 25–29.

Fernandes, L., Casal, S., Pereira, J. A., Pereira, E. L., Saraiva, J. A., & Ramalhosa, E. (2018). Effect of alginate coating on the physico-chemical and microbial quality of pansies (*Viola* × *wittrockiana*) during storage. *Food Science and Biotechnology*, *27*, 987–996.

Gamsjaeger, S., Baranska, M., Schulz, H., Heiselmayere, P., & Musso, M. (2011). Discrimination of carotenoid and flavonoid content in petals of pansy cultivars (*Viola*×*wittrockiana*) by FT-Raman spectroscopy. *Journal of Raman Spectroscopy*, *42*, 1240–1247.

González-Barrio, R., Periago, M. J., Luna-Recio, C., Javier, G.-A. F., & Navarro-González, I. (2018). Chemical composition of the edible flowers, pansy (*Viola wittrockiana*) and snapdragon (*Antirrhinum majus*) as new sources of bioactive compounds. *Food Chemistry*, *252*, 373–380.

Guimarães, R., Barros, L., Carvalho, A. M., & Ferreira, I. C. F. R. (2010). Studies on chemical constituents and bioactivity of *Rosa micrantha*: An alternative antioxidants source for food, pharmaceutical, or cosmetic applications. *Journal of Agricultural and Food Chemistry*, *58*, 6277–6284.

Department Health, (1994). *Nutritional aspects of cardiovascular disease* (Vol. *46*, pp. 37–46). London, England: H. M. Stationary Office.

International Organization for Standardization (2011). *Animal and vegetable fats and oils—Gas chromatography of fatty acid methyl esters—Part 2: Preparation of methyl esters of fatty acids (12966-2)* (1st ed., pp. 1–15). Geneva, Switzerland: International Organization for Standardization.

Kotze, M. J., Jürgens, A., Johnson, S. D., & Hoffmann, J. H. (2010). Volatiles associated with different flower stages and leaves of *Acacia cyclops* and their potential role as host attractants for *Dasineura dielsi* (Diptera: Cecidomyiidae). *South African Journal of Botany*, *76*, 701–709

Langenheim, J. H. (1994). Higher plant terpenoids: A phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, *20*, 1223–1280.

Li, A.-N., Li, S., Li, H.-B., Xu, D.-P., Xu, X.-R., & Chen, F. (2014). Total phenolic contents and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods*, *6*, 319–330.

Lim, T. K. (2014). *Viola* × *wittrockiana*. In T. K. Lim (Ed.), *Edible medicinal and non medicinal plants* (pp. 818–821). The Netherlands: Springer.

Loizzo, M. R., Pugliese, A., Bonesi, M., Tenuta, M. C., Menichini, F., Xiao, J., & Tundis, R. (2016). Edible flowers: A rich source of phytochemicals with antioxidant and hypoglycemic properties. *Journal of Agricultural and Food Chemistry*, *64*, 2467–2474.

Miura, K., Stamler, J., Nakagawa, H., Elliott, P., Ueshima, H., Chan, Q., . . . Zhao, L. (2008). Relationship of dietary linoleic acid to blood pressure. The international study of macro-micronutrients and blood pressure study. *Hypertension*, *52*, 408–414.

Miyoshi, T., Noda, Y., Ohno, Y., Sugiyama, H., Oe, H., Nakamura, K., . . . Ito, H. (2014). Omega-3 fatty acids improve postprandial lipemia and associated endothelial dysfunction in healthy individuals - a randomized cross-over trial. *Biomedicine & Pharmacotherapy*, *68*, 1071–1077.

Park, C. H., Chae, S. C., Park, S.-Y., Kim, J. K., Kim, Y. J., Chung, S. O., . . . Park, S. U. (2015). Anthocyanin and carotenoid contents in different cultivars of chrysanthemum (*Dendranthema grandiflorum* Ramat.) flower. *Molecules*, *20*, 11090–11102.

Pires, T. C. S. P., Dias, M. I., Barros, L., & Ferreira, I. C. F. R. (2017). Nutritional and chemical characterization of edible petals and corresponding infusions: Valorization as new food ingredients. *Food Chemistry*, *220*, 337–343.

Ramel, A., Martinez, J. A., Kiely, M., Bandarra, N. M., & Thorsdottir, I. (2010). Moderate consumption of fatty fish reduces diastolic blood pressure in overweight and obese European young adults during energy restriction. *Nutrition*, *26*(2), 168–174.

Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J., & Vabkova, J. (2012). Edible flowers—a new promising source of mineral elements in human nutrition. *Molecules*, *17*, 6672–6683.

Sakata, Y., & Uemoto, S. (1976). Studies on the flower colours of the sweet pea. I. Anthocyanidin pigmentation in flowers of spring-flowering sweet pea. *Journal of the Japanese Society for Horticultural Science*, *45*, 181–186.

Salem, N., Msaada, K., Hamdaoui, G., Limam, F., & Marzouk, B. (2011). Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). *Journal of Agricultural and Food Chemistry*, *59*, 4455–4463.

Schmitzer, V., Veberic, R., Osterc, G., & Stampar, F. (2010). Colour and phenolic content changes during flower development in groundcover rose. *Journal of the American Society for Horticultural Science*, *135*(3), 195–202.

Shidfar, F., Keshavarz, A., Hosseyni, S., Ameri, A., & Yarahmadi, S. (2008). Effects of omega-3 fatty acid supplements on serum lipids, apolipoproteins and malondialdehyde in type 2 diabetes patients. *Eastern Mediterranean Health Journal*, *14*, 305–313.

Singer, P., Jaeger, W., Berger, I., Barleben, H., Wirth, M., Richter-Heinrich, E., . . . Gödicke, W. (1990). Effects of dietary oleic, linoleic and alpha-linolenic acids on blood pressure, serum lipids, lipoproteins and the formation of eicosanoid precursors in patients with mild essential hypertension. *Journal of Human Hypertension*, *4*(3), 227–233.

Skowyra, M., Calvo, M. I., Gallego, M. G., Azman, N. A. M., & Almajano, M. P. (2014). Characterization of phytochemicals in petals of different colours from *Viola* × *wittrockiana* Gams. and their correlation with antioxidant activity. *Journal of Agricultural Science*, *6*, 93–105.

Sood, S., Vyas, D., & Nagar, P. K. (2006). Physiological and biochemical studies during flower development in two rose species. *Scientia Horticulturae*, *108*, 390–396

Sotelo, A., López-García, S., & Basurto-Peña, F. (2007). Content of nutrient and antinutrient in edible flowers of wild plants in Mexico. *Plant Foods for Human Nutrition*, *62*, 133–138.

Tang, J., Wang, C. K., Pan, X., Yan, H., Zeng, G., Xu, W., . . . Tan, N. (2010). Isolation and characterization of cytotoxic cyclotides from *Viola tricolor*. *Peptides*, *31*(8), 1434–1440.

Vieira, P. M. (2013). Avaliação da composição química, dos compostos bioativos e da atividade antioxidante em seis espécies de flores comestíveis (Master Thesis). Universidade Estadual Paulista Press, São Paulo, Brazil.

Vukics, V., Toth, B. H., Ringer, T., Ludanyi, K., Kery, A., Bonn, G. K., & Guttman, A. (2008). Quantitative and qualitative investigation of the main flavonoids in heartsease (*Viola tricolor* L.). *Journal of Chromatographic Science*, *46*, 97–101.

Weryszko-Chmielewska, E., & Sulborska, A. (2012). Diversity in the structure of the petal epidermis emitting odorous compounds in *Viola* × *wittrockiana* Gams. *Acta Scientiarum Polonorum Hortorum Cultus*, *11*(6), 155–167

Zhao, H.-X., Zhang, H.-S., & Yang, S.-F. (2014). Phenolic compounds and its antioxidant activities in ethanolic extracts from seven cultivars of Chinese jujube. *Food Science and Human Wellness*, *3*, 183–190.