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ORIGINAL PAPER

Borage, calendula, cosmos, Johnny Jump up, and pansy flowers: volatiles, bioactive compounds, and sensory perception

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Abstract

The aim of the present work was to study the main volatile and bioactive compounds (monomeric anthocyanins, hydrolysable tannins, total flavonoids, and total reducing capacity) of five edible flowers: borage (*Borage officinalis*), calendula (*Calendula arvensis*), cosmos (*Cosmos bipinnatus*), Johnny Jump up (*Viola tricolor*), and pansies (*Viola × wittrockiana*), together with their sensory attributes. The sensory analysis (10 panelists) indicated different floral, fruity, and herbal odors and taste. From a total of 117 volatile compounds (SPME–GC–MS), esters were most abundant in borage, sesquiterpenes in calendula, and terpenes in cosmos, Johnny Jump up, and pansies. Some bioactive and volatile compounds influence the sensory perception. For example, the highest content of total monomeric anthocyanins (cosmos and pansies) was associated with the highest scores of colors intensity, while the floral and green fragrances detected in borage may be due to the presence of ethyl octanoate and 1-hexanol. Therefore, the presence of some volatiles and bioactive compounds affects the sensory perception of the flowers.

Keywords Edible flowers · Volatile compounds · Sensory analysis · Bioactive compounds

Introduction

Edible flowers are becoming more popular in recent years due to the interest of consumers and professional chefs. Flowers not only look great, but also add color, aroma, and flavor to drinks and dishes. It is known that fragrance/aroma and appearance are attributes that affect consumers' preferences, being important quality factors. Usually, flavors and fragrances of flowers are analyzed through their volatile essential oils [1]. Currently, there are some studies that have applied solid-phase microextraction (SPME) method to analyze volatile compounds in fresh flowers [2–5], having a more clear perception of the real flower volatiles by avoiding the interferences of newly formed compounds induced by extraction condition or enzymatic action. Edible flowers have a complex flavor, without single compound that accounts for a distinctively flavor. The characteristic flavor of a flower is mainly due to the association of several volatile constituents, which are mostly made up of terpenes, esters, alcohols, carbonyls, and alkane compounds [2, 4, 6]. However, there are others nonvolatile chemical constituents, such as phenolic compounds, sugars, and organic acids, that have a variable impact on the volatility of aroma compounds and taste and consequently on their sensory perception [7]. Thus, the present work had two objectives. The first one was to quantify the main bioactive compounds [monomeric anthocyanins, flavonoids, total reducing capacity (TRC) and hydrolysable tannins] and volatile compounds by headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC–MS) present in five common fresh edible flowers (borage, pansies, Johnny Jump

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up, calendula, and cosmos), together with their organoleptic appreciation. The second objective was to determine possible relationships between the volatile and phenolic compounds identified in the five flowers with the tasters' sensory perception.

Materials and methods

Samples

White borage (*Borage officinalis*), yellow calendula (*Calendula arvensis)*, purple cosmos (*Cosmos bipinnatus)*, purple Johnny Jump up (*Viola tricolor*), and red pansies (*Viola × wittrockiana*) flowers were obtained from a Portuguese store, located in the Northeast of Portugal, that sells edible flowers. In Fig. 1, the five studied flowers are presented. Around 20 g of each flower was used to perform the analyses described below.

Volatile compounds

Headspace solid-phase microextraction (HS-SPME) fibers

For the HS-SPME, a fiber coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 µm) was selected, based on a preliminary assay conducted with other fiber (PDMS; 100 µm), both from Supelco (Bellefonte, USA). The selection of the fiber was based on the highest qualitative (number of volatiles extracted) and quantitative data (peak areas) obtained for a sample (data not shown).

HS-SPME

The HS-SPME was carried out according to the methodology applied by Almeida et al. [8], with some modifications. First, fresh petals of borage, calendula and cosmos (0.20–0.30 g), whole pansy flowers and four flowers of Johnny Jump up (0.70–1.00 g) (cut a few mm below the calix, being the pedicels wrapped in aluminum foil to minimize water loss) were placed in 50 ml vials (except pansies in 100 ml vials). Then, 4-methyl-2-pentanol was added as internal standard (10 ppm in methanol), being the vials immediately sealed with a polypropylene cap with silicon septum. The volatiles were released at room temperature (± 25 °C) during 5 min. After that, the 30/50 µm DVB/CAR/PDMS fiber was exposed during 30 min at room temperature for volatiles adsorption, and then inserted into the injection port of the GC system for thermal desorption and reconditioning (10 min at 280 °C). For each sample of flower, the HS-SPME analysis was performed in quintuplicate.

Gas chromatography–mass spectrometry (GC–MS) analysis

A Shimadzu GC-2010 Plus gas chromatographer equipped with a mass spectrometer Shimadzu GC/MS-QP2010 SE detector was used for volatiles determination. A TRB-5MS (30 m × 0.25 mm × 0.25 µm) column (Teknokroma, Spain) was used. The injector was set at 220 °C and the manual injections were made in splitless mode, with helium (Praxair, Portugal) at a linear velocity of 30 cm/s and a total flow of 24.4 mL/min as mobile phase. The oven temperatures were the following: 40 °C (1 min); 2°C/min until 220 °C (30 min). The ionization source was maintained at 250 °C with ionization energy of 70 eV and with an ionization current of 0.1 kV. All mass spectra were acquired by electron ionization in the *m/z* 35–500 range. The full-scan MS spectra fragments were compared with those obtained from a database (NIST 11), and with those of commercial standards acquired from diverse producers (see Table 1). For qualitative purposes, the areas of the chromatographic peaks were determined integrating the re-constructed chromatogram from the full-scan chromatogram using for each compound the ion base (*m/z* intensity 100%). For semi-quantification purposes, volatile amounts were calculated by the ratio of each individual base ion peak area to the area of the internal standard base ion peak area and converted to mass equivalents on the basis on the internal standard mass added.

Bioactive compounds

Extraction

Fresh samples were extracted with water:acetone (6:4; v/v), at 37 °C, for 30 min under agitation (IKA, RCT Model B, Staufen, Germany) at a frequency of 1000 rpm, following the methodology used by Li et al. [9]. The extracts were filtered, concentrated in the rotary evaporator (Stuart RE3022C, Staffordshire, United Kingdom), frozen at – 18 °C, and lyophilized (48 h, Coolsafe, Lynge, Denmark). The obtained extracts were redissolved in water:acetone (6:4; v/v) to a concentration of 50 mg extract/mL and preserved under freezing until further analysis. Each extraction treatment was performed in triplicate.

Monomeric anthocyanins, total flavonoids, hydrolysable tannins, and total reducing capacity

The total monomeric anthocyanins, total flavonoids and hydrolysable tannins contents, as well as the total reducing capacity (TRC) of the edible flowers extracts were determined following the methodologies used by Fernandes et al. [10]. All measurements were performed in triplicate. The results for monomeric anthocyanins were expressed in mg cyanidin-3-glucoside/g dried weight (mg Cy 3-glu/g

Fig. 1 Visual appearance of the five edible flowers

Edible flowers	
Borage (*Borago officinalis* L.)	
Johnny Jump up (*Viola tricolor*)	
Pansies (*Viola × wittrockiana*)	
Calendula (*Calendula arvensis*)	
Cosmos (*Cosmos bipinnatus*)	

DW), flavonoids in mg of quercetin equivalent/g dried weight (mg QE/g DW), hydrolysable tannins in mg of tannic acid equivalent/g dried weight (mg TAE/g DW) and TRC in mg gallic acid equivalent/g dried weight (mg GAE/g DW).

Sensory analysis

For sensory analysis, ten tasters (six females and four males, from 29 to 45 years) from the Polytechnic Institute of Bragança, Portugal (including teachers, students, and other staff) agreed to participate in the evaluation sessions. The panel is subject to periodic training and updates in sensory analysis,

Table 1 Volatile profile of five edible flowers, expressed in μg/100 g of flower (mean ± standard deviation)

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (m/z)[c]	ID[d]	Edible flowers* (μg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
Alcohols	(*Z*)-3-Hexen-1-ol	Moldy, earthy	867	859	67	S/MS	n.d	32.8 ± 14.5	n.d	14.1 ± 3.0	n.d
	1-Hexanol	Fruity, floral, herbal, sweet	871	870	56	S/MS	85.5 ± 22.9	n.d	n.d	6.5 ± 1.7	2.83 ± 2.89
	1-Octen-3-ol	Herbal, spicy carrot	980	979	57	S/MS	67.8 ± 8.0	n.d	n.d	n.d	n.d
	2-Ethyl-1-hexanol	Citrus, green, rose	1032	1033	57	MS	29.4 ± 5.4	n.d	n.d	n.d	n.d
	(*E*)-2-Octen-1-ol	Green	1071	1066	57	MS	n.d	n.d	n.d	n.d	0.53 ± 0.31
	1-Octanol	Floral, herbal, green, fatty	1073	1068	41	S/MS	n.d	n.d	n.d	n.d	0.53 ± 0.30
	2-Phenylethanol	Floral, spicy, honey, lilac, rose	1107	110	91	MS	n.d	n.d	n.d	7.0 ± 2.2	n.d
	Σ of alcohols						183	32.8	n.d	27.6	3.89
Aldehydes	Hexanal	Fruity, herbal, grassy	798	801	44	S/MS	n.d	7.94 ± 5.17	n.d	n.d	n.d
	(*E*)-2-Hexenal	Green, apple-like	859	859	41	S/MS	n.d	3.06 ± 0.84	n.d	0.76 ± 0.20	n.d
	Benzaldehyde	Fruity, woody almond, burnt sugar	958	960	77	S/MS	n.d	n.d	n.d	3.83 ± 1.49	n.d
	Decanal	Floral, green, fatty, lemon orange peel	1204	1203	43	S/MS	n.d	0.42 ± 0.16	n.d	n.d	0.16 ± 0.06
	Phenylacetaldehyde	Floral, hyacinth	1041	1042	91	S/MS	n.d	n.d	n.d	18.8 ± 10.8	n.d
	(*E*)-2-Octenal	Fresh cut grass	1058	1054	41	MS	n.d	n.d	n.d	n.d	4.09 ± 1.60
	Nonanal	Citrus, floral, fruity, lavender, melon	1102	1102	57	S/MS	3.58 ± 0.60	1.98 ± 0.78	10.0 ± 2.97	n.d	1.80 ± 0.85
	Octanal	Fruity, floral, citrus, fatty	1073	1068	41	S/MS	n.d	n.d	n.d	0.19 ± 0.05	1.57 ± 0.60
	Σ of aldehydes						3.58	13.4	10	23.6	7.62
Aliphatic Hydrocarbons	Undecane	---	1103	1100	57	MS	n.d	n.d	n.d	0.39 ± 0.24	n.d
	Dodecane	Alkane, fusel	1201	1200	57	MS	n.d	0.91 ± 0.26	1.81 ± 0.25	0.39 ± 0.05	0.85 ± 0.38
	Tetradecane	Alkane	1401	1400	57	MS	n.d	n.d	1.16 ± 0.41	1.09 ± 0.18	0.54 ± 0.11
	Pentadecane	Alkane	1499	1500	57	MS	n.d	n.d	n.d	0.20 ± 0.05	n.d
	Σ of aliphatic hydrocarbon							0.91	2.97	2.07	1.39
Esters	Butyl 2-methylbutanoate	Green	799	804	71	MS	n.d	n.d	n.d	2.32 ± 1.37	n.d
	Ethyl butanoate	Fruity	853	854	57	MS	477 ± 71	n.d	n.d	n.d	n.d
	Methyl 2-methylbutanoate	Fruity	853	854	57	S/MS	84.8 ± 9.9	n.d	n.d	n.d	n.d
	Ethyl isovalerate	Fruity	858	858	88	MS	n.d	n.d	n.d	n.d	8.99 ± 4.43
	Ethyl pentanoate	Fruity	902	901	88	MS	24.9 ± 3.8	n.d	n.d	n.d	n.d
	Ethyl 2-methylbutanoate	Acidic	942	948	55	MS	24.1 ± 4.8	n.d	n.d	n.d	n.d
	Ethyl hexanoate	Fruity	999	997	88	MS	101 ± 10	9.43 ± 2.01	1.41 ± 0.13	1.03 ± 0.16	n.d
	Hexenyl acetate	Green	1007	1005	43	S/MS	n.d	n.d	n.d	3.08 ± 0.94	n.d

Table 1 (continued)

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (m/z)[c]	ID[d]	Edible flowers* (µg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
	Hexyl acetate	Fruity, herbal, citrus, green, spicy	1016	1009	43	S/MS	n.d	n.d	7.91 ± 0.66	n.d	85.2 ± 47.8
	Methyl benzoate	Wintergreen, almond floral	1091	1090	105	MS	19.3 ± 7.3	n.d	n.d	n.d	n.d
	Diethyl malonate	Fruity	1091	1090	115	MS	13.8 ± 4.3	n.d	n.d	n.d	n.d
	Ethyl heptanoate	Fruity	1098	1093	88	MS	34.1 ± 6.5	n.d	n.d	0.59 ± 0.13	n.d
	Ethyl benzoate	Fruity, chamomile, minty, lavender, melon	1168	1168	105	MS	832 ± 132	n.d	n.d	24.0 ± 6.0	n.d
	Hexenyl butanoate	Green	1187	1186	67	MS	3.41 ± 0.50	n.d	n.d	n.d	n.d
	Ethyl-(4*E*)-octenoate	Fruity	1189	1186	55	MS	1.59 ± 0.36	n.d	n.d	n.d	n.d
	Methyl salicylate	Minty, sweet	1189	1191	120	MS	n.d	n.d	n.d	7.63 ± 3.13	2.57 ± 1.01
	Hexyl butanoate	Apple peel	1192	1192	43	MS	9.37 ± 1.16	n.d	n.d	n.d	n.d
	Ethyl octanoate	Fruity, floral, green, anise, sweet	1197	1197	88	MS	40.8 ± 7.3	4.82 ± 2.45	n.d	2.09 ± 0.29	n.d
	Thymol methyl ether	Herbal	1235	1235	149	MS	n.d	1.07 ± 0.39	1.66 ± 0.19	n.d	n.d
	Hexyl 2-methylbutanoate	Green	1240	1236	103	MS	n.d	n.d	0.55 ± 0.09	4.22 ± 0.91	n.d
	Ethyl salicylate	Wintergreen, mint	1267	1269	120	MS	n.d	n.d	n.d	16.9 ± 2.53	1.07 ± 0.25
	Ethyl nonanoate	Waxy	1296	1296	88	MS	49.6 ± 16.0	n.d	n.d	n.d	n.d
	Ethyl decanoate	Fruity, grape, waxy	1395	1395	88	MS	9.99 ± 2.94	n.d	n.d	n.d	n.d
	Gerany acetone	Floral	1452	1455	43	MS	n.d	n.d	1.78 ± 0.61	n.d	n.d
	(*E*)-Ethyl cinnamate	Honey, cinnamon	1460	1460	131	MS	7.93 ± 2.21	n.d	n.d	n.d	n.d
	Σ of esters						1734	15.3	13	59.5	97.8
Ketones	3-Octanone	Soap, gasoline	986	983	43	S/MS	26.9 ± 6.53	n.d	n.d	n.d	n.d
	3-Heptanone	Soap	887	890	57	MS	n.d	n.d	n.d	n.d	13.5 ± 10.9
	6-Methyl 5-hepten-2-one	Blackcurrant, boiled fruit, citrus, pepper, woody	988	986	43	S/MS	n.d	n.d	4.82 ± 1.49	0.91 ± 0.31	7.46 ± 2.77
	2-Nonanone	Fruity, green. Baked, fatty	1089	1097	43	S/MS	n.d	n.d	n.d	n.d	8.79 ± 4.42
	Σ of ketones						26.9	n.d	4.82	0.91	29.8
Sesquiterpenes	δ-Elemene	Woody	1335	1338	121	MS	n.d	2.36 ± 0.58	n.d	n.d	n.d
	α-Cubebene	Herbal, wax	1348	1348	161	MS	n.d	6.31 ± 1.61	n.d	n.d	n.d
	α-Ylangene	–	1369	1375	105	MS	n.d	1.77 ± 1.04	n.d	n.d	n.d
	α-Copaene	Woody, spicy	1374	1376	161	MS	n.d	17.7 ± 3.06	n.d	n.d	n.d
	Sesquiterpene-like compound 1		1384		81	MS	n.d	1.19 ± 0.38	n.d	n.d	n.d
	β-Bourbonene	Herbal	1381	1385	81	MS	n.d	21.5 ± 6.5	n.d	n.d	n.d
	β-Copaene	Woody	1387	1391	161	MS	n.d	9.73 ± 1.77	n.d	n.d	n.d

Table 1 (continued)

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (*m/z*)[c]	ID[d]	Edible flowers* (µg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
	β-Cubebene	Citrus, fruity	1387	1391	161	MS	n.d	3.69 ± 0.92	n.d	n.d	n.d
	Sesquiterpene-like compound 2		1392		105	MS	n.d	3.11 ± 1.58	n.d	n.d	n.d
	Longifolene	Woody	1398	1400	161	MS	n.d	n.d	n.d	n.d	0.20 ± 0.05
	α-Gurjunene	Woody, balsamic	1405	1409	204	MS	n.d	5.90 ± 3.37	n.d	n.d	n.d
	α-Caryophyllene	Floral, woody	1415	1419	93	MS	n.d	118 ± 26	n.d	4.23 ± 0.05	n.d
	Sesquiterpene-like compound 3		1425		120	MS	n.d	7.70 ± 2.84	n.d	n.d	n.d
	(*E*)-α-bergamotene	Woody, warm, tea	1434	1434	93	MS	n.d	0.66 ± 0.30	n.d	n.d	n.d
	α-Guaiene	Woody, spicy	1440	1439	105	MS	n.d	n.d	0.40 ± 0.08	n.d	n.d
	Aromadendrene	Woody	1441	1441	161	MS	n.d	0.37 ± 0.36	n.d	n.d	n.d
	Sesquiterpene-like compound 4		1443		161	MS	n.d	3.11 ± 1.86	n.d	n.d	n.d
	β-Caryophyllene	Woody, spicy, sweet	1449	1454	93	S/MS	n.d	26.4 ± 13.9	2.30 ± 0.27	0.72 ± 0.19	1.92 ± 1.20
	Sesquiterpene-like compound 5		1470		161	MS	n.d	2.13 ± 0.42	n.d	n.d	n.d
	Sesquiterpene-like compound 6		1474		161	MS	n.d	9.82 ± 2.03	n.d	n.d	n.d
	γ-Muurolene	Citrus, fruit	1477	1479	161	MS	n.d	22.6 ± 8.2	1.06 ± 0.57	n.d	n.d
	Alloromadendrene	Woody	1481	1483	105	MS	n.d	5.86 ± 2.56	n.d	n.d	n.d
	Germacrene D	Woody, spicy	1486	1485	161	MS	n.d	2.73 ± 0.80	n.d	n.d	n.d
	α-Muurolene	Woody	1496	1500	105	MS	n.d	13.5 ± 1.8	n.d	n.d	n.d
	(*E,E*)-α-Farnesene	Woody, citrus, sweet	1507	1505	41	S/MS	n.d	n.d	n.d	2.36 ± 0.50	0.31 ± 0.09
	Sesquiterpene-like compound 7		1513		161	MS	n.d	0.55 ± 0.19	n.d	n.d	n.d
	Calamenene	Herbal, spicy	1518	1518	159	MS	n.d	10.8 ± 2.4	n.d	n.d	n.d
	δ-Cadinene	Thyme, medicine, woody	1521	1523	161	MS	n.d	31.5 ± 4.4	1.88 ± 1.45	0.08 ± 0.07	n.d
	α-Cadinene	Woody	1534	1538	105	MS	n.d	6.46 ± 1.20	1.44 ± 1.55	n.d	n.d
	α-Calacorene	Woody	1538	1542	157	MS	n.d	1.15 ± 0.25	n.d	n.d	n.d
	Caryophyllene oxide	Woody	1576	1583	41	MS	n.d	n.d	n.d	0.17 ± 0.05	n.d
	Epi-α-cadinol	Herbal	1635	1640	161	MS	n.d	0.70 ± 0.42	n.d	n.d	n.d
	Σ sesquiterpenes						n.d	338	7.08	7.56	2.43
Terpenes	Styrene	Balsamic, gasoline	890	889	104	MS	n.d	7.72 ± 2.16	13.7 ± 2.0	n.d	n.d
	Camphene	Camphor	949	954	93	MS	n.d	n.d	n.d	0.49 ± 0.15	n.d

Table 1 (continued)

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (*m/z*)[c]	ID[d]	Edible flowers* (μg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
	α-Thujene	Woody, green, herbal	929	930	93	MS	n.d	788 ± 97	n.d	1.92 ± 0.48	n.d
	α-Pinene	Fruity, green, woody, camphor, citrus, pine	934	939	93	S/MS	n.d	268 ± 33	n.d	19.6 ± 3.9	9.47 ± 4.34
	β-Pinene	Woody	976	979	93	S/MS	n.d	n.d	n.d	11.4 ± 2.3	3.69 ± 1.47
	Sabinene	Pepper, turpentine, woody	975	975	93	MS	n.d	98.6 ± 8.0	2.91 ± 2.87	5.17 ± 0.82	n.d
	β-Myrecene	Balsamic, fruity, lemon, spicy, sweet	992	990	41	MS	n.d	21.8 ± 8.3	3.10 ± 1.24	106 ± 20	7.32 ± 2.65
	α-Phellandrene	Flowery, citrus, sweet	1001	1002	93	MS	n.d	66.6 ± 14.7	n.d	n.d	n.d
	α-Terpinene	Anise, floral, fruity, minty, oily, peach	1015	1017	121	MS	n.d	11.8 ± 1.2	n.d	0.64 ± 0.19	n.d
	ρ-Cymene	Balsamic, citrus, fruity, herbaceous lemon, spicy	1023	1024	119	MS	42.1 ± 6.6	n.d	36.0 ± 6.6	3.86 ± 0.38	10.4 ± 4.3
	Limonene	Citrus, fruity, minty, orange, peely	1028	1029	68	S/MS	9.11 ± 1.39	40.7 ± 2.5	5.83 ± 0.61	56.9 ± 6.2	188 ± 122
	(*E*)-β-Ocimene	Sweet, tropical fruits	1051	1050	93	MS	n.d	9.29 ± 2.25	n.d	n.d	n.d
	β-Ocimene	Flowery, sweet	1052	1050	93	MS	n.d	n.d	7.70 ± 6.02	10.1 ± 1.8	7.89 ± 4.14
	γ-Terpinene	Fruity, lime	1059	1059	93	MS	4.46 ± 0.97	n.d	n.d	1.55 ± 0.21	n.d
	(*Z)*-Sabinene hydrate	Balsamic	1067	1070	93	MS	n.d	0.76 ± 0.30	n.d	n.d	n.d
	(*E)*-Sabinene hydrate	Woody, balsamic	1060	1068	93	MS	n.d	n.d	6.17 ± 1.22	n.d	n.d
	ρ-Cymenene	Citrus, terpenic, woody, spicy	1088	1088	117	MS	n.d	111 ± 7	19.4 ± 12.0	n.d	n.d
	Linalool	Floral, freesia	1098	1096	71	S/MS	n.d	0.66 ± 0.19	0.91 ± 0.15	n.d	n.d
	δ-Terpinene	Gasoline, turpentine	1106	1059	93	MS	n.d	37.5 ± 1.0	n.d	n.d	n.d
	1,3,8-*p*-Menthatriene	Turpentine	1109	1110	91	MS	n.d	n.d	6.87 ± 4.20	n.d	n.d
	β-Thujone	Thujonic	1112	1114	41	MS	n.d	1.73 ± 1.68	n.d	n.d	n.d
	Perillene	Woody	1117	1114	69	MS	n.d	3.65 ± 1.19	n.d	n.d	n.d
	Alloocimene	Herbal	1130	1132	121	MS	n.d	1.57 ± 0.29	n.d	1.42 ± 0.20	1.24 ± 0.64
	(-)-Champor	Camphor, medicine	1141	1139	95	S/MS	n.d	n.d	1.14 ± 0.50	n.d	4.48 ± 1.24
	Neo-Allo-ocimene	Sweet, herbal	1143	1144	121	MS	n.d	1.23 ± 0.22	3.34 ± 2.22	1.48 ± 0.21	1.47 ± 0.77
	L-Menthone	Minty	1151	1152	112	MS	n.d	n.d	n.d	n.d	0.53 ± 0.19
	Neomenthol	Mentholic, minty sweet	1163	1165	71	MS	n.d	n.d	n.d	n.d	2.44 ± 0.92
	3-Thujen-2-one	----	1170	1171	108	MS	n.d	4.56 ± 2.29	n.d	n.d	n.d
	Menthol	Peppermint, mentholic	1171	1171	71	S/MS	n.d	n.d	1.16 ± 0.41	1.34 ± 0.89	24.6 ± 16.0

Table 1 (continued)

Chemical class	Compound	Sensory description	LRI[a]	LRI lit[b]	QI (m/z)[c]	ID[d]	Edible flowers* (µg/100 g of flower)				
							Borage	Calendula	Cosmos	Johnny Jump up	Pansy
	Terpin-4-ol	Fruity, herbaceous, licorice, musty, spicy, sweet, terpenic, woody	1174	1177	148	MS	n.d	1.08 ± 0.24	n.d	n.d	n.d
	Isomenthol	Mentholic	1180	1182	71	MS	n.d	n.d	n.d	n.d	0.75 ± 0.25
	Terpinolene	Fruity, herbal, pine sweet, woody	1083	1088	93	MS	n.d	2.85 ± 0.10	n.d	0.78 ± 0.11	n.d
	Neoisomenthol	Menthol	1186	1186	71	MS	n.d	n.d	n.d	n.d	0.77 ± 0.23
	Estragole	Licorice, anise	1194	1195	148	MS	n.d	0.54 ± 0.07	n.d	n.d	n.d
	(*Z*)-Cadina-1(6),4-diene	----	1460	1463	161	MS	n.d	12.0 ± 3.7	0.59 ± 0.43	n.d	n.d
	(*E*)-Cadina-1,4-diene	----	1528	1534	119	MS	n.d	1.88 ± 0.56	n.d	n.d	n.d
	Σ of terpenes						55.7	1494	109	223	263
Other compounds	ρ-Xylene	Geranium	872	872	91		n.d	n.d	17.8 ± 2.7	n.d	18.4 ± 12.1
	Veratrole	----	1146	1148	138		n.d	n.d	n.d	0.29 ± 0.11	n.d
	Σ of others compounds						n.d	n.d	17.8	0.29	18.4

n.d. not detected

*Values are from semi-quantification using 4-methyl-2-pentanol as internal standard

[a]LRI—linear retention index obtained

[b]LRI Lit—linear retention index reported in the literature [37]

[c]Quantification ions

[d]Identification method (S—identified with standard; MS—identified by comparing mass spectrum with database NIST 11)

especially for the evaluation of olive oils, cheeses, and table olives. To evaluate the sensory profile of the five flowers under study in the present work, the flowers were examined without any condiments, bread, crackers, etc. After a careful evaluation of the perceived flavors (minimum three flowers of each species/panelist), the tasters were asked to fill out a questionnaire aimed at determining the performances of the edible flowers. Different organoleptic characteristics (color, spiciness, sweetness, astringency, bitterness, taste, and odor) were included in the evaluation scheme and were expressed in a scale of 1–10.

Statistical analysis

The SPSS Statistic software, version 18.0 (SPSS Inc., Chicago, USA), was used for the statistical treatment of the data. The normality and variance homogeneity were checked by Shapiro–Wilk and Levene tests, respectively. As the data followed a normal distribution, analyses of variance (ANOVA) or ANOVA Welch were carried out to evaluate if there were significant differences ($p < 0.05$) between samples. ANOVA was applied when homogeneity of variances was observed, while ANOVA Welch was applied for the other cases. In addition, significant post hoc analyses were performed (Tukey HSD test if variances in the different groups were identical or Games-Howell test if they were not). All analyses were performed in triplicate.

Results and discussion

Volatile compounds

The volatile composition of the five edible flowers is described in Table 1. In total, 117 volatile compounds were identified, belonging to different chemical classes. Calendula flowers presented the highest number of identified compounds (62), followed by Johnny Jump up (42), pansy (34), and cosmos (29). Borage showed the lowest diversity of compounds identified (24). The volatile compounds were distributed by seven chemical classes, namely, alcohols (7); aldehydes (8); aliphatic hydrocarbons (4); esters (25); ketones (4); sesquiterpenes (32); and terpenes (36).

In general terms, the five edible flowers analyzed showed differences in the volatile profiles considering qualitative and quantitative results. Terpenes were the major chemical class in almost all edible flowers studied (values between 55.7 and 1494 µg/100 g flower). This was expected, because these compounds have important roles in plants, such as pollinator attraction, direct and indirect defense against insects, bacteria, fungi, and in intra/inter-plant signaling [11]. Mazza and Cottrell [6] also observed that terpenes were the most identified volatile compounds (82–91%) for flowers and stems of three *Echinacea* species. Flamini et al. [12] also detected monoterpenes in high proportion, namely, 90.5 and 93.3% in whole flowers and petals of *Citrus deliciosa*, respectively. Within the 36 terpenes identified, limonene was detected in all studied flowers. However, the most abundant identified terpene was different for each edible flower. In more detail, for borage and cosmos, the main terpene was ρ-cymene; in calendula, it was α-thujene followed by α-pinene; in Johnny Jump up, it was β-myrecene followed by limonene; and in pansy, it was limonene.

Esters were the second chemical class in terms of diversity in borage (16 compounds) and Johnny Jump up (nine compounds) flowers. Between the esters identified, ethyl benzoate was the most abundant in both flowers (832 and 24.0 µg/100 g flower, respectively). It is reported that ethyl benzoate has a pleasant odor that could be described similar to wintergreen or mint, and it is frequently used in pharmacy, cosmetic, and food industry [13].

Sesquiterpenes were the second chemical class most abundant in calendula concerning the number of the compounds quantified, being 28 compounds detected, but seven of those were just tentatively identified as sesquiterpene-like compounds. α-Caryophyllene (118 µg/100 g flower) was the major sesquiterpene, followed by δ-cadinene (31.5 µg/100 g flower), β-caryophyllene (26.4 µg/100 g flower), and γ-muurolene (22.6 µg/100 g flower). In the other flowers, β-caryophyllene was always detected, except in borage; and it was the most abundant in cosmos and pansy. On contrary, α-caryophyllene was the most abundant sesquiterpene in Johnny Jump up flowers. This particular compound is one of the 12 most common volatile compounds detected in floral scents. Furthermore, some sesquiterpenes were only detected in one flowers' species, such as: caryophyllene oxide in Johnny Jump up, longifolene in pansies, and α-guaiene in cosmos. In calendula, several sesquiterpenes were detected solely in this flower, such as β-bourbonene and α-muurolene.

Some alcohols were also identified, such as 1-hexanol and (*Z*)-3-hexen-1-ol, the most abundant. (*Z*)-3-Hexen-1-ol is produced in small amounts by the plants and it acts as an attractant to many predatory insects [14].

Aldehydes were present in flowers in small amounts. Nonanal was the most abundant in cosmos and borage, phenylacetaldehyde in Johnny Jump ups and (*E*)-2-octenal in pansies. Nonanal is an attractant for some insects and repellent to others depending on its concentration [15–17].

Ketones and aliphatic hydrocarbons were the compounds with lower representativeness (number of compounds identified) in the edible flowers studied, although Johnny Jump up and pansies were the ones that presented the major number of aliphatic hydrocarbons and ketones, respectively. Among ketones, 6-methyl-5-hepten-2-one was the only compound identified in three flowers (cosmos, Johnny Jump ups, and

Table 2 Total reducing capacity, monomeric anthocyanins, flavonoids, and hydrolysable tannins of five edible flowers expressed in dry weight

Flowers	Parameters			
	TRC (mg GAE/g DW)	Hydrolysable tannins (mg TAE/g DW)	Flavonoids (mg QE/g DW)	Total monomeric anthocyanins (mg Cy 3-glu/g DW)
Borage	5.6 ± 0.6[b]	74.7 ± 10.4[b]	40.4 ± 5.5[b]	0.15 ± 0.05[a]
Calendula	1.1 ± 0.2[a]	3.7 ± 1.8[a]	0.35 ± 0.10[a]	0.07 ± 0.01[a]
Cosmos	12.4 ± 1.1[c]	82.9 ± 7.4[b]	44.5 ± 3.3[b]	4.18 ± 0.09[d]
Johnny Jump up	15.8 ± 0.8[d]	79.2 ± 14.8[b]	68.9 ± 8.5[c]	0.64 ± 0.17[b]
Pansy	18.0 ± 1.0[e]	78.4 ± 6.7[b]	98.8 ± 3.5[d]	1.47 ± 0.06[c]

Values are expressed as: mean ± standard deviation

Values with the same letter in the same column are not statistically different ($p > 0.05$)

pansies) but at low levels. This compound is formed from the degradation of carotenoids and reported to contribute to off-flavors [18]. Dodecane was the aliphatic hydrocarbon present in all studied flowers, except in borage.

Regarding other compounds, veratrole was detected in Johnny Jump up and ρ-xylene in cosmos and pansies.

Total reducing capacity, hydrolysable tannins, total flavonoids, and monomeric anthocyanins

Table 2 shows the total reducing capacity (TRC), hydrolysable tannins, total flavonoids, and monomeric anthocyanins contents determined in the five studied edible flowers. Significant differences among them ($p < 0.05$) were observed. Pansies showed the highest values of TRC (18.0 mg GAE/g DW) and flavonoids (98.8 mg QE/g DW), while cosmos presented the highest contents of hydrolysable tannins (82.9 mg TAE/g DW) and monomeric anthocyanins (4.18 mg Cy 3-glu/g DW). Nevertheless, Johnny Jump ups, pansies, and borage also presented high values of hydrolysable tannins, not being significantly different from cosmos. On contrary, calendula always presented the lowest values of all studied bioactive compounds. Intermediary values of TRC and monomeric anthocyanins were detected in Johnny Jump ups and of hydrolysable tannins and flavonoids in borage. When expressing our results in fresh weight, cosmos, Johnny Jump ups, and pansies presented the highest TRC. Pansies also had the highest content of flavonoids, while cosmos showed the highest contents of hydrolysable tannins and monomeric anthocyanins, followed by pansies. In our work, the values were equal to 11.6, 1.39, and 0.91 mg Cy 3-glu/100 g FW for pansies, borage, and calendula, respectively. Similar results were reported by Benvenuti et al. [19]. These authors detected the following values for red pansies (12.4 mg Cy 3-glu/100 g FW), borage (1.43 mg Cy 3-glu/100 g FW), and calendula (0.47 mg Cy 3-glu/100 g FW).

Sensory analysis

Figure 2 and Table 3 show the sensory profiles of the five studied edible flowers. The radar plots, for odors and tastes detected by the panelists, are shown in Fig. 2. The odors detected in the flowers were divided into six classes, namely, floral (e.g., carnation, lilies, marigold, orange blossom, orchid, pollen, rose, and violet), fruity (e.g., banana skin, fig, mandarin, peach, and plum), herbal (e.g., green grass/leaves), marine (sea air), spicy (vanilla), and wood (cedar, acacia). For the taste, five classes were used, namely, floral (lavanda, lilies, petals rose, and pollen), fruity (apple, cherries, chestnut, grape seed, banana skin, walnut, and cabbage), herbal (green grass/leaves and mint, parsley), sweet (honey), and wood (camphor, cedar). It was observed that each flower had its individual sensory characteristics (Fig. 1). Regarding odor, the panelists detected more floral fragrances in Johnny Jump up (fragrances of carnation, lilies, pollen, rose, and violet), cosmos (fragrances of acacia, carnation, pollen, and violet), and pansies (fragrances of lilies, orchid, rose, and violet), while for calendula, a wood odor was detected (fragrance of cedar). In borage, several fragrances were felt such as rose and violet (floral), green leaves (herbal), and sea air (marine). Concerning taste, pansies, and Johnny Jump up flowers showed a fruity flavor. In more detail, pansies tasted more like chestnut and walnut, while Johnny Jump up like cherries and walnut. Cosmos presented a high lavanda taste, followed by parsley, camphor green grass/leaves and mint tastes, a complex mixture of floral, herbal, and wood flavors. Regarding calendula, a high mixture of flavors was also detected by the panel, such as parsley, cabbage, grape seed, rose petals, cedar, cherries, and banana skin. On the other hand, borage showed the smallest range of flavors, being the cabbage, and chestnut flavors the most intense.

Furthermore, each flower was classified according to three sensory attributes including visual appearance, odor,

Fig. 2 Odors and tastes detected by the panel of tasters for each edible flower species

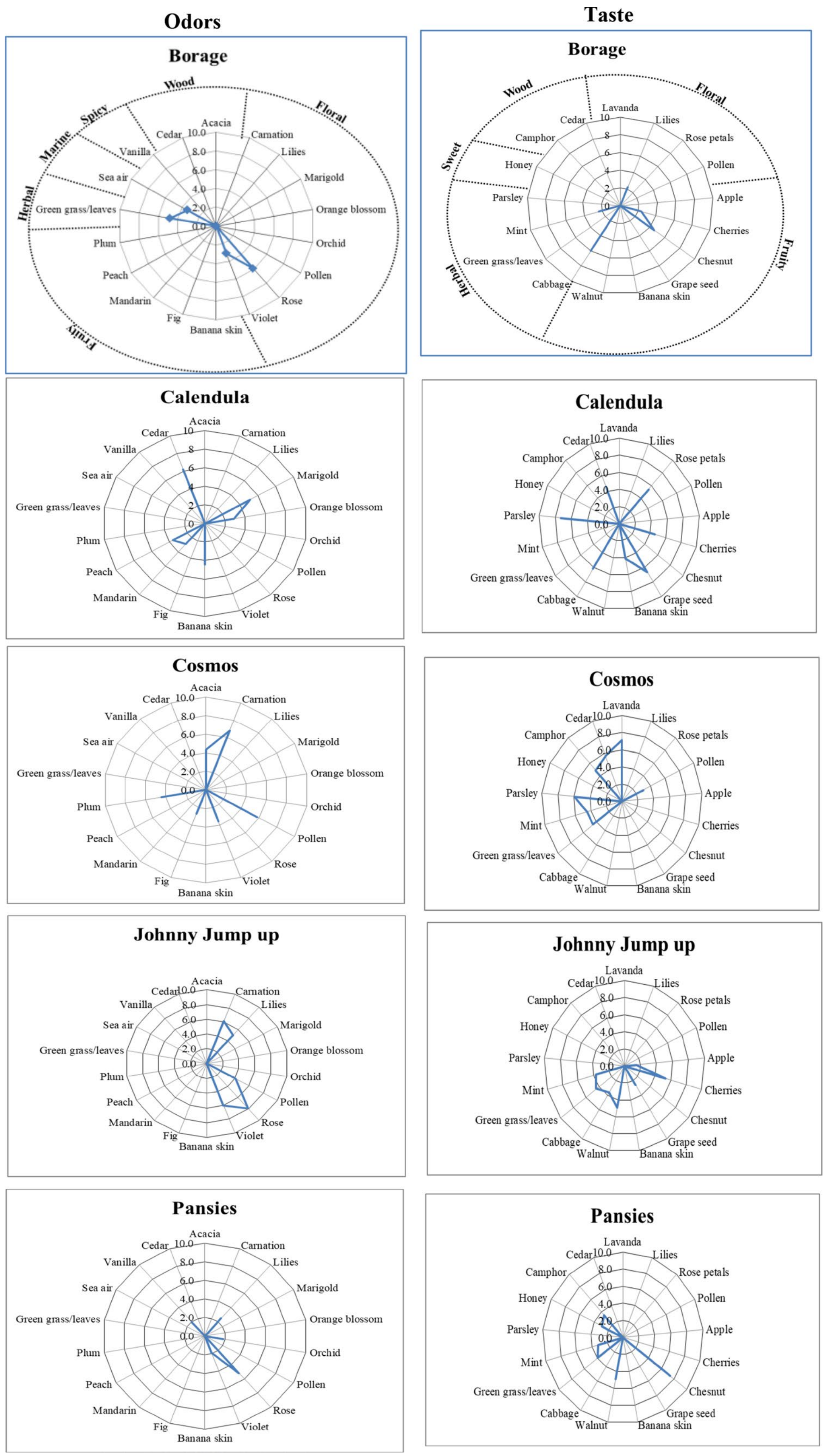

Table 3 Sensory evaluation of the five edible flowers

Sensory attributes	Edible flowers				
	Borage	Calendula	Cosmos	Johnny Jump up	Pansies
Visual appearance					
Colors intensity	6.4 ± 1.1^{a} (5.1–7.8)	6.8 ± 0.5^{a} (6.3–7.3)	8.3 ± 0.7^{b} (7.2–9.4)	7.6 ± 1.1^{a,b} (5.0-8.3)	8.5 ± 0.8^{b} (7.4–9.7)
Physical integrity of the plant	9.4 ± 0.6^{a} (8.5–10)	9.6 ± 0.4^{a} (8.9–10.0)	9.3 ± 0.3^{a} (9.1–10)	9.5 ± 0.8^{a} (7.7–10)	9.5 ± 0.6^{a} (8.3–10)
Odor					
Odor sensation	8.0 ± 0.9^{a} (6.5–9.2)	7.8 ± 0.5^{a} (6.6–8.5)	8.4 ± 0.6^{a} (7.5–9.2)	8.6 ± 0.8^{a} (7.2–9.9)	7.6 ± 2.8^{a} (1.5–9.7)
Odor intensity	6.1 ± 0.9^{a} (4.9–7.8)	8.4 ± 0.7^{b} (7.7–9.8)	7.2 ± 1.4^{a,b} (4.7-9.0)	9.0 ± 0.6^{b} (8.1–9.9)	5.3 ± 2.7^{a} (2.2–8.6)
Taste					
Mouthfeel	5.8 ± 1.1^{a} (4.0-7.2)	6.2 ± 0.7^{a,b} (5.4-7.0)	7.5 ± 1.0$^{b.c}$ (6.3–9.2)	6.8 ± 1.6$^{a,b.c}$ (4.9–9.9)	8.4 ± 0.6^{c} (7.4–9.3)
Persistence	4.2 ± 1.1^{a} (2.9–6.4)	6.6 ± 0.8^{b,c} (5.3–7.6)	7.8 ± 1.0^{c} (6.1–9.2)	5.6 ± 2.0^{a,b} (1.5–7.6)	7.5 ± 1.6^{b,c} (4.6–9.1)
Bitterness	1.6 ± 0.4^{b} (0.9–2.1)	6.4 ± 1.1^{c} (4.4–7.8)	6.3 ± 1.0^{c} (4.6–7.6)	1.5 ± 1.2^{a,b} (0.1–3.7)	0.3 ± 0.4^{a} (0.0–1.0)
Astringency	1.4 ± 0.7^{a} (0.7–2.4)	4.4 ± 1.8^{b} (2.0-7.3)	4.2 ± 1.4^{b} (1.8–5.9)	1.6 ± 1.3^{a} (0.1–4.1)	0.3 ± 0.4^{a} (0.0–1.0)
Spiciness	0.3 ± 0.5^{a} (0.0-1.5)	2.6 ± 0.9^{b,c} (1.2–4.2)	3.6 ± 0.9^{c} (2.7–5.3)	0.3 ± 0.5^{a} (0.0-1.2)	1.7 ± 0.7^{b} (1.0-2.3)
Sweetness	3.5 ± 1.0^{b} (2.1–5.1)	0.5 ± 0.4^{a} (0.0-0.9)	0.7 ± 0.8^{a} (0.0-2.6)	2.6 ± 1.1^{b} (0.4–4.5)	6.4 ± 1.0^{c} (5.2–7.8)

Mean ± standard error (minimum–maximum)

Values with the same letter in the same line are not statistically different ($p > 0.05$)

and taste (Table 3). Regarding visual appearance, two descriptors were evaluated, color intensity, and physical integrity of the plant. Cosmos and pansies were the flowers with the highest scores of colors intensity, while all flowers showed good physical integrity (> 9). According to the panel, all flowers revealed a pleasant odor sensation (> 7.5), with no significant differences between them. On contrary, concerning odor intensity, Johnny Jump up, calendula, and cosmos had the highest scores, while borage and pansies had the lowest. Concerning taste, all flowers had a delightful mouthfeel (> 5), although pansies were distinguished from the others with the highest value (8.4). Cosmos, pansies, and calendula had the most persistent flavor. In more detail, calendula and cosmos originated a more bitter, astringent and spicy taste than borage, Johnny Jump up, and pansies. In contrast, pansies had the sweetest taste, followed by borage and Johnny Jump up. These results were like those referred by Benvenuti et al. [19], who reported that calendula showed higher values of spiciness and bitterness than borage and pansy, as well as, borage and pansy were sweeter than calendula. Thus, our results showed that the five studied flowers have a high sensory biodiversity. This will allow their valorization, because these flowers can make the dishes more attractive and confer a peculiar taste and odor.

Association between sensory attributes and bioactive compounds

Sensory attributes of flowers are dependent on the content of minor components like phenolic and volatile compounds. Furthermore, each single component can contribute to different sensory perceptions. Bioactive compounds, such as phenolics, are plant metabolites and contribute to important organoleptic properties (color, bitterness, and astringency) [20]. Concerning flowers' color, anthocyanins play an important role. By observing Tables 2 and 3, the flowers that presented the highest contents of total monomeric anthocyanins, namely, cosmos and pansies (4.18 and 1.47 mg Cy 3-glu/g DW, respectively), were those that had the highest scores of colors intensity (8.3 and 8.5, respectively).

Phenolic compounds are responsible for the bitterness and astringency of plants, being tannins more likely to be astringent and flavonoids more bitter [21]. In this order, it was expected that pansies were the most bitter flowers followed by Johnny Jump ups, because they had the highest values of TRC and flavonoids. However, the panel reported low scores of bitterness for both flowers. These results can be due to the sweetness caused by the sugars present in the nectar of the flowers (not analyzed), which may be an efficient masking agent of astringency and bitterness caused by the phenolic compounds [22]. In fact, both flowers were described by the panelists to have a sweet taste (6.4 for pansies and 2.6 for Johnny Jump ups).

On contrary, for cosmos, the panelists did not detect large sweet notes (0.7), being bitterness (6.3) and astringency (4.2) the most detected flavors. These results are in accordance with those obtained for some analyzed bioactive compounds. In fact, cosmos presented the highest values of hydrolysable tannins (82.95 mg TAE/g DW) and intermediary values of flavonoids (44.55 mg QE/g DW). Calendula showed the lowest value of TRC (Table 2), but the panelists detected in this flower a great range of tastes and odors, probably because the intensities of fruity and floral aromas/flavors seem to increase when the level of polyphenols decrease [23].

Association between sensory attributes and volatile compounds

The description of the odor of each isolated volatile compound was obtained from the literature [24–29] and is presented in Table 1. In this section, it was analyzed the possible relationship between volatile compounds obtained by GC–MS and the sensory attributes, assessed by the panelists. First, it is known that the volatile compounds present at higher concentration are not necessarily the major contributors of odor [28]. In borage flowers, a high number of esters associated to fruity fragrances were detected [13, 26, 30]. In fact, the panelists detected some green and floral notes, and a fruity taste (chestnut, cabbage, and cherries). Those floral and green fragrances may be due to the presence of some volatile compounds, as ethyl octanoate and 1-hexanol as reported by Śliwińska et al. [30]. Calendula showed woody (cedrus), floral (marigold and orange blossom), and fruity (banana, mandarin and peach) fragrances that were reported by panel (Fig. 2). The woody odor of calendula was probably due to the high levels of sesquiterpenes detected, most of them described as contributing to a wood odor (Table 1). Furthermore, some sesquiterpenes (e.g., calamenene, β-caryophyllene, α-copaene) give the sensory perception of spicy, so probably, it was because of this that the panel detected a spicy taste for this flower (2.6, the second highest). Mandarin and orange blossoms fragrances may be due to the presence of α-pinene. According to the panelist group, cosmos showed floral and fruity odors (Fig. 2). The fruity odor was probably due to the high levels of ρ-cymene (41.7 μg/100 g). Regarding Johnny Jump up and pansies, the panel detected floral fragrances for both flowers. β-Ocimene and 1-hexanol are important floral scents in different flowers [31–34] and their odor was described as floral [30], while β-myrecene is described as sweet and fruity. Therefore, these components might play an important role in the sweet, floral aroma of these two samples. Furthermore, the panel detected in both flowers notes of rose, wherein 2-phenylethanol is one of the principal component of fragrant rose flowers [35, 36]; however, this compound was only detected in Johnny Jump up. The panel also mentioned some mint flavors when they tasted both flowers, probably due to presence of menthol, isomenthol, neomenthol, neoisomenthol, and L-menthone compounds.

Therefore, the volatiles produced from flowers vary significantly among species, contributing to the diverse range in fragrances and aromas found in the plant kingdom.

Conclusions

The flowers analyzed showed statistical differences in their sensory attributes (colors intensity, odor intensity, mouthfeel, persistence, bitterness, astringency, and sweetness) and in the variety of volatiles detected, presenting calendula the highest number of identified compounds (62), followed by Johnny Jump up (42), pansy (34), cosmos (29), and borage (24). Terpenes were the major chemical class in terms of diversity in almost all edible flowers studied. Regarding bioactive compounds, the highest values of TRC and flavonoids were determined in pansies, and hydrolysable tannins and monomeric anthocyanins in cosmos, having calendula the lowest. Some relationships were found between color, bitterness and astringency with the presence of some bioactive compounds. For example, more anthocyanins gave higher intensities of color (pansies and cosmos), more bitterness to a higher content of flavonoids (cosmos), and more astringency to higher levels of tannins (cosmos); however, regarding taste, it was more difficult to take precise conclusions, because some compounds can mask the presence of others. Additional relationships were found between the presence of some volatile compounds and the sensory perception, such as the high number of sesquiterpenes detected in calendula can be associated with the woody notes detected by the panel.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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