

Phytonutritional and aromatic profiles of *Tulbaghia simmleri* Beauv. edible flowers during cold storage

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Abstract: Edible flowers are appreciated due to their aesthetic features, nutritional value and antioxidant properties. *Tulbaghia simmleri* Beauv. (Amaryllidaceae family) flowers are characterized by a pleasant garlic taste and are consumed both as fresh and dried products. The aim of this work was to assess the effect of chilling temperature (+4°C) on the visual quality, nutritional content, and aroma profile of *T. simmleri* flowers after two (T2) and six (T6) days of storage. Colorimetric analysis highlighted a reduction in petal brightness at T6 and hence their darkening, due to a significant increase in *a** coordinate and the decrease in the *b** one. Total polyphenols and flavonoids content remained unchanged until the end of the experiment, while total anthocyanins increased at T2. Flowers antioxidant activity (DPPH assay) decreased progressively during cold storage, while catalase (CAT) and ascorbate peroxidase (APX) activities increased. The aroma profile was analyzed by HS-SPME associated with GC-MS, underlining that fresh flowers were dominated by high content in monoterpenes (around 80%), with 1,8-cineol as main compound (53.1%). Cold storage reduced this class of volatiles while sesquiterpenes and non-terpenes increased; between them, benzyl benzoate reached 12%.

1. Introduction

Edible flowers (EFs) are traditionally consumed since ancient times (Mlcek and Rop, 2011). Some of them are commonly recognised as vegetables (e.g. artichokes, broccoli, capers), while others are still considered

“unusual food” (reviewed in Pires *et al.*, 2019). EFs straightly rely on their colours, shapes, flavours, tastes, and nutrients (e.g. carbohydrates, proteins, vitamins, phytochemical compounds with antioxidant and healthy properties) (Fernades *et al.*, 2017). Their market is constantly expanding, and new species with attractive sensorial features and good storage attitude are required.

Tulbaghia (common name: wild garlic) is a genus of monocotyledonous plants (Amaryllidaceae family) indigenous to South Africa (Lyantagaye, 2011). Herbaceous perennial bulbs, corms or rhizomes characterize its species. *Tulbaghia* spp. flowers, held in umbels in groups of ten or more, are strongly fragrant and characterised by tubular shape (Zschocke and Van Staden, 2000). A raised crown-like structure or a fleshy ring at the centre of the flower tube are distinctive features of this genus (Vosa, 2000). The colours are different, mainly white, pink or mauve. Flowers and rhizomes produce cysteine-derived sulphur compounds (e.g. marasminin), which confer to this organs a pleasant alliaceous smell, especially when bruised or during senescence (Aremu and Van Staden 2013; Kubec *et al.*, 2013). The peculiar aroma and the pungent garlicky taste of flowers make several *Tulbaghia* spp. interesting for the food industry (Kubec *et al.*, 2013).

T. simmleri Beauv. is mainly known as ornamental plant, which flowers consist of six tepals and a central crown of six lobes, fused for more than a third of their length to form a tube. The lobes have pointed tips, giving the crown a fringed edge (Vosa, 2000). In the southern hemisphere, its period of blooming ranges between April to October, even though, with particular climate conditions, it could be extended until early spring (Zschocke and Van Staden, 2000). In the northern hemisphere, however, its period of blooming ranges between October to April. Several bioactive compounds characterize this plant, since it is used to treat fever, colds, headaches, asthma, and tuberculosis in South African traditional medicine (Zschocke and Van Staden, 2000). *T. simmleri* has been severely neglected when compared to the most common *T. violacea*, for which several culinary uses are known, also concerning flowers (Aremu and Van Staden, 2013; Rivas-García *et al.*, 2022). Further investigation on *T. simmleri* worth to be performed, since this species produce deep mauve, long lasting edible flowers, which period of bloom does not overlap the one of *T. violacea* (not available in autumn and winter). This will ensure the availability of EFs

with garlic taste for most of the year. Moreover, Takaidza *et al.* (2018) highlighted good total polyphenolic and flavonoid content, and hence good antioxidant activity, in *T. simmleri* plants, in comparison with other seven *Tulbaghia* species, *T. violacea* included.

Postharvest technologies are common methods to extend EFs shelf-life, as it is generally rather short (2-10 days) (Fernandes *et al.*, 2019, 2020). Flowers are high value products, which must be picked with care, packaged properly to protect them from any mechanical damage, and stored at proper temperature until consumption (Fernandes *et al.*, 2020). Improperly handled/stored edible flowers suffer tissue browning, flower wilt, dehydration, petal discoloration, and abscission. The senescence process is associated with physiological changes and catabolism, which are linked to accelerated respiratory levels, weight reduction, and/or plant hormone response (Kou *et al.*, 2012; Landi *et al.*, 2018). To address these concerns, fresh edible flowers are often stored under low temperatures, generally at chilling ones (4-5°C) (Fernandes *et al.*, 2020). Since different EFs species showed different behaviour at cold storage (Landi *et al.*, 2018; Marchioni *et al.*, 2020 a, 2020 b), postharvest studies should be performed for each flower, in order to elucidate their physiological response to low temperature and hence their shelf-life.

The aim of this work was to evaluate the phytonutritional and aromatic profile of *T. simmleri* EFs stored at 4°C for 0, 2 and 6 postharvest days. Spectrophotometric and chromatographic analyses were performed in order to highlight any changes in polyphenolic content (flavonoids and anthocyanins included), antioxidant activity, and volatile organic compounds (VOCs) during cold storage.

2. Materials and Methods

Plant material and postharvest conditions

Tulbaghia simmleri plants were provided by the Chambre d’Agriculture des Alpes-Maritimes (CREAM, Nice, France) and were grown at Research Centre for Vegetable and Ornamental Crops (CREA, Sanremo, Imperia, Italy, GPS: 43.816887, 7.758900). Details on plant cultivation is reported in Najjar *et al.* (2019). Full open flowers were picked in April, weighed and cold stored as described in Marchioni *et al.* (2020 b), for two (T2) and six (T6) postharvest days. Fresh flowers

were considered as control (T0).

Weight loss and colour determination

Flowers weight was measured (Ohaus[®] analytical Standard Series™ Model AS60S, Ohaus Corporation, Florham Park, N.J. USA) before cold storage (T0) and at the end of each experimental point (T2 and T6) to calculate their weight loss (formula reported in Fernandes *et al.*, 2018). Once flowers had been weighed, their colour was evaluated with a spectrophotometer SP60 series (X-Rite Incorporated, Michigan, USA). L^* (lightness), a^* (redness) and b^* (yellowness) colour coordinates (CIELAB scale, CIE 1976) were measured in different point of at least ten flowers, in order to best describe their colour variations.

Biochemical analyses

Biochemical analyses were performed using frozen samples. Total phenolic, flavonoid and anthocyanins content were determined as reported by Marchioni *et al.* (2020 b). Data were reported as mg gallic acid equivalents (GAEq)/g fresh weight (FW) (polyphenols), mg catechin equivalents (CEq)/g FW (flavonoids), and mg malvin chloride equivalents (MEq)/g FW (anthocyanins). Radical scavenging activity (DPPH assay) of each sample was determined as described by Brand-Williams *et al.* (1995). Data was expressed in IC_{50} , which represent the concentration of the sample able to inhibit by 50% the radical DPPH. All absorbance were read in a UV-1800 spectrophotometer (Shimadzu Corp., Kyoto, Japan).

Enzymatic activities

Frozen flowers (200 mg) were pulverized and homogenized in 2 mL of extraction buffer, consisting of 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 2% (w/v) insoluble polyvinylpyrrolidone (PVPP), as reported by Pistelli *et al.* (2017). Samples were centrifuged at maximum speed for 30 min at 4°C and the supernatant was used for enzyme activities. The soluble protein content was determined according to Bradford (1976) using bovine serum albumin as standard.

Catalase (CAT, EC 1.11.1.6) activity was measured by monitoring the decomposition of hydrogen peroxide (H_2O_2), recording the decline in absorbance per minute at 240 nm (Zhang and Kirkham, 1996). The reaction started by adding 20 μ L of extract to 980 μ L of 8.8 mM H_2O_2 solution in 50 mM sodium phosphate buffer. One unit of CAT is determined as the amount

of enzyme required to detoxify 1 μ mole of H_2O_2 ($\epsilon = 394 \text{ M}^{-1} \text{ cm}^{-1}$) per minute. Data were expressed as unit of CAT per mg of soluble proteins ($\mu\text{mol min}^{-1} \text{ mg}^{-1}$).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following the decrease in absorbance at 290 nm ($\epsilon = 2.7 \text{ mM}^{-1} \text{ x cm}^{-1}$) due to enzymatic ascorbate oxidation (Nakano and Asada, 1981). The reaction started by the addition of 50 mM H_2O_2 solution to the reaction mixture (20 μ L of extract, 0.15 mM disodium EDTA and 0.37 mM ascorbic acid in 50 mM sodium phosphate buffer). A unit of APX is defined as the amount needed to oxidize 1 μ mole of ascorbic acid per minute. Data were expressed as unit of APX per mg of soluble proteins ($\mu\text{mol min}^{-1} \text{ mg}^{-1}$).

Spontaneous emission analysis

The spontaneous emission analysis was performed as reported in our previous work (Marchioni *et al.*, 2020 b). Briefly, and after the chosen storage time had elapsed (0, 2 and 6 days at 4°C), 1g of *T. simmleri* was properly weighted to be sealed in a 25 mL glass flask and kept at laboratory temperature (around 21°C) for 15 min (equilibration time). Once the time expired, the 100 μ m polydimethylsiloxane PDMS fiber (Supelco, Bellefonte, PA, USA), was exposed to the flask headspace for 10 min, to be than transferred into the GC-MS instrument.

Statistical analysis

The normal distribution of the residuals and the homogeneity of variance was determined and then data were statistically analyzed by one-way analysis of variance (ANOVA) (Past3, version 3.15), using Tukey Honestly Significant Difference (HSD) with a cut-off significance of $p < 0.05$ (letters).

3. Results and Discussion

Weight loss and chromatic changes during cold storage

The visual quality of *T. simmleri* flowers has been almost entirely maintained up to the sixth days of cold storage (T6) (Fig. 1, Table 1). The main changes observed during postharvest treatment were the decrease in flowers fresh weight, brightness (L^*) and bluish parameter (b^*), along with the increase in the reddish parameter (a^*) (Table 1). Taken together, these variations resulted in a slight darkening of the petals at the end of the experiment, without any evi-

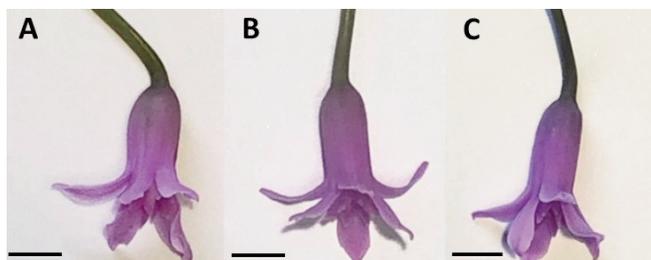


Fig. 1 - Visual appearance of *T. simmleri* flowers after different times of cold storage (4°C): freshly picked flowers (A); after 2 days of cold storage (T2) (B); and after 6 days of cold storage (T6) (C). Bar scale: 1 cm.

dent loss of flower firmness.

The decrease in fresh weight is due to the loss of cell turgor, which is correlated to flower shape. Significant water loss can determine decreased floral diameter, as well as petals curling and crumpling (Kou *et al.*, 2012; Ahmad and Thair, 2016; Marchioni *et al.*, 2020 b). Nevertheless, the weight loss in *T. simmleri* flowers was very limited (around 7%), showing, therefore, a good aptitude to cold storage. Moreover, the latter was observed to reduce the brightness of seven different EFs (Landi *et al.*, 2018), as well as *T. simmleri* flowers (Table 1). This decrease in L^* values is indicative of tissue darkening, commonly associated with the oxidation of phenolics and

Table 1 - Weight loss and chromatic changes of *T. simmleri* flowers at 0 (T0), 2 (T2), and 6 (T6) postharvest days (storage at 4°C)

Parameters	Days		
	0	2	6
Weight loss (%)	0 c	3.21 ± 0.04 b	7.34 ± 0.69 a
L^*	56.01 ± 1.25 a	55.54 ± 1.19 a	49.45 ± 0.68 b
a^*	22.71 ± 0.64 c	25.78 ± 0.65 b	27.84 ± 0.46 a
b^*	-14.16 ± 1.08 a	-19.73 ± 0.69 b	-20.02 ± 0.51 b

Data are reported as mean ± standard error (weight loss, n = 4; L^* , a^* , b^* , n = 15). Different letters indicate statistically significant differences ($p < 0.05$; Tukey's HSD test).

Table 2 - Antioxidant compounds, radical scavenger activity (DPPH assay), catalase (CAT) and ascorbate peroxidase (APX) activities of *T. simmleri* flowers at 0 (T0), 2 (T2), and 6 (T6) postharvest days (storage at 4°C)

Parameters	Days		
	0	2	6
Total polyphenols (mg GAEq/g FW)	1.22 ± 0.01 a	1.30 ± 0.04 a	1.32 ± 0.03 a
Total flavonoids (mg CEq/g FW)	0.30 ± 0.01 a	0.32 ± 0.01 a	0.29 ± 0.01 a
Total anthocyanins (mg MEq/g FW)	0.21 ± 0.02 b	0.29 ± 0.01 a	0.24 ± 0.02 a
DPPH assay (IC_{50} mg/ml)	4.20 ± 0.28 a	3.46 ± 0.19 a	5.22 ± 0.09 b
CAT activity ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	12.68 ± 0.41 b	9.05 ± 0.24 c	21.25 ± 0.27 a
APX activity ($\mu\text{mol min}^{-1} \text{mg}^{-1}$)	0.66 ± 0.04 b	0.64 ± 0.04 b	0.83 ± 0.04 a

Data are reported as mean ± standard error (n = 6). Different letters indicate statistically significant differences ($P < 0.05$; Tukey's HSD test).

their polymerization into dark brown pigments, as a result of the activities of polyphenol oxidase (PPO), peroxidase and phenylalanine ammonia lyase (PAL) (Landi *et al.*, 2018; Hu and Shen, 2021). The same process could also be responsible for the changes in the color coordinates a^* and b^* , which turn towards darker hues (Table 1).

Antioxidant compound and enzyme activities

Polyphenols are considered as the most important and widest natural compounds with antioxidant activity (Cavaiuolo *et al.*, 2013). Thanks to their bioactive potential, these molecules can help to prevent chronic degenerative diseases, cardiovascular disorders, and different types of cancer (Pires *et al.*, 2019; Skrajda-Brdak *et al.*, 2020). Postharvest treatment should maintain unaltered flowers polyphenols concentration to guarantee health benefit until flowers consumption. Our results satisfied this statement, because no changes were observed up to T6 for polyphenol and flavonoids amounts (Table 2). Indeed, a short increase in the total anthocyanins content was quantified already after 2 days (T2) that could be correlated to the interchange between bluish and reddish parameters (Table 1). Despite this positive trend, it should be noted that *T. simmleri* fresh flowers are characterized by low amount of phenolic compound than other well-known and currently consumed EFs (Li *et al.*, 2014; Chen *et al.*, 2018). Moreover, higher quantities of polyphenols and flavonoids were also reported in other species of the same genus, such as *T. cominsii* and *T. violacea*, probably connected to the use of different extraction methods (Landi *et al.*, 2018; Rivas-García *et al.*, 2022). Nevertheless, maintaining the levels of phenolic compounds in *T. simmleri* flowers could indicate that this species did not show substantial signs of decay up to the end of the experiment. As regards total anthocyanins content, their increase was previ-

ously observed also in other EFs stored at low temperature, but the regulatory mechanisms in flowers are still under debate (Shvarts *et al.*, 1997; Landi *et al.*, 2015; Marchioni *et al.*, 2020 b).

Senescence and flowers exposure to low temperatures are tightly associated with a rise in reactive oxygen species (ROS) level in the cells, whose production is accompanied by the activation of several enzymes involved in ROS scavenging (Cavaiuolo *et al.*, 2013; Darras, 2020). Polyphenolic compounds also take part to this process, as demonstrated by the reduction of flowers antioxidant activity observed at T6 (Table 2). In this work, the attention was paid to the ROS scavenging enzymes that use hydrogen peroxide (H_2O_2) as substrate, namely catalase (CAT) and ascorbate peroxidase (APX). *T. simmleri* flowers showed that CAT activity is higher than the one of APX (Table 2), suggesting a greater involvement of CAT in H_2O_2 inactivation. Moreover, both the enzymes increased their activity at T6 (Table 2). To the best of our knowledge, very few papers investigated ROS scavenging enzymes activity in EFs stored at chilling temperature as single postharvest treatment. In fact, Chrysargyris *et al.* (2018, 2019) combined the conservation at 5°C with preharvest salinity treatment and modified atmosphere packaging to observe the storage aptitude of *Tagetes patula* and *Petunia × hybrida* flowers. Nevertheless, in agreement with our results, APX activity was lower than the one of CAT in *T. patula* flowers, after both 7 and 14 postharvest days (Chrysargyris *et al.*, 2018). CAT activity was also investigated by Rizzo *et al.* (2019), highlighting different trend depending on the species and the polypropylene (PP) film used. In the control thesis (comparable with our experiment), CAT activity increases significantly after 6 days of cold storage only in half out of the four studied flowers (*Malva sylvestris* and *Papaver rhoeas*), similarly to what we observed for *T. simmleri*.

Aroma profile

Monoterpenes were the main class of compounds, regardless the storage time and their percentage, that represented at least 50% of the identified fraction (Table 3). Interesting to note is the drastically decrease in oxygenated hydrocarbons content which was of 77% (passing from 0- to 2-day conservation) and 60% (passing from 0- to 6-day conservation) respectively. On the contrary, this decrease was somehow compensated by the increase in the monoterpene hydrocarbons after 2-day storage (an increase of about 2-folds) and by non-terpene compounds after

6-day storage (an increase of about 2.5-folds).

In detail of composition, the fresh flower (T0) was rich in linalool and 1,8-cineol and these compounds almost completely disappear after 2 days of storage. A decrease of linalool content was observed also in papaya “Golden” fruit stored at low temperature (Gomes *et al.*, 2016). Interestingly is also the increase of limonene content, about 5-folds, from T0 and T2 (3.01% vs 14.78%, respectively), the same compound conserved the latter percentage even at T6. Worthy to note, the presence of benzyl-benzoate in the flowers is only noticeable after 2- and 6-days of refrigeration, and its quantity is tripled during this time.

This work reported for the first time the chemical composition of spontaneous emission of the studied species. Also noteworthy is the absence of sulfur compounds. Almost similar behavior has been seen in *T. violacea*, where such compounds were present in a negligible amount, which were around 1.2% in leaves and do not exceed 4% in roots detected using the same analysis technique (HS-SPME) (Staffa *et al.*, 2020). Rhizomes’ essential oil (EO) of a South African species of *T. violacea* was also reported to be rich in 2,4-dithiapentne, which represent more than the half of the identified fraction (Soyingbe *et al.*, 2013). Hydrocarbons were the major compounds in the hexane extract of *T. violacea* calli from Cairo (Egypt) (55.0%), while the flowers were rich in oxygenated compounds (74.6%) (Eid and Metwally, 2017). On the contrary, the EO from the same species studied by the same team but published two year before underline the prevalence of sulfur compounds in both leaves and flowers and represented 79.7% and 57.5%, respectively (Eid, 2015).

4. Conclusions

Cold storage can reduce some biochemical reactions, although stress conditions increase the reactive species of oxygen (ROS) inside plant tissues. *Tulbaghia simmleri* flowers maintain almost unaltered their visual quality, and their content in antioxidant compounds, up to 6 postharvest days. Moreover, cells counteract ROS production increasing CAT and APX activity. The aroma profiles changed during the cold treatment, even if monoterpenes remained the most represented class of volatile compounds. Looking at the main characteristics of the flowers we can conclude that *T. simmleri* showed a good aptitude to chilling temperature, suggesting the need to test longer period of storage.

Table 3 - Aroma profile of *T. simmleri* flowers detected by headspace solid phase microextraction (HS-SPME) at 0 (T0), 2 (T2), and 6 (T6) postharvest days

N°	Class	Component	L.R.I	Days		
				0	2	6
1	nt	(E)-3-hexen-1-ol	866	2.37 ± 0.10		
2	mh	α-Thujene	932		0.20 ± 0.00	tr
3	mh	α-Pinene	939	0.19 ± 0.02	3.36 ± 0.83	1.78 ± 0.12
4	mh	Camphene	953		0.38 ± 0.08	0.19 ± 0.01
5	nt	Benzaldehyde	961		0.93 ± 0.18	
6	mh	Sabinene	976		0.53 ± 0.11	0.26 ± 0.00
7	nt	1-octen-3-ol	978	4.89 ± 0.16		
8	mh	β-Pinene	980		1.18 ± 0.27	0.59 ± 0.02
9	nt	3-Octanone	988	2.90 ± 0.16		
10	om	2,3-dehydro-1,8-cineole	991	0.96 ± 0.08		
11	mh	Myrcene	992		1.48 ± 0.61	
12	nt	3-Octanol	993	2.09 ± 0.13		0.80 ± 0.21
13	mh	δ-3-Carene	1011		0.44 ± 0.00	
14	mh	α-Terpinene	1018	0.18 ± 0.04	1.03 ± 0.37	0.84 ± 0.08
15	mh	p-Cymene	1026	0.12 ± 0.01	7.26 ± 2.92	4.72 ± 0.70
16	mh	Limonene	1031	3.10 ± 0.08	14.74 ± 6.42	14.80 ± 1.13
17	om	1,8-Cineole	1033	53.10 ± 0.08		10.38 ± 0.25
18	om	(Z)-β-ocimene	1033		0.20 ± 0.06	0.14 ± 0.03
19	mh	(E)-β-ocimene	1040		0.62 ± 0.00	0.38 ± 0.08
20	nt	Phenyl acetaldehyde	1043	1.30 ± 0.04		
21	mh	γ-Terpinene	1062	0.63 ± 0.08	5.74 ± 0.12	3.82 ± 0.02
22	om	cis-Sabinene hydrate	1068	0.82 ± 0.16		
23	mh	Terpinolene	1088	0.32 ± 0.15	1.23 ± 0.39	1.05 ± 0.02
24	mh	Linalool	1098	15.51 ± 0.10	1.32 ± 0.68	0.92 ± 0.04
25	nt	Phenyl ethyl alcohol	1110		1.31 ± 0.03	
26	om	trans-Limonene oxide	1139		1.24 ± 0.06	
27	om	trans-Pinocarveol	1140			0.83 ± 0.10
28	om	Camphor	1143		1.89 ± 0.44	1.95 ± 0.14
29	om	Menthone	1154		0.46 ± 0.03	0.51 ± 0.01
30	om	Isomenthone	1164		0.40 ± 0.18	0.22 ± 0.01
31	om	Borneol	1165		0.76 ± 0.06	0.59 ± 0.02
32	om	δ-Terpineol	1167	tr		
33	om	trans-linalool oxide	1172	0.47 ± 0.05		0.32 ± 0.02
34	om	neo-Menthol	1174		0.76 ± 0.14	
35	om	cis-Pinocamphone				0.71 ± 0.06
36	om	4-Terpineol	1177	0.24 ± 0.08	1.57 ± 0.40	1.19 ± 0.09
37	om	α-Terpineol	1189	5.27 ± 0.08	0.79 ± 0.15	0.26 ± 0.04
38	nt	Decanal	1204	0.64 ± 0.06		
39	om	Verbenone	1205			0.30 ± 0.05
40	om	Lilac alcohol B	1210	1.08 ± 0.11		
41	nt	Methyl 4-nonenoate		0.36 ± 0.14		
42	om	trans-Carveol	1217	0.32 ± 0.08		
43	om	Methyl carvacrol	1244		0.68 ± 0.30	0.68 ± 0.12
44	om	Linalyl acetate	1257		2.14 ± 0.33	1.95 ± 0.35
45	om	Isobornyl acetate	1285		2.11 ± 0.49	1.98 ± 0.35
46	om	Myrtenyl acetate	1325	1.43 ± 0.16	0.30 ± 0.00	
47	om	Methyl perillate		0.15 ± 0.07		
48	sh	α-Cubebene	1351		0.23 ± 0.00	
49	sh	α-Longipinene	1352			1.98 ± 0.35
50	sh	α-Copaene	1376		0.63 ± 0.11	0.27 ± 0.07
51	sh	β-Caryophyllene	1418	0.99 ± 0.06	2.48 ± 0.49	2.89 ± 0.21
52	sh	α-Guaiene	1439			0.35 ± 0.04
53	sh	Aromandrene	1442		0.12 ± 0.00	0.17 ± 0.02
54	ac-12	(E)-geranyl acetone	1453	tr		

Data are reported as mean ± standard deviation (SD) (n=2).

... to be continued

Table 3 - Aroma profile of *T. simmleri* flowers detected by headspace solid phase microextraction (HS-SPME) at 0 (T0), 2 (T2), and 6 (T6) postharvest days

N°	Class	Component	L.R.I	Days		
				0	2	6
55	sh	α -Humulene	1454		0.21 \pm 0.00	0.22 \pm 0.01
56	sh	Alloaromandrene	1461		0.30 \pm 0.06	0.43 \pm 0.01
57	sh	Viridiflorene	1493		0.57 \pm 0.06	0.66 \pm 0.06
58	sh	(<i>E,E</i>)- α -farnesene	1508	tr	1.72 \pm 0.10	
59	sh	<i>trans</i> - γ -cadinene	1513			0.55 \pm 0.02
60	om	Geranyl isobutyrate	1514	tr		
61	sh	<i>trans</i> -Calamenene	1532		1.35 \pm 0.14	
62	os	Caryophellene oxide	1581		0.15 \pm 0.00	2.36 \pm 0.02
63	sh	Cadalene	1674	0.30 \pm 0.07	0.59 \pm 0.08	0.44 \pm 0.06
64	nt	Benzyl benzoate	1762		12.58 \pm 2.85	35.30 \pm 0.02
		monoterpene hydrocarbons (mh)		18.95 \pm 0.02	37.55 \pm 0.67	24.80 \pm 1.83
		oxygenated monoterpenes (om)		63.82 \pm 0.01	14.46 \pm 3.01	25.32 \pm 0.66
		<i>Total monoterpenes</i>		82.77 \pm 0.01	52.01 \pm 3.68	50.12 \pm 0.17
		sesquiterpenes hydrocarbons (sh)		1.29 \pm 0.13	7.90 \pm 0.64	9.67 \pm 0.52
		oxygenated sesquiterpenes (os)		-	0.15 \pm 0.00	0.55 \pm 0.02
		<i>Total sesquiterpenes</i>		1.29 \pm 0.13	7.97 \pm 0.75	10.22 \pm 0.54
		non terpenes (nt)		15.54 \pm 0.39	14.81 \pm 3.00	35.73 \pm 0.08
		<i>Total identified</i>		98.59 \pm 0.26	74.79 \pm 0.06	96.06 \pm 0.71

Data are reported as mean \pm standard deviation (SD) (n=2).

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