

Article

# Floral Scent Evaluation of Three Cut Flowers Through Sensorial and Gas Chromatography Analysis

Danilo Aros<sup>1,\*</sup>, Nicole Garrido<sup>1</sup>, Constanza Rivas<sup>2</sup>, Marcela Medel<sup>1</sup>, Carsten Müller<sup>3</sup>, Hilary Rogers<sup>3</sup> and Cristina Úbeda<sup>4</sup>

- <sup>1</sup> Faculty of Agricultural Sciences, University of Chile, Santa Rosa 11315, La Pintana, Santiago 7510157, Chile; nicole.garrido@ug.uchile.cl (N.G.); mmedel@uchile.cl (M.M.)
- <sup>2</sup> Vicerrectoría de Investigación y Doctorados, Universidad San Sebastián, Lota 2465, Santiago 7510157, Chile; constanzarivas@u.uchile.cl
- <sup>3</sup> School of Biosciences, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK; mullerct@cardiff.ac.uk (C.M.); rogershj@cardiff.ac.uk (H.R.)
- <sup>4</sup> Instituto de Ciencias Biomédicas, Universidad Autónoma de Chile, Av. Independencia 1027, Independencia, Santiago 7510157, Chile; c\_ubeda@us.es
- \* Correspondence: daros@uchile.cl; Tel.: +56-2-29785728

Received: 5 December 2019; Accepted: 8 January 2020; Published: 16 January 2020



MDF

Abstract: The main function of floral scent is to attract and guide pollinators, but it is also an important character in the ornamental plant industry. Several studies have considered the chemical evaluation of floral scent during vase life, but only a few have considered sensorial analysis of this character, which is a very important quality trait for the marketing of ornamental plants. This study focused on assessing the floral scent of three fragrant cut flowers of high economic importance: Lilium, chrysanthemum, and freesia. Eighty individuals were included in a sensorial analysis where the attributes of floral scent liking and intensity were evaluated. The composition of the floral scent was analyzed through the collection of headspace followed by gas chromatography-mass spectrometry (GC-MS). The floral scents of oriental lily and freesia were perceived as more intense, compared to chrysanthemum. A total of 28 volatile compounds were detected and the monoterpenes  $\beta$ -pinene (40.7  $\pm$  1.8 µg·L<sup>-1</sup>),  $\beta$ -cis-ocimene (5552  $\pm$  990 µg·L<sup>-1</sup>), and linalool (11,800  $\pm$  220 µg·L<sup>-1</sup>) were the major volatile organic compounds (VOCs) present in chrysanthemum, lilium, and freesia, respectively. The results presented in this study confirm that the concentration and abundance of volatile compounds is not directly related to the human perception of floral scent.

Keywords: floral scent; GC-MS; vase life; sensorial analysis; monoterpenes; cut flowers

## 1. Introduction

Floral scent plays a crucial role in the pollination syndrome since its main function is to attract and guide pollinators [1,2]. Floral scent is also important in the marketing of ornamental plants and has been described as one of the most important characteristics during the vase life of cut flowers for consumers [3]. However, this characteristic has been insufficiently developed in new cultivars of flowers since the heredity of floral scent is rather complicated, being easily lost and acquired across generations [4]. Moreover, negative selection has been performed by breeders due to the apparent correlation that exists between the presence of floral scent and shorter vase life of the flower [5].

The determination of the composition of floral scent is normally performed through the collection of headspace followed by gas chromatography-mass spectrometry (GC-MS) analysis through which different volatile compounds are separated and identified. By this method, floral scent has been described as a complex mixture of small (100–250 D) volatile organic compounds. Among these,

monoterpenoid and sesquiterpenoid, phenylpropanoid, and benzenoid compounds have been commonly found as being emitted by flowers [6,7]. A total of 1719 volatile organic compounds (VOCs) present in 991 species and isolated by head-space collection have been reviewed, considering 270 published studies [8].

On the other hand, scent can also be assessed through sensory analysis, which is based on the perception by our olfactory system and is commonly used to identify consumer preferences [9], since this is the only method able to evaluate hedonic attributes. Several studies have found that our olfactory system is much more sensitive and complex than any other technique for the evaluation of scent [10]. Whereas through GC-MS specific VOCs can be identified, sensorial analysis allows the evaluation of the fragrance as a whole bouquet. Moreover, sensorial analysis has been used to demonstrate the positive effect of floral scent on human health through the analysis of psychophysiological responses of subjects exposed to the fragrance of Japanese plum blossom [11]. More recently, studies have combined an olfactory system with GC-MS, resulting in olfactometry (GC-MS/O), which allows a qualitative assessment of odorous compounds [12] from products, such as meat [13] and fruits [14].

Although several studies have considered the chemical evaluation of floral scent [15–17], very little information has been published related to sensorial analysis of this important characteristic for the marketing of ornamental plants. Most of the sensorial analyses have been performed to evaluate food [18] and beverages, such as juice and wine [19,20].

This study aimed to assess the floral scent of three fragrant cut flowers of high economic importance: Lilium, chrysanthemum, and freesia, considering both sensorial and GC-MS analysis.

## 2. Materials and Methods

#### 2.1. Plant Material

Cut flowers of oriental lily (*Lilium* spp. cv. 'Sweetness'), freesia (*Freesia* x hybrida cv. 'Oberon'), and chrysanthemum (*Chrysanthemum* sp.), harvested from local growers, were purchased at the Santiago Flower Market (Santiago, Chile). Floral scent was evaluated both sensorially and using GC-MS at anthesis. For the sensorial analysis, flower stems were trimmed to 60 cm for freesia and chrysanthemum, and 80 cm for lily, and placed in 2-L graduated cylinders (Figure 1A). For the GC-MS evaluation, flower stems were trimmed to 5 cm and introduced into 1-L glass jars. Individual flowers of oriental lily and chrysanthemum were used while an inflorescence with 4 to 5 open flowers was used for freesia (Figure 1B).



**Figure 1.** Flowers of oriental lily (*Lilium* spp. cv. 'Sweetness'), freesia (*Freesia* x hybrid cv. 'Oberon'), and chrysanthemum (*Chrysanthemum* spp. cv. 'Marble') (from left to right) used for the sensorial analysis (**A**) and GC-MS analysis (**B**).

### 2.2. Sensory Analysis of Floral Scent

For the sensorial analysis, 80 individuals, including males and females with no restriction on age, were part of a 'non-trained' panel that performed the sensorial analysis carried out at the Laboratory

of Sensorial Analysis, Faculty of Agricultural Sciences, University of Chile. They were recruited via email and using advertising posters, and their participation was absolutely voluntary.

The floral scent from fresh cut flowers of three species (oriental lily, freesia, and chrysanthemum) was evaluated. Each sample was identified by a three-digit numerical code and presented to the evaluators as previously described (Figure 1A).

Individuals were firstly asked to complete a survey regarding general information about themselves (i.e., age, sex, income, flower purchasing frequency, and consumption habits) and about what they appreciate more when buying or looking at flowers. Five characters were presented (flower color and size, floral scent, stem length, and vase life) and the assessment was performed using the following Likert scale: 'Strongly agree', 'agree', 'neutral', 'disagree', and 'strongly disagree'.

After the first survey, individuals were asked to evaluate floral scent liking and intensity by randomly approaching each flower and sniffing at an approximate distance of 10 cm. They were asked to wait about 1 min in between the evaluation of each sample. Assessment of floral scent liking was performed using a survey with the following hedonic scale: 'Like extremely'; 'like very much'; 'like moderately'; 'like slightly'; 'neither like nor dislike'; 'dislike slightly'; 'dislike moderately'; 'dislike very much', and 'dislike extremely'. Floral scent intensity was also assessed with the following scale: 'Extremely high'; 'wery high'; 'moderately high'; 'slightly high'; 'neither high nor low'; 'slightly low'; 'moderately low'; 'very low'; and 'extremely low'.

## 2.3. Evaluation of VOCs Using GC-MS

The collection of VOCs from oriental lily, freesia, and chrysanthemum was performed as previously described [3,21] using three replicates for each species. Briefly, samples were presented as previously described (Figure 1B), enclosed in 1-L glass jars, with 100 mL of distilled water. The collection of VOCs from the headspaces over the flowers was performed using solid phase microextraction (SPME). A 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)-coated solid-phase microextraction (SPME) silica fiber (StableFlex fibre, Sigma Aldrich, Gillingham, UK) was exposed to the headspace for 30 min. This exposure time was established after testing 20, 30, and 40 min of exposure time, with 30 min being the best exposure time of the fiber to the sample for the higher recovery of volatile compounds (data not shown). Desorption of the collected VOCs was performed using the injection port of the gas chromatograph (GC 6890, Agilent, California, CA, USA) and exposing the fiber at 240 °C for 2 min. VOCs were separated using a 30 m, 0.25 mm ID capillary column over 0.25  $\mu$ m HP-5MS (Agilent) and using the following temperature program: Initial temperature 40 °C for 5 min, first step increase 6 °C/min to 80 °C, holding this temperature for 5 min, and second step increase 4 °C/min up to 170 °C. Electron Impact mass spectra were recorded in full scan mode from 35 to 500 m/z (70 eV, MSD 5975, Agilent) coupled to a GC (GC 6890, Agilent). Ten microliters of an internal standard composed of 2 µL of 4-methyl-2-pentanol in 10 mL of ethanol were injected regularly to ensure that the conditions of the instrument were consistent in time. Tentative identification was achieved by comparison of the mass spectra with the NIST mass spectra library (v. 2.0, 2011), and also comparing them with the Kovats found in the Flavornet (www.flavornet.org) and VCF (www.vcf-online.nl) databases. For this purpose, an n-alkanes solution (C6-C30) (Sigma Aldrich, Gillingham, UK) was injected in the same conditions as previously described to calculate Kovats retention indices.

Data were expressed as the relative area, calculated by dividing the peak area of the target ion (major ion) of each compound by the peak area of the target ion of the internal standard (major ion). Moreover, the major compound of each species,  $\beta$ -cis-ocimene (lily),  $\beta$ -pinene (chrysanthemum), and linalool (freesia) was quantified by constructing calibration curves with 5 points (known concentrations) (Appendix A). Calibration was performed using the same conditions previously described for the GC-MS analysis, collecting the headspace of increasing volumes of each pure compound diluted in ethanol. The volume of flowers was calculated and substituted by adding the same volume of water to the sampling jars. Data in Table 3 were expressed in the concentration ( $\mu$ g·L<sup>-1</sup>) obtained from calibration curves with reference standards (relative area vs. concentration).

## 2.4. Statistics

For the sensorial analysis, the hedonic scale (i.e., 'like extremely' = 9, 'dislike extremely' = 1) as well as the Likert scale (i.e., 'strongly agree' = 5, 'strongly disagree' = 1) was translated into scores, and the standard deviation (STEDV) and standard error (SE) were calculated. Analysis of variance (ANOVA) was performed using SPSS 17.0 (IBM, North Castle, NY, USA) for Windows, using Tukey's HSD (honestly significant difference) test for multiple pairwise comparisons with a significance level of 0.05.

# 3. Results

## 3.1. Sensory Analysis

Individuals that participated in the sensory analysis were mostly female (64%), below the age of 31 years old (73%), and the majority of them declared they bought flowers only occasionally (53%). Flower color (4.84) and floral scent (4.27) were the most appreciated characters, showing significant differences to vase life (3.98), stem length (3.74), and flower size (3.68) (Table 1).

**Table 1.** Distribution of the population (n = 80) that participated in the sensorial analysis of three cut flowers, considering age, sex, purchase frequency, and their opinion about the character most appreciated when buying or looking at flowers. Different small letters indicate significant differences in ANOVA followed by a Tukey's post hoc test ( $p \le 0.05$ ).

	(%)		
Age			
< 31 years old	73		
31–45 years old	19		
> 45 years old	9		
Sex			
Female	64		
Male	36		
Purchase Frequency			
Weekly	4		
Every 2 weeks	8		
Monthly	16		
Occasionally	52		
Never	20		
	Likert scale (1 to 5)		
Character Most Appreciated			
Flower size	3.68 a		
Flower colour	4.84 c		
Floral scent	4.27 b		
Stem length	3.74 a		
Vase life	3.98 a		

Sensory analysis performed by the non-trained panel composed of 80 individuals showed that the floral scent of freesia (6.91) obtained the highest liking score compared to the floral scent of oriental lily (6.11) and chrysanthemum (5.95). Regarding intensity, floral scents of oriental lily and freesia were perceived as more intense, with scores close to 'moderately high' (7.14 and 6.95, respectively), compared to chrysanthemum that only reached a value of 3.96, which is close to 'slightly low' (Figure 2).



**Figure 2.** Floral scent liking and intensity of chrysanthemum, freesia, and oriental lily, evaluated through sensorial analysis ( $\pm$ SE, *n* = 80). The scale ranged from 'like extremely/extremely high' (= 9) to 'dislike extremely/extremely low' (= 1). Different letters (small = liking, capital = intensity) indicate significant differences in ANOVA followed by a Tukey's post hoc test (*p* ≤ 0.05).

## 3.2. VOCs Analysis.

Analysis of the VOCs by GC-MS identified a total of 28 volatile compounds, most of them mono and sesquiterpenes. The highest number of compounds were detected in chrysanthemum (21 VOCs), followed by freesia (14 VOCs) and oriental lily (14 VOCs) (Table 2). The monoterpenes  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -myrcene, and D-limonene were detected in all three species, although with different relative abundances. The major volatile compounds detected were  $\beta$ -pinene, linalool, and  $\beta$  cis-ocimene for chrysanthemum, freesia, and oriental lily, respectively (Figure 3). While chrysanthemum showed the lowest value for the relative area (sum total = 1221), oriental lily showed the highest value (sum total = 84,791) (Table 2). **Table 2.** List of volatile organic compounds (VOCs) detected through GC-MS from the headspace collection of freesia, oriental lily, and chrysanthemum flowers, indicating the retention time (min), aromatic description (www.flavornet.com), and relative areas ( $\pm$ STDEV, *n* = 3) normalized to 4-methyl-2-pentanol. ID: identification reliability. A, mass spectrum and Linear Retention Index (LRI) agreed with standards; B, mass spectrum agreed with mass spectral data base and LRI agreed with the literature data (Flavornet and Pherobase); C, tentatively identified, mass spectrum agreed with mass spectral database.

Kovats	ID	Volatile Compound	Aromatic Description –	Relative Area			
				Chrysanthemum	Freesia	Oriental Lily	
928	С	xylene	plastic	$51.1 \pm 0.4$	$45.3 \pm 13.51$	$155 \pm 31$	
940	В	origanene	wood, green, herb	nd *	nd	$163 \pm 38$	
950	В	α-pinene	pine, turpentine	$78.4 \pm 12.5$	$7.40 \pm 0.77$	$719 \pm 159$	
976	В	camphene	camphor, mothball, oil, warm	$93.7 \pm 8.2$	nd	nd	
995	С	cumene	solvent	$38.9 \pm 2.2$	$23.8 \pm 5.7$	$150 \pm 51$	
1006	А	β-pinene	pine, resin, turpentine, wood	$186 \pm 9$	$9.03 \pm 1.91$	$218 \pm 64$	
1008	В	mesitylene	pesticide	$45.2 \pm 12.9$	$24.5 \pm 0.8$	$77.8 \pm 7.3$	
1024	В	D-limonene	lemon, orange	$43.83 \pm 12.62$	$37.9 \pm 10.3$	$779 \pm 118$	
1028	В	eucalyptol	pine, eucalyptus, herbal, camphor	nd	nd	$2447\pm 665$	
1036	С	β-terpinene	lemon	$24.29 \pm 5.46$	nd	$184 \pm 13$	
1046	В	trans-β-ocimene	herbaceous, weak floral, green, terpenic	$10.9 \pm 1.1$	nd	$1180\pm48$	
1054	А	β-cis-ocimene	warm herbaceous, green, terpenic	nd	$10.2\pm1.6$	35,931 ± 6234	
1062	-	Unidentified terpene 1	ni**	$21.1 \pm 3.3$	$19.8 \pm 1.6$	$3887 \pm 578$	
1085	В	γ-terpinene	gasoline, turpentine, bitter, resin	$6.94 \pm 1.09$	$4.26 \pm 1.39$	nd	
1101	А	linalool	flower, lavender, bergamot, coriander	nd	982 ± 256	nd	
1108	В	nonanal	fat, citrus, green, pungent	$12.5 \pm 1.7$	nd	nd	
1110	В	chrysanthenone	ni	$63.6 \pm 18.6$	nd	nd	
1155	В	camphor	camphor, earth, pine, spice	$159 \pm 30$	nd	nd	
1171	С	methyl benzoate	prune, lettuce, herb, sweet	nd	nd	$2916\pm918$	
1345	С	farnesane	ni	$107 \pm 27$	$135 \pm 12$	nd	
1445	В	dihydro-β-ionone	woody cedar, berry seedy, oily	nd	$47.5 \pm 14.8$	nd	
1446	В	caryophyllene	balsamic, hop, wood, spice	$41.3 \pm 9.7$	nd	nd	
1453	В	α-bergamotene	wood, warm, tea	$25.6 \pm 1.3$	nd	nd	
1490	В	β-ionone	seaweed, violet, flower, raspberry	nd	$412 \pm 118$	nd	
1496	В	α-farnesene	wood, sweet, citrus, floral	$17.9 \pm 0.5$	nd	$48.2\pm7.8$	
1512	С	(E)-β-famesene	citrus, green, floral, fresh	$94.8 \pm 5.1$	nd	nd	
1539	В	δ-cadinene	thyme, medicine, wood	$32.5 \pm 3.7$	nd	nd	
1544	-	Unidentified terpene 2	ni	$54.5 \pm 10.8$	$3.24 \pm 1.15$	nd	
		Sum total		1221	3278	84,791	
			* nd = not detected; **	ni = no information			



**Figure 3.** Chromatograms obtained from the analysis through GC-MS to detect the floral scent of (**A**) chrysanthemum, (**B**) oriental lily, and (**C**) freesia, showing the highest peak in each case (β-pinene, β-cis-ocimene, and linalool, respectively).

The lowest concentration of the major VOC was found in chrysanthemum ( $\beta$ -pinene) at 40.7 ± 1.8 µg L<sup>-1</sup> while higher concentrations were observed for  $\beta$  cis-ocimene (5552 ± 990 µg L<sup>-1</sup>) and linalool (11,800 ± 220 µg L<sup>-1</sup>), the major VOCs present in lilium and freesia, respectively (Table 3). The odor activity value (OAV) can be calculated as the ratio between the concentration of an individual compound and its odor detection threshold (ODT). Thus, an OAV above 1 means that this is an aroma active compound and therefore it contributes to the overall aroma of that particular sample. As shown in Table 3, the OAV is clearly higher than 1 in the case of ocimene and linalool, suggesting that these may be the main impact odorants of lilium and freesia flowers, respectively. However, in the case of chrysanthemum, the concentration of  $\beta$ -pinene is below the ODT and therefore it would not be an impact odorant of this flower.

**Table 3.** The concentration of major compounds detected in the floral scent of chrysanthemum, lilium, and freesia, showing their odor detection threshold (ODT) and odor activity value (OAV).

Major Compound	Flower	Concentration (µg·L <sup>−1</sup> )		ODT *	OAV
β-pinene	Chrysanthemum	40.7	±1.80	1500	0.03
β-cis-ocimene	Oriental lily	5552	±990	34	163.29
Linalool	Freesia	11,800	±220	1	11,800

\* ODT values were taken from data published by Tamura et al. (2001).

Following terpenes, benzenoid compounds (3) were numerically the next most abundant class of VOCs detected in all three species evaluated while volatile compounds belonging to the groups of ketones (2), aldehydes (1), and esters (1) were exclusively detected in freesia, chrysanthemum, and oriental lily, respectively (Figure 4).



■ Oriental lily ■ Freesia ■ Chrysanthemum

## 4. Discussion

Data on the purchase frequency showed that the majority of the participants buy flowers only occasionally (53%). This result is similar to a previous study performed in Wales (UK), in which participants also declared that they only buy flowers for special occasions (74%) [3]. In another study, Taiwanese consumers showed a purchasing frequency of 1 to 2 (37.9%) and 3 to 14 (39.4%) times per year [22]. It is perhaps surprising that the results are not very different among these three studies,

**Figure 4.** Number of VOCs detected by GC-MS analysis in flowers of oriental lily, freesia, and chrysanthemum, clustered by chemical groups.

considering that the per capita consumption of flowers is higher in the UK and Taiwan compared to Chile [23], where our study was carried out.

Flower color and scent were the two characters most appreciated by consumers, which is in agreement with previous results [3]. In terms of the most appreciated characters, the results show no differences between Chilean and British evaluators. Furthermore, this finding also confirms that, considering the consumer's opinion, floral scent is an important character to study in flowers.

The floral scent of freesia obtained the highest liking score by the evaluators, which could be associated to the highest intensity being perceived in this species. This association is supported by the data from chrysanthemum, which reached the lowest values for both the intensity and liking of the floral scent. Moreover, a previous study also suggested a positive correlation between floral scent liking and intensity [3]. However, the association between scent liking and intensity is not completely clear as both negative and positive correlations between these two characters have been reported using synthetic odors [24], everyday odors [25], and ambient scents [26]. The association between intensity and liking could also depend on the floral species evaluated, as an inverted U-shaped function has been described [27], suggesting, for example, that the high intensity of oriental lily could be associated to a negative effect on its liking appreciation.

Cross-modal associations between different sensory modalities have been described [28], particularly for the association between smell and sight, including associations between odors and colors [29] and odors and abstract symbols [30]. Thus, it is very likely that the appreciation of floral scent liking and intensity was modulated by the color and general appearance of the flowers displayed in this experiment.

Freesia was particularly well evaluated in terms of floral scent liking (6.91), compared to other scented flowers, such as segregating lines of alstroemeria, which in a previous study were scored closer to 6 (using the same hedonic scale) [3]. Moreover, the same study showed that the highest intensity observed for the scented alstroemerias was only close to 6, indicating a higher value for this character in both freesia and oriental lily.

The major volatile compounds detected were  $\beta$ -pinene, linalool, and  $\beta$  cis-ocimene for chrysanthemum, freesia, and oriental lily, respectively (Figure 3). These three VOCs are present in the floral scent of more than 50% of the families of seed plants according to a review previously published [8]. As expected, most of the compounds detected as part of the floral scent in the three species analyzed were terpenes. In particular monoterpenes were the most abundant compounds, followed by sesquiterpenes (Figure 4). Terpenoid compounds are the largest group of plant natural products, with a wide variety of different structures playing different ecological roles [31], and many of them have been found in the floral scents of different plant species, acting mainly as attractors of pollinators [32]. The human perception threshold of these compounds is commonly very low; therefore, they are usually impact aroma compounds in the flowers [33].

The volatile profile of some species of *Chrysanthemum* spp. have been described [34–36] and several of the compounds detected in our study were also reported previously. In particular camphor,  $\alpha$ -pinene, and  $\beta$ -pinene were reported as the main volatile compounds of the essential oil of *Chrysanthemum coronarium* flowers [34], which are the same major VOCs we detected for this species. Moreover, caryophyllene, a sesquiterpene previously reported in the scent of several flowers [8], in our study, was only observed in the flowers of chrysanthemum. This sesquiterpene, together with the previously described camphor, has been described as one of the impact aroma compounds in chrysanthemum [35,36]. In contrast, in freesia, a lower number of volatile compounds were detected. Among them, linalool was the most abundant. This compound has been found in over 50% of plant families [8], and in freesia, the importance of linalool in the aroma of this species has been reported [37]. Previous studies concluded that based on the stable inheritance of linalool emission and the higher amount compared to other VOCs in the bouquet, linalool may have an important influence on the aroma of freesias [38]. The purpose of the production of linalool is not clear, but it might be to attract insects, or be related to a defense mechanism since linalool is relatively toxic to animals and microorganisms [39]. Also, in this

flower, we observed a high amount of  $\beta$ -ionone, a carotenoid-derived compound [40] that has been found in several species, such as *Osmanthus fragrans* [41], petunia [42], and gladiolous [43]. Particularly in freesia,  $\beta$ -ionone has been described as the key compound influencing human perception of freesia's floral scent [44]. This occurs because  $\beta$ -ionone shows extreme sensitivity differences depending on the odorant receptors of the evaluators [45].

In comparison with chrysanthemum and freesia, the sum total of the relative area of VOCs was 70 and 25 times higher in the oriental lily than in the other two species, respectively. In the lily,  $\beta$ -cis-ocimene, a monoterpene, was the most abundant compound, followed by unidentified terpene 1, methyl benzoate, and eucalyptol.  $\beta$ -cis-ocimene has been detected as the major compound in oriental lilies (cv. 'Marco Polo', 'Siberia', and 'Sorbonne'), whereas this compound contributed little to the sum total of compounds in Asiatic and Longiflorum x Asiatic (LA) cultivars [15]. A previous study also described  $\beta$ -cis-ocimene, eucalyptol, and linalool as the three major monoterpenes in all the oriental cultivars of lilium analyzed to date [46]. Moreover,  $\beta$ -cis-ocimene has been described as a key floral volatile in plants, playing an important role as an attractant of a wide spectrum of pollinators [47].

Another VOC found in the lilium bouquet was origanene ( $\alpha$ -thujene), a terpene described as having a 'wood/green/herb' scent, and which was not detected in the other two species tested here. This compound has previously been described in Bromeliaceae and *Montanoa tomentosa* flowers [48,49]. Also, the quantities of D-limonene and unidentified terpene 1 were much higher in oriental lily than in the other two flowers. These two monoterpenes (origanene and D-limonene) are very common compounds present in floral scent, present in more than 70% of families of plants [8], and have both been described as important contributors to the floral scent in attracting pollinators [50].

As mentioned above, the scent of chrysanthemum flowers contained the highest number of volatile compounds, followed by freesia and oriental lily, but oriental lily showed the highest total amount of volatiles followed by freesia and chrysanthemum.

The highest score for floral scent liking was observed in the evaluation of freesia, which was close to 7 ('like moderately'), compared to chrysanthemum and oriental lily, with values close to 6 ('like slightly'). This might be due to the aromatic description of the major VOC of freesia, linalool, which is described as 'flower/lavender', whereas the major VOCs of oriental lily and chrysanthemum are descried as 'warm herbaceous/green/terpenic' ( $\beta$ -cis-ocimene) and 'pine/resin/turpentine' ( $\beta$ -pinene), respectively. Thus, the association the evaluators made between freesia and a 'flower/lavender' fragrance could have induced them to appreciate the floral scent of this species more.

Sensorially, the floral scent of oriental lily did not show significant differences in terms of intensity with freesia (Figure 2), despite the fact that its relative area total sum of the VOCs (84,791) was about 25 times higher than the total sum of the VOCs of freesia (3278) (Table 2). This result suggests that when analyzing floral scent, the total VOC abundance is not necessarily associated to intensity perception. VOCs only participate in the aroma that is perceived by humans if they are above a certain odor threshold [51] and it is possible that the compounds detected in oriental lily were not more highly perceived by the evaluators, despite their higher concentration compared to freesia. In fact, linalool, the major VOC present in freesia, has been reported to have a lower ODT (1.0) compared to  $\beta$ -cis-ocimene (34) and  $\beta$ -pinene (1500) [33], the major VOCs of oriental lily and chrysanthemum, respectively. However, it has been reported that odor receptors may participate by interacting among aroma compounds, showing no-effect, a masking effect, additive action, and a synergistic effect [52,53]. Furthermore, even compounds at sub-threshold concentrations have been found to interact with other VOCs to contribute to the aroma [54].

### 5. Conclusions

The results presented in this study confirmed that the concentration and abundancy of volatile compounds is not directly related to the human perception of floral scent, as many other factors, some of them still unrevealed, are involved during the interaction between chemical compounds and odor receptors. While through GC-MS specific volatile compounds were identified, sensorial analysis

allowed an evaluation of the fragrance as a whole bouquet. Although our olfactory system can be more sensitive than analytical tools, we were unable to identify these compounds independently. Finally, if the main goal is to satisfy consumer demand, for example, by obtaining new scented cultivars, then the best methodology would be sensorial analysis, as hedonic evaluation is exclusive for human beings. If we are focused on detecting an optimum point of quality from a floral scent perspective, further analysis should be performed throughout the vase life, taking into consideration the effect of cold storage as well.

Author Contributions: Conceptualization, D.A., C.U. and M.M.; methodology, C.U., M.M. and C.M.; formal analysis, N.G., C.R., D.A. and C.U.; data curation, N.G. and C.R.; investigation, N.G. and C.R.; writing—original draft preparation, D.A., H.R. and C.M.; writing—review and editing, D.A., C.U., H.R. and C.M.; visualization, D.A., C.U., H.R. and C.M.; supervision, D.A., C.U. and H.R.; project administration, D.A.; funding acquisition, D.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by FONDECYT Initiation into Research N°11130325 and FONDEQUIP N°EQM130129, Government of Chile.

Acknowledgments: The authors would like to thank to Héctor Morales for his valuable technical support during the GC-MS experiments.

Conflicts of Interest: The authors declare no conflict of interest.

## Appendix A

The concentration of the major volatile compounds of each species ( $\beta$ -pinene, linalool, and  $\beta$ -cis-ocimene) was calculated by constructing calibration, finding  $R^2 > 0.93$  for all the three VOCs analyzed.



**Figure A1.** Calibration curves of the three major compounds detected in flowers of chrysanthemum, freesia, and oriental lily.

## References

- Dudareva, N.; Klempien, A.; Muhlemann, J.K.; Kaplan, I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytol.* 2013, 198, 16–32. [CrossRef] [PubMed]
- 2. Raguso, R.A. Wake up and smell the roses: The ecology and evolution of floral scent. *Annu. Rev. Ecol. Evol. Syst.* **2008**, *39*, 549–569. [CrossRef]

- 3. Aros, D.; Spadafora, N.; Venturi, M.; Núñez-Lillo, G.; Meneses, C.; Methven, L.; Müller, C.; Rogers, H. Floral scent evaluation of segregating lines of *Alstroemeria caryophyllaea*. *Sci. Hortic.* **2015**, *185*, 183–192. [CrossRef]
- Amrad, A.; Moser, M.; Mandel, T.; de Vries, M.; Schuurink, R.C.; Freitas, L.; Kuhlemeier, C. Gain and loss of floral scent production through changes in structural genes during pollinator-mediated speciation. *Curr. Biol.* 2016, 26, 3303–3312. [CrossRef] [PubMed]
- Borda, A.M.; Clark, D.G.; Huber, D.J.; Welt, B.A.; Nell, T.A. Effects of ethylene on volatile emission and fragrance in cut roses: The relationship between fragrance and vase life. *Postharvest Biol. Technol.* 2011, 59, 245–252. [CrossRef]
- Dudareva, N.; Pichersky, E. Biochemical and molecular genetic aspects of floral scents. *Plant Physiol.* 2000, 122, 627–634. [CrossRef]
- 7. Dudareva, N.; Pichersky, E. (Eds.) Biology of Floral Scent; CRC Press: Boca Raton, FL, USA, 2006.
- Knudsen, J.T.; Eriksson, R.; Gershenzon, J.; Ståhl, B. Diversity and distribution of floral scent. *Bot. Rev.* 2006, 72, 1–120. [CrossRef]
- 9. Veramendi, M.; Herencia, P.; Ares, G. Perfume odor categorization: To what extent trained assessors and consumers agree? *J. Sens. Stud.* **2013**, *28*, 76–89. [CrossRef]
- 10. Hinterholzer, A.; Schieberle, P. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flavour Fragr. J.* **1998**, *13*, 49–55. [CrossRef]
- 11. Jo, H.; Rodiek, S.; Fujii, E.; Miyazaki, Y.; Park, B.J.; Ann, S.W. Physiological and psychological response to floral scent. *HortScience* **2013**, *48*, 82–88. [CrossRef]
- 12. Brattoli, M.; Cisternino, E.; Dambruoso, P.R.; De Gennaro, G.; Giungato, P.; Mazzone, A.; Palmisani, J.; Tutino, M. Gas chromatography analysis with olfactometric detection (GC-O) as a useful methodology for chemical characterization of odorous compounds. *Sensors* **2013**, *13*, 16759–16800. [CrossRef] [PubMed]
- 13. Wu, W.; Tao, N.P.; Gu, S.Q. Characterization of the key odor-active compounds in steamed meat of *Coilia ectenes* from Yangtze River by GC-MS-O. *Eur. Food Res. Technol.* **2014**, 238, 237–245. [CrossRef]
- 14. Úbeda, C.; San-Juan, F.; Concejero, B.; Callejón, R.M.; Troncoso, A.M.; Morales, M.L.; Ferreira, V.; Hernández-Orte, P. Glycosidically bound aroma compounds and impact odorants of four strawberry varieties. *J. Agric. Food Chem.* **2012**, *60*, 6095–6102. [CrossRef]
- 15. Kong, Y.; Sun, M.; Pan, H.T.; Zhang, Q.X. Composition and emission rhythm of floral scent volatiles from eight lily cut flowers. *J. Am. Soc. Hortic. Sci.* **2012**, *137*, 376–382. [CrossRef]
- 16. Yue, Y.; Yu, R.; Fan, Y. Characterization of two monoterpene synthases involved in floral scent formation in *Hedychium coronarium. Planta* **2014**, 240, 745–762. [CrossRef] [PubMed]
- 17. Li, Y.; Wan, Y.; Sun, Z.; Li, T.; Liu, X.; Ma, H.; Li, Z. Floral scent chemistry of *Luculia yunnanensis* (Rubiaceae), a species endemic to China with sweetly fragrant flowers. *Molecules* **2017**, *22*, 879. [CrossRef] [PubMed]
- 18. Amerine, M.A.; Pangborn, R.M.; Roessler, E.B. *Principles of Sensory Evaluation of Food*; Elsevier: Amsterdam, The Netherlands, 2013.
- 19. Andreu-Sevilla, A.J.; Mena, P.; Martí, N.; Viguera, C.G.; Carbonell-Barrachina, Á.A. Volatile composition and descriptive sensory analysis of pomegranate juice and wine. *Food Res. Int.* **2013**, *54*, 246–254. [CrossRef]
- 20. Del Barrio-Galán, R.; Medel-Marabolí, M.; Peña-Neira, Á. Effect of different aging techniques on the polysaccharide and phenolic composition and sensory characteristics of Syrah red wines fermented using different yeast strains. *Food Chem.* **2015**, *179*, 116–126. [CrossRef]
- 21. Aros, D.; Gonzalez, V.; Allemann, R.K.; Müller, C.T.; Rosati, C.; Rogers, H.J. Volatile emissions of scented Alstroemeria genotypes are dominated by terpenes, and a myrcene synthase gene is highly expressed in scented Alstroemeria flowers. *J. Exp. Bot.* **2012**, *63*, 2739–2752. [CrossRef]
- 22. Huang, L.C.; Yeh, T.F. Floral consumption values for consumer groups with different purchase choices for flowers. *HortTechnology* **2009**, *19*, 563–571. [CrossRef]
- 23. Rabobank. World floriculture map 2015. Rabobank Ind. Note 2015, 475, 1-4.
- 24. Doty, R.L. An examination of relationships between the pleasantness, intensity, and concentration of 10 odorous stimuli. *Percept. Psychophys.* **1975**, 17, 492–496. [CrossRef]
- Distel, H.; Ayabe-Kanamura, S.; Martínez-Gómez, M.; Schicker, I.; Kobayakawa, T.; Saito, S.; Hudson, R. Perception of everyday odors—Correlation between intensity, familiarity and strength of hedonic judgement. *Chem. Senses* 1999, 24, 191–199. [CrossRef] [PubMed]
- 26. Leenders, M.A.; Smidts, A.; El Haji, A. Ambient scent as a mood inducer in supermarkets: The role of scent intensity and time-pressure of shoppers. *J. Retail. Consum. Serv.* **2016**, *48*, 270–280. [CrossRef]

- 27. Moskowitz, H.R. Intensity and hedonic functions for chemosensory stimuli. *Chem. Senses Nutr.* **1977**, 1, 71–101.
- 28. Spence, C. Crossmodal correspondences: A tutorial review. *Atten. Percept. Psychophys.* **2011**, *73*, 971–995. [CrossRef]
- 29. Schifferstein, H.N.; Tanudjaja, I. Visualising fragrances through colours: The mediating role of emotions. *Perception* **2004**, *33*, 1249–1266. [CrossRef]
- 30. Seo, H.S.; Arshamian, A.; Schemmer, K.; Scheer, I.; Sander, T.; Ritter, G.; Hummel, T. Cross-modal integration between odors and abstract symbols. *Neurosci. Lett.* **2010**, *478*, 175–178. [CrossRef]
- 31. Degenhardt, J.; Köllner, T.G.; Gershenzon, J. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry* **2009**, *70*, 1621–1637. [CrossRef]
- 32. Schiestl, F.P. The evolution of floral scent and insect chemical communication. *Ecol. Lett.* **2010**, *13*, 643–656. [CrossRef]
- 33. Tamura, H.; Boonbumrung, S.; Yoshizawa, T.; Varanyanond, W. The volatile constituents in the peel and pulp of a green Thai mango, Khieo Sawoei cultivar (*Mangifera indica* L.). *Food Sci. Technol. Res.* **2001**, *7*, 72–77. [CrossRef]
- 34. Alvarez-Castellanos, P.P.; Bishop, C.D.; Pascual-Villalobos, M.J. Antifungal activity of the essential oil of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. *Phytochemistry* **2001**, *57*, 99–102. [CrossRef]
- Usami, A.; Ono, T.; Marumoto, S.; Miyazawa, M. Comparison of volatile compounds with characteristic odor in flowers and leaves of nojigiku (*Chrysanthemum japonense*). J. Oleo Sci. 2013, 62, 631–636. [CrossRef] [PubMed]
- 36. Wu, Y.; Yan, Z.; Zhao, J.; Zhu, Y. Headspace solid-phase microextraction and gas chromatography-mass spectrometry of volatile components of *Chrysanthemum morifolium* Ramat. *Trop. J. Pharm. Res.* **2016**, *15*, 2241–2244. [CrossRef]
- Wongchaochant, S.; Inamoto, K.; Doi, M. Analysis of flower scent of freesia species and cultivars. *Acta Hortic.* 2005, 673, 595–601. [CrossRef]
- 38. Fu, Y.; Gao, X.; Xue, Y.; Hui, Y.; Chen, F.; Su, Q.; Wang, L. Volatile compounds in the flowers of Freesia parental species and hybrids. *J. Integr. Plant Biol.* **2007**, *49*, 1714–1718. [CrossRef]
- 39. Bruneton, J. Pharmacognosy, Phytochemistry, Medicinal Plants; Lavoisier publishing: Paris, France, 1995.
- 40. Urlacher, V.B.; Makhsumkhanov, A.; Schmid, R.D. Biotransformation of β-ionone by engineered cytochrome P450 BM-3. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 53–59. [CrossRef]
- 41. Baldermann, S.; Kato, M.; Fleischmann, P.; Watanabe, N. Biosynthesis of α-and β-ionone, prominent scent compounds, in flowers of *Osmanthus fragrans*. *Acta Biochim. Pol.* **2012**, *59*, 79–81. [CrossRef]
- 42. Simkin, A.J.; Underwood, B.A.; Auldridge, M.; Loucas, H.M.; Shibuya, K.; Schmelz, E.; Clark, D.G.; Klee, H.J. Circadian regulation of the PhCCD1 carotenoid cleavage dioxygenase controls emission of β-ionone, a fragrance volatile of petunia flowers. *Plant Physiol.* **2004**, *136*, 3504–3514. [CrossRef]
- 43. Suzuki, K.; Oyama-Okubo, N.; Nakayama, M.; Takatsu, Y.; Kasumi, M. Floral scent of wild Gladiolus species and the selection of breeding material for this character. *Breed. Sci.* **2008**, *58*, 89–92. [CrossRef]
- 44. Wooding, S. Olfaction: It makes a world of scents. Curr. Biol. 2013, 23, R677–R679. [CrossRef]
- 45. Jaeger, S.R.; McRae, J.F.; Bava, C.M.; Beresford, M.K.; Hunter, D.; Jia, Y.; Atkinson, K.R.; Chheang, S.L.; Jin, D.; Peng, M.; et al. A Mendelian trait for olfactory sensitivity affects odor experience and food selection. *Curr. Biol.* **2013**, *23*, 1601–1605. [CrossRef]
- 46. Johnson, T.S.; Schwieterman, M.L.; Kim, J.Y.; Cho, K.H.; Clark, D.G.; Colquhoun, T.A. Lilium floral fragrance: A biochemical and genetic resource for aroma and flavor. *Phytochemistry* **2016**, *122*, 103–112. [CrossRef]
- 47. Farré-Armengol, G.; Filella, I.; Llusià, J.; Peñuelas, J. β-Ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules* **2017**, *22*, 1148. [CrossRef]
- Robles-Zepeda, R.E.; Lozoya-Gloria, E.; López, M.G.; Villarreal, M.L.; Ramírez-Chávez, E.; Molina-Torres, J. Montanoa tomentosa glandular trichomes containing kaurenoic acids chemical profile and distribution. Fitoterapia 2009, 80, 12–17. [CrossRef]
- 49. Hilo de Souza, E.; Massarioli, A.P.; Moreno, I.A.; Souza, F.V.; Ledo, C.A.; Alencar, S.M.; Martinelli, A.P. Volatile compounds profile of Bromeliaceae flowers. *Rev. Biol. Trop.* **2016**, *64*, 1101–1116. [CrossRef]

- 50. Byers, K.J.; Vela, J.P.; Peng, F.; Riffell, J.A.; Bradshaw, H.D. Floral volatile alleles can contribute to pollinator-mediated reproductive isolation in monkeyflowers (Mimulus). *Plant J.* **2014**, *80*, 1031–1042. [CrossRef]
- 51. Toropov, A.A.; Toropova, A.P.; Cappellini, L.; Benfenati, E.; Davoli, E. Odor threshold prediction by means of the Monte Carlo method. *Ecotoxicol. Environ. Saf.* **2016**, *133*, 390–394. [CrossRef]
- 52. Saison, D.; De Schutter, D.P.; Uyttenhove, B.; Delvaux, F.; Delvaux, F.R. Contribution of staling compounds to the aged flavour of lager beer by studying their flavour thresholds. *Food Chem.* **2009**, *114*, 1206–1215. [CrossRef]
- Zhu, J.; Chen, F.; Wang, L.; Niu, Y.; Xiao, Z. Evaluation of the synergism among volatile compounds in Oolong tea infusion by odour threshold with sensory analysis and E-nose. *Food Chem.* 2017, 221, 1484–1490. [CrossRef]
- 54. Atanasova, B.; Thomas-Danguin, T.; Langlois, D.; Nicklaus, S.; Chabanet, C.; Etiévant, P. Perception of wine fruity and woody notes: Influence of peri-threshold odorants. *Food Qual. Prefer.* **2005**, *16*, 504–510. [CrossRef]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).