Floral scent: from sensorial to instrumental evaluation

D. Aros[1,a], N. Garrido[1], M. Suazo[1], M. Medel[1] and C. Úbeda[2]

[1]Faculty of Agricultural Sciences, University of Chile, Santiago, Chile; [2]Biomedical Sciences Institute, Faculty of Sciences, Autonomous University of Chile, Santiago, Chile.

Abstract

Floral scent plays a crucial role in the interactions between plants and their environment, and is composed of several to many volatile compounds. From the aesthetic point of view, floral scent is one of the characters most appreciated in flowers, although it is a very variable trait that is frequently acquired and lost throughout evolution. Thus, this character is not often included as a target for ornamental plant-breeding programmes. Several methodologies have been described for floral scent evaluation, both sensorial and instrumental. Considering the importance of this character for the flower market industry, this study focused on the evaluation of floral scent of three scented cut-flowers: *Lilium* 'Sweetness', *Freesia* 'Oberon' and 'semi-double' chrysanthemum. Sensorial evaluation was performed by 78 untrained individuals, and floral scent composition was evaluated by using GC-MS. Freesia showed the highest acceptability, although it was the sample that contained the smallest number of volatile compounds. Chrysanthemum presented the largest number of volatile compounds. For the oriental lily, the sensorial panel designated it as the one with the highest intensity of scent. This was in agreement with the analytical results, because this flower showed relative volatile abundance over a thousand times that of chrysanthemum and freesia. Sensorial and instrumental evaluation showed different results; therefore, it is important to combine and associate these two techniques in order to achieve valuable evaluation of floral scent.

Keywords: gas chromatography, mass spectrometry, oriental lily, freesia, chrysanthemum

INTRODUCTION

The main function of floral scent is to attract and guide pollinators (Jürgens et al., 2003; Reinhard et al., 2004), and it plays a crucial role in fertilization and consequently in production of seeds and fruits (Free, 1970). The admiration and sensual pleasure to mankind generated by flower fragrance has produced a new commercial commodity, even though it is known that floral scent has evolved in plants for other reasons (Vainstein et al., 2001). Both the composition and number of volatiles in the fragrance determine a scent's appeal to humans, and may provoke big differences in perception (Burdock, 1995). From the aesthetic point of view, floral scent is one of the characters most appreciated in flowers, although it is a very variable trait that is frequently acquired and lost throughout evolution. Thus, this character is not often included as a target for ornamental plant-breeding programmes (Aros et al., 2015).

Many investigations have been performed in order to determine the composition of floral scent through collection of headspace and then gas chromatography and mass spectrometry (GC-MS) analysis, by which different volatile compounds are separated and identified. These studies have described floral scent as a complex mixture composed primarily of terpenoid, phenylpropanoid and benzenoid compounds (Dudareva and Pichersky, 2000). Furthermore, Knudsen et al. (1993) have described more than 700 plant volatile compounds observed in 441 taxa.

Another methodology carried out to study floral scent is through sensorial analysis, which is focused on the perception that our olfactory system is able to detect. Sensorial

[a]E-mail: daros@uchile.cl

analysis is widely used to evaluate food products, and little is known about this technique applied to flowers. Sensorial analysis has been performed by untrained panels to evaluate freesia, lily, rose (Morinaka et al., 2001, 2002) and alstroemeria (Aros and Rogers, 2013; Aros et al., 2015), giving interesting results for both liking and intensity of floral scent.

In some cases, our olfactory system can be more sensitive than analytical tools such as GC-MS (Hinterholzer and Schieberle, 1998), although we lack precise words to characterize specific scents, and this is recognized as a disadvantage of sensorial analysis (Burdock, 1995).

Considering the importance of floral scent for the flower market industry, this study focused on the evaluation of this character on three cut-flower species using both sensorial analysis and GC-MS.

MATERIALS AND METHODS

Plant material

Floral scent of oriental lily (*Lilium* 'Sweetness'), freesia (*Freesia* × *hybrida* 'Oberon') and semi-double chrysanthemum (*Chrysanthemum* sp.) was evaluated through sensorial analysis and GC-MS evaluation. Cut flowers were obtained from the market and, for the sensorial analysis, floral stems were trimmed at 60 cm for freesia and chrysanthemum, and 80 cm for oriental lily (Figure 1). For GC-MS evaluation, floral stems were all trimmed at about 5 cm in order to fit one flower in a 1-L glass jar. All the evaluations were performed at anthesis stage described with the maximum floral scent output.

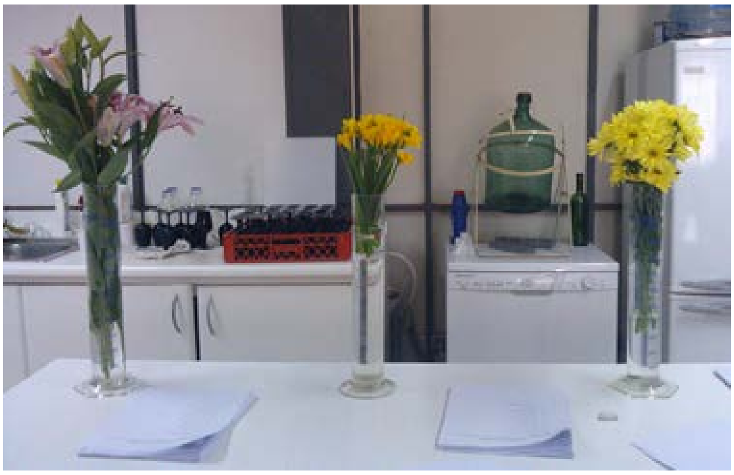

Figure 1. Cut flowers used for floral scent evaluation through sensorial analysis and GC-MS. From left to right: oriental lily (*Lilium* 'Sweetness'), freesia (*Freesia* × *hybrida* 'Oberon') and a 'semi-double' chrysanthemum (*Chrysanthemum* sp.).

Sensorial analysis

Sensorial analysis was performed at the Laboratory of Sensorial Analysis, Faculty of Agricultural Sciences, University of Chile, Santiago, Chile. Seventy-eight untrained individuals participated in the evaluation of floral scent liking. Individuals were asked to evaluate floral scent of oriental lily, freesia and chrysanthemum by ticking a box on a survey, using the following hedonic scale: 'like extremely'; 'like very much'; 'like moderately'; 'like slightly'; 'neither like nor dislike'; 'dislike slightly'; 'dislike moderately'; 'dislike very much'; 'dislike extremely'.

The hedonic scale was translated into scores (i.e. 'like extremely' = 9, 'dislike extremely' = 1), and the standard deviation (STEDV) and standard error (SE) were calculated.

GC-MS analysis

Instrumental evaluation was performed at the Laboratory of Chromatography, Faculty of Agricultural Sciences, University of Chile. The method employed for the extraction and analysis of the samples was solid phase microextraction (SPME). Single flowers of chrysanthemum and oriental lily and inflorescences of freesia were enclosed in a 1-L glass jar with 200 mL extra-pure Milli-Q water (Merck Millipore, USA). For 30 min, the sample was kept hermetically enclosed in the jar in order to saturate the headspace with the floral scent. Afterwards, a fibre (57330-U, Supelco) was exposed to the headspace for another 30 min to collect the volatile compounds. The fibre was subsequently desorbed into the injector of a gas chromatograph (Agilent, HP 6890) set at 220°C. GC analysis continued for 39 min using the following thermal profile: 5 min at 40°C and then increasing temperature at a rate of 6°C min^{-1} until a final temperature of 170°C was reached. MS analysis was carried out with an Agilent MSD 800 in full-scan mode between 35.00 and 500.00 *m/z*.

Peaks displayed in the chromatograms were analysed through Masslab Software and compared with the NIST Library (version 1.2) in order to identify each individual volatile organic compound (VOC) by matching the spectrum of the sample with the spectrum of the library. Relative area under the peak was registered in order to estimate the concentration of the detected VOCs. The compounds were semi-quantified by means of relative area with respect an internal standard of 4-methyl 2-pentanol.

RESULTS AND DISCUSSION

Sensorial analysis

Sensorial analysis showed that freesia was the highest rated by the evaluators, reaching an average liking close to 7 (‘like moderately’). Oriental lily and chrysanthemum were below 6 (‘like slightly’) on average (Figure 2).

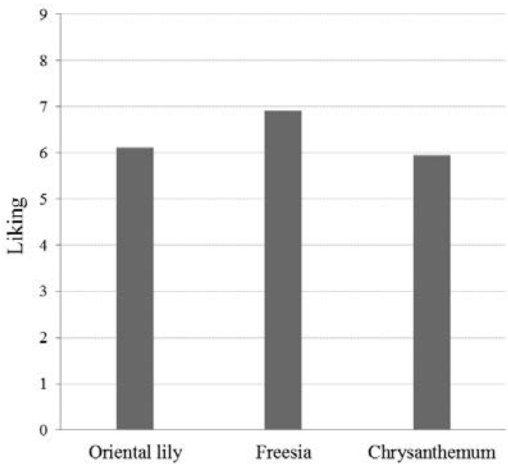

Figure 2. Floral scent liking evaluated through sensorial analysis of oriental lily (*Lilium* ‘Sweetness’), freesia (*Freesia* × *hybrida* ‘Oberon’) and a ‘semi-double’ chrysanthemum (*Chrysanthemum* sp.).

Previous studies have reported similar values for segregating lines of *Alstroemeria caryophyllaea* that showed liking values ranging between 5.9 and 6.9 (Aros et al., 2015). Another study performed by Aros (2010) showed freesia with a liking value of 7.2, slightly higher than the result obtained in the present study (6.91). In the same study, peony and *Alstroemeria* ‘Sweet Laura’ reached values of 7.1 and 7.0, respectively.

Sensorial analysis has been described as being affected by ethnic group (Distel et al., 1999), sex (Larsson et al., 2004) and age (Fusari and Ballesteros, 2008); therefore, is not surprising to find differences in floral scent liking of the same species (i.e. freesia) between the present study and those performed previously (Aros, 2010; Aros et al., 2015).

GC-MS analysis

Large numbers of VOCs were identified in oriental lily and chrysanthemum, showing 17 and 16 VOCs, respectively (Figure 3), whereas freesia only showed six VOCs. The monoterpenes *β*-pinene, linalool and *β*-*cis*-ocimene were the major VOCs detected in the floral scent of oriental lily, freesia and chrysanthemum, respectively (Table 1). These compounds are characterized as being citric, floral, green aromas. Furthermore, the largest amount of VOC was observed in oriental lily, reaching relative values up to 1500 and 8000 times those found in freesia and chrysanthemum, respectively (Table 1).

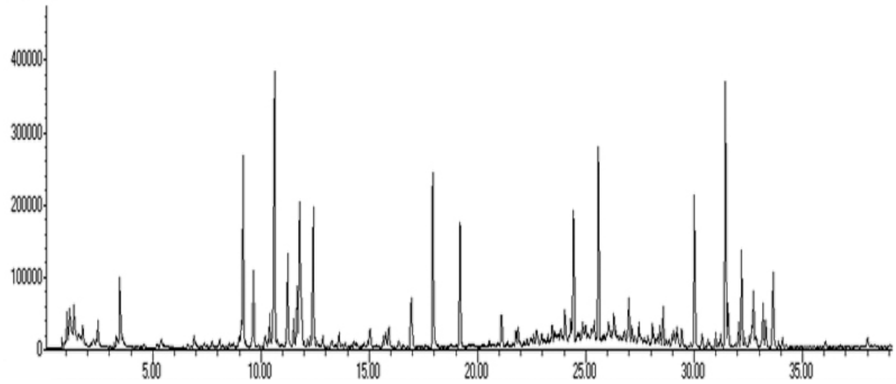

Figure 3. Chromatogram obtained from GC-MS analysis assayed in *Chrysanthemum* sp.

Table 1. Floral scent evaluation of oriental lily, freesia and chrysanthemum through GC-MS analysis.

Flower	GC-MS analysis			Aromatic description of major VOCs
	No. of VOCs	Major VOC	Relative area	
Oriental lily	17	*β*-*cis*-Ocimene	15539.8	Herbaceous, spicy, green
Freesia	6	Linalool	10.4	Citric, fruits, floral, sweet, lemon, lavender, rose
Chrysanthemum	16	*β*-Pinene	2.0	Fresh, pungent, humid, green, sweet, pine, resin, rosin, wood

Previous studies performed by Park et al. (2014) identified phenylacetaldehyde (35.2%), benzaldehyde (20.6%), camphor (4.7%), 2-phenylethyl alcohol (3.8%), bornyl acetate (1.7%) and benzyl alcohol (2.3%) as the major VOCs in chrysanthemum. In the present study, only camphor, at a lower concentration, was detected. Linalool was previously detected as the major VOC in freesia (Fu et al., 2007), confirming the result obtained in this study. In oriental lily, Oyama-Okubo et al. (2011) identified seven of the VOCs detected in the present study: benzaldehyde, benzyl alcohol, *β*-*trans*-ocimene, *β*-*cis*-ocimene, *p*-cresol, iso-eugenol and *α*-farnesene. Another study performed in *Lilium auratum* (Morinaga et al., 2009) observed 10 VOCs, among which *p*-cresol, iso-eugenol, *β*-*trans*-ocimene and *β*-*cis*-ocimene coincided with those detected in this study.

CONCLUSIONS

Both sensorial analysis and GC-MS evaluation provide valuable information for the characterization of floral scent. While specific volatile compounds were identified through GC-MS, the sensorial analysis allowed an evaluation of the fragrance as a whole bouquet. Hence, sensorial analysis data could be seen as more subjective and well appreciated from the market (i.e., liking), and GC-MS evaluation provides more objective and quantitative information (i.e., identification and quantification of VOCs).

ACKNOWLEDGEMENTS

We acknowledge funding from FONDECYT Initiation into Research no. 11130325, CONICYT, Government of Chile.

Literature cited

Aros, D. (2010). Floral scent evaluation of *Alstroemeria*. PhD thesis (Cardiff, UK: Cardiff University).

Aros, D., and Rogers, H. (2013). Floral scent as a new attribute for *Alstroemeria*: evaluation of new fragrant lines of *A. caryophyllaea.* Acta Hortic. *1002*, 207–213 http://dx.doi.org/10.17660/ActaHortic.2013.1002.25.

Aros, D., Spadafora, N., Venturi, M., Núñez-Lillo, G., Meneses, C., Methven, L., Müller, C.T., and Rogers, H. (2015). Floral scent evaluation of segregating lines of *Alstroemeria caryophyllaea.* Sci. Hortic. (Amsterdam) *185*, 183–192 http://dx.doi.org/10.1016/j.scienta.2015.01.014.

Burdock, G.A. (1995). Fenaroli's Handbook of Flavor Ingredients, 3rd edn (Boca Raton, FL, USA: CRC Press).

Distel, H., Ayabe-Kanamura, S., Martínez-Gómez, M., Schicker, I., Kobayakawa, T., Saito, S., and Hudson, R. (1999). Perception of everyday odors – correlation between intensity, familiarity and strength of hedonic judgement. Chem. Senses *24* (*2*), 191–199 http://dx.doi.org/10.1093/chemse/24.2.191. PubMed

Dudareva, N., and Pichersky, E. (2000). Biochemical and molecular genetic aspects of floral scents. Plant Physiol. *122* (*3*), 627–634 http://dx.doi.org/10.1104/pp.122.3.627. PubMed

Free, J. (1970). Insect Pollination of Crops (London, UK: Academic Press).

Fu, Y., Gao, X., Xue, Y., Hui, Y., Chen, F., Su, Q., and Wang, L. (2007). Volatile compounds in the flowers of *Freesia* parental species and hybrids. J. Integr. Plant Biol. *49* (*12*), 1714–1718 http://dx.doi.org/10.1111/j.1744-7909.2007.00571.x.

Fusari, A., and Ballesteros, S. (2008). Identification of odors of edible and nonedible stimuli as affected by age and gender. Behav. Res. Methods *40* (*3*), 752–759 http://dx.doi.org/10.3758/BRM.40.3.752. PubMed

Hinterholzer, A., and Schieberle, P. (1998). Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. Flavour Fragrance J. *13* (*1*), 49–55 http://dx.doi.org/10.1002/(SICI)1099-1026(199801/02)13:1<49::AID-FFJ691>3.0.CO;2-S.

Jürgens, A., Witt, T., and Gottsberger, G. (2003). Flower scent composition in *Dianthus* and *Saponaria* species (Caryophyllaceae) and its relevance for pollination biology and taxonomy. Biochem. Syst. Ecol. *31* (*4*), 345–357 http://dx.doi.org/10.1016/S0305-1978(02)00173-4.

Knudsen, J.T., Tollsten, L., and Bergstrom, G. (1993). Floral scents - a checklist of volatile compounds isolated by head-space techniques. Phytochemistry *33* (*2*), 253–280 http://dx.doi.org/10.1016/0031-9422(93)85502-I.

Larsson, M., Nilsson, L.G., Olofsson, J.K., and Nordin, S. (2004). Demographic and cognitive predictors of cued odor identification: evidence from a population-based study. Chem. Senses *29* (*6*), 547–554 http://dx.doi.org/10.1093/chemse/bjh059. PubMed

Morinaga, S.I., Kumano, Y., Ota, A., Yamaoka, R., and Sakai, S. (2009). Day–night fluctuations in floral scent and their effects on reproductive success in *Lilium auratum.* Popul. Ecol. *51* (*1*), 187–195 http://dx.doi.org/10.1007/s10144-008-0097-1.

Morinaka, Y., Handa, T., Takeuchi, H., Ayabe, S., and Saito, S. (2001). Validity of the sensory evaluation scales for fresh flower scent cultivars. J. Jpn. Soc. Hortic. Sci. *70* (*5*), 636–649 http://dx.doi.org/10.2503/jjshs.70.636.

Morinaka, Y., Takatsu, Y., and Hayashi, M. (2002). Sensory evaluation of floral scent in freesia (*Freesia hybrida* hort.) cultivars. J. Jpn. Soc. Hortic. Sci. *71* (*5*), 702–709 http://dx.doi.org/10.2503/jjshs.71.702.

Oyama-Okubo, N., Nakayama, M., and Ichimura, K. (2011). Control of floral scent emission by inhibitors of phenylalanine ammonia-lyase in cut flower of *Lilium* cv. 'Casa Blanca'. J. Jpn. Soc. Hortic. Sci. *80* (*2*), 190–199 http://dx.doi.org/10.2503/jjshs1.80.190.

Park, S.J., Subramaniyam, M., Moon, M.-K., Jeon, B.-B., Lee, E.-J., Han, S.-H., and Woo, C.-S. (2014). Sleep quality and skin-lightening effects of white mother chrysanthemum aroma. In Industrial Applications of Affective Engineering, J. Watada, H. Shiizuka, K.-P. Lee, T. Otani, and C.-P. Lim, eds. (Cham, Switzerland: Springer International), p.241–252.

Reinhard, J., Srinivasan, M.V., and Zhang, S. (2004). Olfaction: scent-triggered navigation in honeybees. Nature *427* (*6973*), 411 http://dx.doi.org/10.1038/427411a. PubMed

Vainstein, A., Lewinsohn, E., Pichersky, E., and Weiss, D. (2001). Floral fragrance. New inroads into an old commodity. Plant Physiol. *127* (*4*), 1383–1389 http://dx.doi.org/10.1104/pp.010706. PubMed