Industrial Crops and Products 52 (2014) 671–678

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Optimization of fragrance extraction: Daytime and flower age affect scent emission in simple and double narcissi

Fabioloa Ruíz-Ramón, Diego J. Águila, Marcos Egea-Cortines, Julia Weiss*

Genetics, ETSIA, Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, Spain

ARTICLE INFO

Article history:
Received 7 August 2013
Received in revised form 21 October 2013
Accepted 21 November 2013

Keywords:
Narcissus sp
Volatile organic compounds
Headspace solid-phase microextraction
Gas chromatography–mass spectrometry
Circadian rhythm
Double flower

ABSTRACT

The fragrance of Narcissus flowers is used for luxury perfumes because of its delicate odor. Fragrance productivity is relatively low and an increase in fragrance yield is therefore an important issue. This study investigated the development of scent emission and scent profile of narcissus cultivars of simple and double flower architecture. Headspace solid-phase microextraction (HS-SPME) and capillary gas chromatography/mass spectrometry (GC–MS) were applied to analyze flower scent in dependence of daytime and flower age. The major scent compounds identified were those typical for *Narcissus* species, monoterpenes and benzenoids, here in form of cis-β-ocimene and benzyl acetate. The double flower cultivar, with 11 major compounds had a more complex scent profile than the simple flower cultivar with 6 major volatiles. Both cultivars showed circadian emission patterns and produced significantly less scent during the night than during the day with a reduction of 40% in double flowers and 37% in single flowers. Four-day old flowers produced 37% and 59% less volatiles in double and simple flowers compared to freshly cut flowers. Volatile composition varied among cultivars, daytime and flower age, however benzyl acetate and cis-β-ocimene continuously formed the major compounds. Compound flowers with doubled perianth structures produced double amount of scent compared to simple flowers independent of daytime. The drastic differences in volatile production depending on daytime, flower age and flower architecture should be taken into account when using narcissus flowers for the production of absolute fragrance extracts.

1. Introduction

The flower odor of narcissus, a monocotyledon of the Amaryllidaceae family, is very popular in the fragrance industry. The most valued species for the perfume industry are, most importantly *Narcissus poeticus*, next to *Narcissus tazetta* and *Narcissus jonquilla* (Ferri et al., 2009) as well as descendant hybrid varieties (Van Dort et al., 1993). Several authors analyzed the pattern of volatiles emitted by a variety of different species, either searching for most interesting odors (Van Dort et al., 1993; Ehret et al., 1992) or in order to improve the knowledge about floral biology, pollination, and systematics of the genus *Narcissus* (Dobson et al., 1997). Dobson et al. (1997) analyzed nine species native to southern Spain using headspace collection and GC–MS analysis and classified them into three fragrance types based on the identity of their major volatiles. One group was dominated by fatty-acid derived acetates, a second group by monoterpenes but lacking trans-β-ocimene and a third group was dominated by monoterpenes and mainly trans-β-ocimene.

Delicate fragrances, like those from narcissus, are extracted by solvent extraction in order to achieve, first a concrete followed by an absolute extract most faithful to the scent. In case of narcissus, fragrance is extracted via hexane or petroleum ether solvent from flowers resulting in 0.2–0.3% concrete or by supercritical CO_2 extraction resulting in a yield of 0.41% (Ehret et al., 1992; Ferri et al., 2009). In case of solvent extraction, it takes 1000 kg of flowers to produce 2 kg of concrete or 750 g of absolute and the relative low productivity of absolute from narcissus makes it an expensive product, restricted to luxury perfumes (Remy, 2004).

The selection of high scent producing varieties with a favorable volatile composition can be speeded up using headspace absorption to estimate quality and productivity of absolutes. It was shown that volatile analysis by headspace solid-phase microextraction achieves similar profiles as solvent extraction with the advantage of being fast and sample-saving (Xie et al., 2012). Furthermore, quantification of volatile compounds by headspace solid-phase microextraction vs. liquid–liquid extraction with dichloromethane from food beverage revealed no significant differences in the accuracy of recovery of target compounds between the different methods (Caldeira et al., 2007). These investigations indicate that

* Corresponding author at: Department of Genetics, ETSIA, Instituto de Biotecnología Vegetal, Universidad Politécnica de Cartagena, Paseo Alfonso XIII 48, 30203 Cartagena, Spain. Tel.: +34 968325777; fax: +34 968325433.
E-mail address: Julia.weiss@upct.es (J. Weiss).

0926-6690/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2013.11.034

analysis of quantity and composition of volatiles by headspace extraction is a valuable measure for the quality of absolutes.

Volatile production and composition is not constant during flowering time. The analysis of headspace volatiles from several ornamentals including tea rose, narcissus, osmanthus, and spearmint showed that dramatic chemical changes take place after harvest leading to the typical scent pattern (Mookherjee et al., 1989). In case of Narcissus, flower bud opening lasts 24 h and scent emission starts to increase during the first 2 h after bud opening, reaching maximal intensity when flowers are fully open (Reuveni et al., 1999).

Scent emission follows a circadian controlled rhythmic pattern in several species with peak emissions at certain times during the day, as observed in *Antirrhinum majus* (Kolosova et al., 2001), *Rosa hybrida* L. (Helsper et al., 1998), *Rosa damascena* (Picone et al., 2004) or petunia (J.C. Verdonk et al., 2003). Narcissus varieties attract day-active insects, including diurnal lepidoptera (moths and butterflies) bees and flies (summarized by Dobson et al., 1997) which may hint to daytime scent emission.

Within the flower, volatiles are mainly produced by the corolla. In some plants volatiles can be produced in stamens as in *Ranunculus acris* (Bergström et al., 1995) or the pistil in case of Clarkia (Pichersky et al., 1994a). Volatiles may be produced in petals either by specialized cells in specialized positions, as the conical cells in the inner epidermis of *A. majus* (Kolosova et al., 2001) or by both the inner and outer petal epidermis as found in *R. hybrida* (Baudino et al., 2007). Perianth structures of Narcissus cultivars are divided into trumpet shaped, large cupped and small-cupped varieties, depending on the size relation between perianth and corona, a crown-like structure between petals and stamens. An additional group is formed by the double narcissi, with doubled flower parts, either perianth or corona (Dana and Lerner, 2001). In Narcissus, scent is emitted only from the corona (Willmer, 2011).

We have analyzed the scent production in two narcissus varieties differing in floral architecture, taking into account time of the day and floral age in order to optimize scent yields. Our aim was to obtain a comprehensive profile of volatiles emitted by *Narcissus* that could also explain the differences perceived by humans between simple and double flowers.

2. Materials and methods

2.1. Plant material and harvest

Narcissus flowers of local varieties of simple and double flower cultivars were harvested from plantations situated in the Mediterranean southeast of Murcia, Alhama de Murcia, Spain. The plantations were established for the cut flower production. Flowers were harvested at flower opening and kept in water in a Sanyo MRL350 growth chamber under a regime of 16 h fluorescent light at a photosynthetically active photon flux density of 250 $\mu E\, s^{-1}\, m^{-2}$ and 8 h darkness with day/night temperatures of 22/15 °C until scent collection.

Samples corresponding to day-time scent emission were taken at 10:00 a.m. and those corresponding to night-time emission at 10:00 p.m. As dawn was approaching 6 a.m., these timings corresponded to roughly subjective time 4 h and subjective time 16 h, defining subjective time 0 as the time when an organism initiates the day, coinciding with dawn (Egea-Cortines et al., 2013).

2.2. Analysis of volatile compounds

The floral scent constituents were collected by HS-SPME. Separation, and qualitative identification and quantification was performed by capillary gas chromatography/mass spectrometry (GC–MS) as described previously (Manchado-Rojo et al., 2012). Three flowers per cultivar and sampling time were placed inside Falcon tubes for 12 h (DeltaLab, www.deltalab.es). Volatiles from the headspace within the tube was extracted using a Twister bar (Gerstel GmbH & Co. KG, http://www.gerstel.de/) which consists in a magnetic stir bar of 10 mm length coated with 0.5 mm polydimethylsiloxane that had previously been conditioned.

Chromatographic peak identification is based on library matching applying the Standard Reference Database 1A NIST 2005, version 2.0 (National Institute of Standards and Technology, http://www.nist.gov/rsd/nist1a.cfm).

The relative contribution of volatile compounds was calculated based on the integrated area of particular peaks relative to the total integrated peak area.

3. Results

3.1. Volatile profile in Narcissus varieties with simple and compound flower

The chromatography profile showed a total of between 90 and 100 peaks for both varieties, the majority contributing only marginally to the total volatile emission (Fig. 1). Table 1 summarized the 21 major volatiles accounting with a relative abundance over 2% of the total emission. The two varieties analyzed presented diverging profiles in these major compounds, although both varieties showed as principle compounds benzyl acetate and

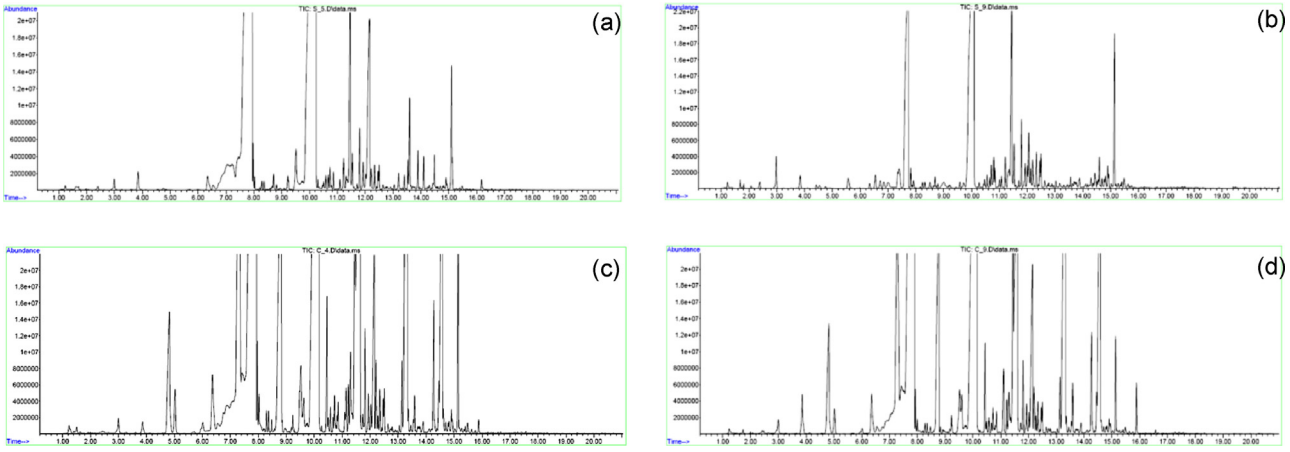

Fig. 1. Gas chromatograms of representative samples of (a) simple flower day, (b) simple flower night, (c) double flower day and (d) double flower night.