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Analytical Methods

Characterisation of aroma active compounds of Spanish saffron by gas chromatography–olfactometry: Quantitative evaluation of the most relevant aromatic compounds

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ABSTRACT

The aromatic composition of a saffron sample from Valle de Jiloca (Teruel, Spain) was evaluated for the first time by gas chromatography–olfactometry (GC–O). Volatiles released by 10 g of saffron sample were collected in a trapping system consisting of LiChrolut EN resins and eluted with dichloromethane/methanol (95:5). GC–O revealed that the aroma emitted by this kind of saffron is due to at least twenty different aroma molecules. From an olfactometric point of view, the most important aroma compounds of this saffron sample were safranal (modified frequency value [MF] 93%), followed by 2,3-butanedione, hexanal, E-2-nonenal and an odorant with a characteristic aroma of burnt curry that could not be identified. All of them had MF values higher than 70%. An estimate was made of the levels of these aromatic molecules detected by GC–O. Safranal and isophorone, both volatiles with aromatic descriptors of "saffron" were quantified using a headspace microextraction (HS-SPME) method.

1. Introduction

Saffron is the commercial name of the dried stigmas of *Crocus sativus L.* flowers. Saffron is considered to be one of the most expensive spices and is cultivated in many countries, including Spain, Greece, India, China and Iran. Saffron is used mainly as a spice, food colorant and herbal medicine, in which it is used as an analgesic (Ríos, Recio, Giner, & Manez, 1996). In recent years it has been demonstrated that saffron has distinctive anticancer activities (Nair, Pannikar, & Pannikar, 1991; Ríos et al., 1996).

Many extraction processes have been used to extract the chemical components of saffron, such as hydrodistillation (HD), micro-simultaneous hydrodistillation–extraction (MSDE) (Rodel & Petrzika, 1991; Tarantilis & Polissiou, 1997), vacuum headspace (VHS) (Tarantilis & Polissiou, 1997), supercritical fluid extraction (SFE) (Zougagh, Rios, & Valcarcel, 2006), thermal desorption (TD) (Alonso, Salinas, EstebanInfantes, & SanchezFernandez, 1996; Carmona et al., 2006), extraction with organic solvent (Tarantilis, Polissiou, & Manfait, 1994), solid-phase microextraction (SPME) (D'Auria, Mauriello & Rana, 2004) and ultrasonic solvent extraction (USE) (Jalali-Heravi, Parastar, & Ebrahimi-Najafabadi, 2009; Kanakis, Daferera, Tarantilis, & Polissiou, 2004).

Many papers have been published on saffron volatile composition (Carmona et al., 2006; Carmona, Zalacain, Salinas, & Alonso, 2007; Du, Wang, Hu, & Yao, 2008; Kanakis et al., 2004; Maggi et al., 2009; Rodel & Petrzika, 1991; Tarantilis & Polissiou, 1997; Zougagh et al., 2006). All of them have focused on identifying the major compounds present in this spice using gas chromatography coupled to mass spectrometry (GC–MS). However, information on the range of the levels of some of these major compounds is provided in only some papers (Alonso et al., 1996; Jalali-Heravi et al., 2009; Kanakis et al., 2004). In the paper published by Kanakis et al. (2004), 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde, or safranal, and 4-hydroxy-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, HTCC, were quantified in Greek saffron. Alonso et al. (1996) quantified only the safranal content present in saffron samples from La Mancha (Spain), whereas Jalali-Heravi et al. (2009) quantified forty compounds identified by GC–MS in Iranian saffron. The information obtained by GC–MS analysis is interesting and useful for knowing the qualitative profile, but incomplete because it is necessary to evaluate which compounds present are odour-active and contribute to the saffron aroma.

In order to locate and rank the odorants that are most important aromatically, olfactometric study (GC–O) is required. Few papers have undertaken GC–O studies of saffron samples. Rodel & Petrzika (1991) were the first to use GC–O to evaluate saffron aroma. In their first paper on the topic, they only confirmed the highly intense and characteristic odour associated with the peak

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generated by safranal, which is considered to be the impact compound of saffron. However, this study revealed that many other unidentified compounds contribute to the complete aroma of saffron. Cadwallader, Baek, and Cai (1997) carried out the second and most important olfactometric study of saffron samples using aroma extract dilution analysis (AEDA). They concluded that a compound tentatively identified as 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one has an extremely important role in saffron aroma, even greater than that of safranal. Recently, Maggi et al. (2009) analysed different Spanish saffron samples by GC–O. The aim was to assign aroma descriptors to the nineteen volatile saffron constituents identified by GC–MS. In conclusion, the only study that reveals complete olfactometric information in detail (gas chromatographic retention data estimated on both polar and non-polar columns, olfactory descriptors, chemical identities of the compounds responsible for these aromas and a concentration range of the most important odorant) was that of Cadwallader et al. (1997).

Therefore, the first aim of this study was to examine the aromatic profile of a saffron sample from Teruel (Spain) by GC–O for the first time and determine the key odorants. The second aim was to provide quantitative or semi-quantitative information about all of the compounds considered relevant in saffron according to the previous olfactometric study.

2. Materials and methods

2.1. Samples

The saffron sample used in this study was obtained from a saffron farming area in the province of Teruel (Aragón, Spain). A large amount of saffron was provided by La Carrasca S.A. The samples were kept at ambient temperature and protected from light until analysis.

2.2. Reagents

Solvents: dichloromethane and methanol were purchased from Merck (Darmstadt, Germany); water was purified in a MilliQ system from Millipore (Bedford, MA). Resins: Lichrolut® EN resins (non-polar resins) and polypropylene cartridges (0.8 cm internal diameter, 3 ml internal volume) were supplied by Merck (Darmstadt, Germany).

Standards: The standards used for identifications were supplied by Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), PolyScience (Niles, IL), Lancaster (Strasbourg, France) and Panreac (Barcelona, Spain). An alkane solution (C_8–C_{28}), 20 mg L^{-1} in dichloromethane, was used to calculate the linear retention index (LRI) of each analyte. A standard of (*Z*)-2-nonenal was not found, but this aldehyde is present in commercial (*E*)-2-nonenal at a concentration of 5–10% (Ferreira et al., 2009; Valim, Rouseff, & Lin, 2003).

2.3. Gas chromatography–olfactometry

2.3.1. Preparation of extracts

Saffron volatiles were collected using a purge and trap system (Campo, Ferreira, Escudero, & Cacho, 2005). The Lichrolut® EN cartridge (400 mg) was placed on top of a bubbler flask containing 10 g of saffron. This sample was purged by a stream of nitrogen at ambient temperature for 4 h. Volatile saffron constituents released in the headspace were trapped in the cartridge containing the sorbent and were further eluted with 3.2 mL of dichloromethane containing 5% methanol.

2.3.2. GC–O analysis

All sniffing experiments were carried out in a Trace gas chromatograph from ThermoQuest (San Jose, CA), equipped with a flame ionisation detector (FID) and a sniffing port (ODO-1 from SGE, Ringwood, Australia) connected by a flow splitter to the column exit. The columns used for this study were a DB-Wax column from J&W (Folsom, CA), 30 m × 0.32 mm ID, 0.5-μm film thickness, and an HP-5MS column from Agilent (Santa Clara, CA), 30 m × 0.25 mm ID, 0.25-μm film thickness. A constant pressure of 52 kPa was maintained throughout the analysis time. The carrier was H_2. One microlitre was injected in splitless mode for 1 min splitless time. Injector and detector were both kept at 250 °C. The temperature program was 40 °C for 5 min, which was then raised by 4 °C min^{-1} to 100 °C, followed by 6 °C min^{-1} to 220 °C, and finally kept at 220 °C for 20 min. To prevent condensation of high-boiling compounds on the sniffing port, the port was heated with a laboratory-made rheostat.

The olfactometric strategy used in this study combined measurements of intensity and frequency of detection and has been widely used in previous papers published by members of our laboratory (Cullere et al., 2010; Escudero, Campo, Farina, Cacho, & Ferreira, 2007).

In this GC–O study, assessments were carried out by a panel of six expert judges belonging to our laboratory. The saffron extract was concentrated to 200 μL, and the extract was smelled once by each panellist. Sniffing time was approximately 40 min and each judge took part in one session per day. Panellists were asked to score the intensity of each aromatic stimulus using a 4-point scale (0 = not detected, 1 = weak, 2 = clear but not intense note, 3 = intense note). The signal obtained was modified frequency (*MF*(%)), a parameter that was calculated with the formula proposed by Dravnieks (1985):

$$MF(\%) = (F(\%) \times I(\%))^{1/2}$$

where *F*(%) is the detection frequency of an aromatic attribute expressed as a percentage of total number of judges and *I*(%) is the average intensity expressed as percentage of the maximum intensity.

2.4. Gas chromatography–mass spectrometry (GC–MS)

2.4.1. Quantification of safranal and isophorone (HS-SPME-GC–MS)

The quantification method was designed and published by D'Auria et al. (2004). This methodology is based on HS-SPME-GC–MS analysis and uses a 100-μm PDMS-SPME fibre, purchased from Supelco-Spain (Madrid, Spain). The fibre was maintained over the sample (0.1 g) in a 20-mL vial at 36 °C for 20 min. The analyses were performed with a CP-3800 chromatograph coupled to a Saturn 2200 ion trap mass spectrometric detection system from Varian (Palo Alto, CA). A DB-Wax-ETR capillary column (J&W Scientific) of 60 m × 0.25 mm I.D., film thickness 0.25 μm, was used, preceded by a 3 m × 0.25 mm uncoated pre-column from Supelco (Bellefonte, PA).

Helium was the carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature was initially 40 °C for 5 min and was then raised at 4 °C min^{-1} to 100 °C, followed by a rate of 6 °C min^{-1} to 220 °C and finally held at this temperature for 30 min. The MS parameters were: MS transfer line and chamber ionisation temperature 200 °C and trap emission current 80 μA. The global run time was recorded in full scan mode (*m*/*z* 45–200 mass range). The chromatographic data were analysed with Varian Saturn GC–MS Version 5.2 software. The injection was in split mode 1/80 at a temperature of 250 °C. A desorption time of 0.4 min was used. The detector was held at 230 °C.