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Analytical Methods

Determination of low molecular weight volatiles in *Ficus carica* using HS-SPME and GC/FID

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ABSTRACT

Ficus carica L. is one of the earliest cultivated fruit trees, having an important consumption in Mediterranean countries. In this work, the volatile compound profiles of two characteristic Portuguese white varieties ("Pingo de Mel" and "Branca Tradicional") was determined by HS-SPME and GC/FID. Leaves, pulps and peels, submitted to freezing and lyophilisation treatments, were analysed.

The two varieties presented a similar profile composed of eight volatile compounds: acetaldehyde, ethyl acetate, methanol, ethanol, hexanal, limonene, (*E*)-2-hexenal and octanal. The total volatile content was different among the vegetal materials, following the order leaves > peels > pulps. Methanol and ethanol are the major compounds in all samples.

The developed procedure revealed to be rapid, sensitive, reproducible and accurate. The detection limit values were low, and the method precise. The recovery values for acetaldehyde, ethyl acetate, methanol and ethanol were generally high, suggesting that it will be most suitable for compounds with low molecular weight. Due to its rapidity and low cost, this technique can be useful in the quality control of fig fruit and leaves.

1. Introduction

Fig (*Ficus carica* L.) is a tree belonging to the Moraceae family, one of the first plants cultivated by humans as a seasonal food. It is an important constituent of the Mediterranean diet (Solomon et al., 2006). Previous studies with the fruits indicated that they are a source of minerals, vitamins and dietary fibre; they are fat and cholesterol-free and contain a high number of amino acids (Solomon et al., 2006; Vinson, Zubik, Bose, Samman, & Proch, 2005). Figs. also present sugars and organic acids that influence their quality, and exhibit one of the highest concentrations of polyphenols among the commonly consumed fruits and beverages, which contribute positively to human health (Veberic, Colaric, & Stampar, 2008; Vinson et al., 2005; Oliveira et al., 2009).

The formation of volatile compounds in fruits is a dynamic process, and generally the typical flavour of most of them is not present at harvest but develops after the ripening process. Volatile compounds present in fresh and processed fruits significantly affect their flavour and aroma quality, which is formed by a complex group of chemical substances (Riu-Aumatell, Castellari, López-Tamames, Galassi, & Buxaderas, 2004). The variability in aroma compounds has been reported to depend on climatological conditions, cultivar, maturity and technological factors, like harvest, post-harvest treatments, processing and storage conditions (Douillard & Guichard, 1990; Rizzolo, Polesello, & Polesello, 1992; Botondi, De Santis, Bellicontro, Vizovitis, & Mencarelli, 2003; Lin, Rouseff, Barros, & Naim, 2002).

Gas chromatography (GC) methodologies have been described for the analysis of the volatile fraction of several matrices, using different techniques (Portari, Marchini, & Jordão, 2008; Ferrari, Arado, Nardo, & Giannuzzi, 2003). The determination of volatile compounds use headspace (HS) sampling coupled to GC as the preferred technique (Portari et al., 2008). The direct sample injection in packed columns (Ferrari et al., 2003) became obsolete and was gradually substituted by headspace techniques in capillary columns (Wasfi, Al-Awadhi, Al-Hatali, Al-Rayami, & Al-Katheeri, 2004) and, more recently, by the selective headspace injection using solid-phase microextraction (SPME) fibres (Riu-Aumatell et al., 2004; Dong, Mei, & Chen, 2006).

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SPME involves the adsorption of analytes onto a fused silica fibre, coated with a suitable stationary phase, and their subsequent desorption immediately before chromatographic analysis (Pawliszyn, 2000). The target analytes can be adsorbed on the fibre by immersing it in the sample or by exposing it to the sample headspace, in which case matrix interferences can be drastically reduced (Riu-Aumatell et al., 2004). Methods using SPME technique save preparation time, solvent purchase and disposal costs and can improve the detection limits (Dong et al., 2006). Actually, the SPME technique has been successfully applied to the analysis of volatile and semi-volatile organic compounds from environmental, biological and food samples (Barroso et al., 2005; Guedes de Pinho et al., 2008, 2009), namely fruits (Ibañez, Lopez-Sebastian, Ramos, Tabera, & Reglero, 1998).

Some works have reported the presence of volatile compounds in *F. carica* (Gibernau, Buser, Frey, & Hossaert-McKey, 1997; Grison, Edwards, & Hossaert-McKey, 1999; Grison-Pigé, Hossaert-McKey, Greeff, & Bessière, 2002; Buttery, Flath, Mon, & Ling, 1986). However, to our knowledge there is no report comparing the volatile composition of several materials from *F. carica*. In addition, no previous study involved two characteristic Portuguese white varieties: “Pingo de Mel” and “Branca Tradicional”.

This study was designed to determine low molecular weight volatiles in leaf, peel and pulp of the two above mentioned *F. carica* varieties, subjected to different treatments (frozen and lyophilised), by HS-SPME coupled to GC/FID.

2. Materials and methods

2.1. Standards and reagents

All chemicals used were of analytical grade: acetaldehyde, ethyl acetate and limonene (Fluka, Milwaukee, WI), methanol and acetonitrile (Merck, Darmstadt, Germany), ethanol (Panreac, Barcelona, Spain), hexanal and octanal (Sigma–Aldrich, Steinheim, Germany), (*E*)-2-hexenal, 1-propanol, 1-butanol and 3-methyl-1-butanol (SAFC, St. Louis, USA).

Stock standard solutions of the analytes (10 g/l) were prepared in water, as well as a stock solution of the internal standard (IS) acetonitrile (1 g/100 ml).

2.2. Plant material

Ficus carica (“Pingo de Mel” and “Branca Tradicional” varieties) leaves and fruits were collected from the Mirandela region (Northeast Portugal), in September 2008. Peel and pulp were separated. All materials were frozen (−20 °C) and an aliquot was subsequently lyophilised. Frozen and lyophilised samples were analysed separately. Voucher specimens were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Porto University.

2.3. Headspace solid-phase microextraction (HS-SPME) procedure

2.3.1. SPME fibres

HS-SPME experiments were performed using a manual fibre holder and a 65 µm PDMS-DVB fibre, obtained from Supelco (Bellefonte, PA, USA). Fibre was conditionated by inserting it into the GC injector; temperature and time were applied according to the procedure recommendation of Supelco: 250 °C for 30 min.

2.3.2. Volatiles extraction

One gram of sample was mixed with 20 µl of IS solution and 4980 µl of distiled water. The mixtures were stirred at 200 rpm, at 40 °C for 10 min.

The fibre was then exposed to the headspace for 20 min, with agitation (200 rpm), at 40 °C. After it was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption. After 1 min the fibre was removed and conditioned in another GC injection port (10 min at 250 °C).

2.4. Gas chromatography with flame ionisation detection (GC/FID) analysis

The GC used was a Focus GC ThermoFinnigan Model, with a FID. The injection port of the chromatographer was equiped with a glass liner (5-mm i.d.) appropriate for SPME analysis.

The analysis were performed with a CPWax 57 CB (WCOT Fused Silica), 25 m × 0.25 mm i.d., DF = 0.2 µm column, from Varian (Palo Alto, CA). The temperature of the FID and of the injector was 220 °C. The oven temperature was programmed at 38 °C (for 3 min), followed by an increase of 5 °C/min until 195 °C. The carrier gas was helium, at a flow of 1.5 ml/min. The analysis was performed in split mode with a ratio of 1:7.

Compounds were identified by comparing their retention times with those of authentic compounds analysed under the same conditions, and by comparison of the retention indices (as Kovats indices) with literature data.

3. Results and discussion

3.1. HS-SPME volatiles extraction

HS-SPME methodologies require an accurate time, controlled sample heating, and adequate sample weight to obtain a good partitioning of the volatile compounds between liquid and headspace vapour. Thus, a preliminary assay with an aqueous solution of frozen sample was performed, using different conditions.

3.1.1. Effect of extraction temperature

A PDMS-DVB fibre was used to establish the experimental conditions for HS-SPME analysis of *F. carica* volatile compounds. In this experiment the temperature ranged from 30 to 60 °C, for 30 min. Although, in a general way, areas obtained were higher for 30 °C, in this work the volatile compounds were extracted at 40 °C, once this temperature is slightly superior to that of the human palate (∼35 °C), where mastication occurs (Beaulieu & Grimm, 2001), constituting a better approach to the situation of ingestion of these matrices. On the other hand, it is easier to mantain constant and homogeneous higher temperatures such as 40 °C than lower ones like 30 °C.

3.1.2. Effect of sample weight

Experiments were performed using distinct sample weights, from 0.5 to 1.5 g. The sample volume was 5 ml. In Fig. 1, we can observe that a higher sample amount did not always result in increased extraction of compounds. In fact, during the laboratory experiment, we had some difficulties in the homogenisation of some samples, namely pulps and peels, when we used a mass of 1.5 g and for this reason we have chosen to work with 1.0 g of each material to homogenise the sample.

3.1.3. Effect of fibre exposure duration

Equilibrium times between 5 min and 1 h before SPME analysis have usually been reported (Pérez, Sánchez-Brunete, Calvo, & Tadeo, 2002). In this work, the effect of exposure time on extraction efficiency was evaluated by sampling in headspace after 10, 20, 30, 40 and 50 min. As the extraction time may be influenced by both temperature and stirring speed (Dong et al., 2006), equilibrium