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Effect of sample freezing on the SPME performance in the analysis of chiral volatile compounds in foods

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Abstract

The sensitivity of SPME/GC/MS for the analysis of chiral volatile compounds in food matrices was intended to be improved. To that end, a new approach based on the freezing/defrosting of the sample prior to the extraction is described. The defrosting time was carefully optimised, obtaining different optimal values according to the matrix studied. Relative standard deviations from three replicates of the overall procedure were lower than 12% in all cases. By applying the method proposed, peak areas for the compounds of interest up to nine times higher were obtained in some cases. This improvement in the sensitivity allowed to increase the reliability in the identification of volatile components as well as to detect certain chiral minor compounds in foods. The results shown in the present work suggest the usefulness of sample freezing in food quality studies by increasing the sensitivity achievable in SPME analysis.

Keywords: SPME; Freezing; Volatile; Chirality; Food quality

1. Introduction

In food and beverage industry, aroma has become one of the most valuable attributes, not only to ensure the consumer acceptance but also to evaluate the food quality. In this respect, the volatile components of some products have been used to establish the geographical origin (Mauriello, Moio, Genovese, & Ercolini, 2003) as well as to detect fraudulent additions (Carstensen & Schwack, 2002). Equally, since years ago, some authors have associated the compounds occurring in the aroma of some beverages with storage effects (Tatum, Nagy, & Berry, 1975) and certain manufacturing processes (Moshonas & Shaw, 1989). However, when the volatile fraction is to be studied, a sample preparation step prior to the analytical determination is usually required to isolate the compounds of interest from the major constituents in the matrix. This implies, in most cases, the application of laborious and time-consuming procedures which can eventually bring about the partial loss of the analytes.

Besides, when working with food matrices, many of the aroma compounds are chiral compounds, which must be separated into their corresponding enantiomers. The consideration of stereochemical aspects in food analysis is of great interest because of both the different biological activity shown by the enantiomers making up a specific chiral compound and the usefulness of the enantiomeric purity in quality studies (Armstrong, Chang, & Li, 1990; Marchelli, Dossena, & Palla, 1996; Stalcup, Ekborg, Gasper, & Armstrong, 1993). Nevertheless, the consideration of chirality makes even more complicated the development of an analytical method suitable for determining volatile compounds. This difficulty results from the fact that, for chiral components, each compound can appear as two peaks, which may lead, on the one hand, to overlapping with other matrix components and, on the other, to a decrease in the sensitivity.

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In this regard, solid phase microextraction (SPME) fulfils the requirements to analyse highly volatile chiral compounds in foods. It is a simple and rapid technique which minimizes the sample handling and, consequently, the loss of volatile compounds. Also, it allows to attain the selectivity demanded to isolate effectively some compounds from complex matrices by using the appropriate coating. However, as far as sensitivity is concerned, the small dimension of the fibre and, as a consequence, the small amount of material on it (typically less than 0.5 μl) results in low extraction efficiencies (Baltussen, Sandra, David, & Cramers, 1999). This may be an important limitation when analytes present at very low concentrations, such as some minor enantiomers, are intended to be detected.

We have earlier applied the SPME methodology to the analysis of chiral volatile compounds in edible oils (Ruiz del Castillo, Flores, Herraiz, & Blanch, 2003), fruit beverages (Ruiz del Castillo, Caja, Blanch, & Herraiz, 2003; Ruiz del Castillo, Caja, & Herraiz, 2003; Ruiz del Castillo, Flores, Blanch, & Herraiz, 2004) and aromatic plants used in the manufacture of food flavours (Ruiz del Castillo, Blanch, & Herraiz, 2004). As a result of these works, we have evaluated the authenticity of these matrices on the basis of the enantiomeric ratio of certain chiral components. However, in those cases in which the compounds of interest occurred at particularly low concentrations, the sensitivity achievable in the analysis was not enough for the reliable determination of the target compounds (e.g., minor enantiomers).

The objective of this work was to improve the sensitivity of SPME for volatile compounds by increasing the amount of analytes extracted at equilibrium. To this aim the effect of the sample freezing/thawing prior to the extraction was studied in oils, juices and fruits.

2. Materials and methods

2.1. Materials

(E)-5-methylhept-2-en-4-one (filbertone) standard was supplied by Haarman and Reimer (Holzminden, Germany) whereas terpene were purchased from Sigma–Aldrich (Dorset, UK). All of them were obtained as racemic mixtures, except α-pinene, limonene and terpinen-4-ol, which were provided as the pure (+)-enantiomer, with enantiomeric purities of 94%, 96% and 98%, respectively. The elution order for racemic mixtures was obtained from previous studies (Blanch, Caja, Ruiz del Castillo, & Herraiz, 1999; Ruiz del Castillo & Dobson, 2002; Ruiz del Castillo et al., 2003). The samples included in this work (considering 4 virgin and 3 refined hazelnut oils, 2 fruit juices and 3 soft fruits) were obtained from different suppliers and countries.

2.2. Solid-phase microextraction

A SPME holder (Supelco, Bellefonte, PA, USA) was utilized to carry out the experimentation. A fused silica fibre coated with a 65 μm layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB) was used to trap filbertone from hazelnut oil and volatile terpenes from berries. A 100 μm layer of polydimethylsiloxane (PDMS) was the coating chosen to retain terpenes from juices. Prior to use, both fibres were properly conditioned in the injector of the gas chromatograph by applying the temperature and time recommended by the supplier.

Before performing the extraction, a 1.0 ml volume of either oil or juice (or 1.0 g of sample in the case of the fruit) was placed into a 5.0 ml vial and frozen in a refrigerator at −20 °C for 12 h before thawing. To avoid the loss of volatile components, the vial was previously sealed with a plastic film. Subsequently, the sample was allowed to defrost at room temperature for 5, 1 and 15 min, depending on whether oil, juice or fruit was the matrix studied. This way, all samples were analysed in a semi-thawed state. In order to accomplish the extraction, the SPME-fibre was immediately exposed to the headspace of the sample for 5 min at 70 °C for the oils, 2 min at 60 °C for the juices and 20 min at 55 °C for the soft fruits. To allow the semi-thawed state of the sample to be maintained during the extraction, no equilibrium period was established. Constant sample stirring was essential at all times to facilitate the release of the compounds of interest for oil and juice samples. The experimental variables (i.e., extraction time and temperature, sample volume and SPME coating type) used in the extraction were chosen as result of the optimisation of the method carried out in previous work (Ruiz del Castillo & Dobson, 2002; Ruiz del Castillo, Caja, & Herraiz, 2003; Ruiz del Castillo, Flores et al., 2003). The selection of experimental values was based on those that provided the highest peak areas for the investigated compounds. Finally, the retained components were thermally desorbed into the GC injector at 250 °C for 5 min in all cases and analyzed by GC as described below.

2.3. GC-MS analysis of extracts obtained by SPME

A Hewlett-Packard model 6890 gas chromatograph fitted with a FID operated at 250 °C and coupled to an Agilent 5989A quadrupole MS instrument (Palo Alto, CA) was used to carry out the analyses. The source and the quadrupole temperatures were 230 and 100 °C, respectively. The identification of the target compounds in samples was carried out by comparison of retention times with those of the standard run under identical experimental conditions as well as of the mass spectra obtained with those contained in the Wiley library and with those provided by the standard. The GC separation was

performed on a 25 m × 0.25 mm i.d. fused silica column coated with a 0.25 μm layer of permethylated β-cyclodextrin (Chirasil-β-Dex, Chrompack). Helium was used as the carrier gas at an initial flow rate of 1 ml/min and splitless mode was employed in all cases. The injector was kept at 250 °C throughout the experimentation and the GC column was first programmed at 3 °C/min from 45 °C (5 min) to 90 °C and, then, at 5 °C/min to 150 °C for the oil samples, at 2 °C/min from 40 °C (10 min) to 170 °C for the juices and at 5 °C/min from 40 °C (3 min) to 70 °C, subsequently, at 2 °C/min to 95 °C and, finally, at 7 °C/min to 200 °C for the soft fruits. Data acquisition from the MS was performed using the HP G1701BA ChemStation (revision B.01.00) that enables the control of both the GC and the MS systems.

3. Results and discussion

The study described in the present work was initially carried out on hazelnut oil as previous research performed in the laboratory on this matrix had proved the need for a sensitivity improvement of SPME when low levels of the target compounds were to be detected (Flores, Ruiz del Castillo, Blanch, & Herraiz, submitted for publication). On the other hand, we have proposed the presence of filbertone, the flavour impact component of hazelnuts, as a chiral marker to identify the adulteration of olive oil with hazelnut oil (Blanch, Caja, Ruiz del Castillo, & Herraiz, 1998). However, the harsh conditions usually applied over the refining process may lead to partial losses and, generally speaking, to the decrease in the concentration of volatile compounds. Consequently, the detection of filbertone in refined hazelnut oil is specially difficult. For this reason, we considered in this study both virgin and refined hazelnut oils.

The defrosting time prior to the extraction was optimised in the present work by testing 1, 3, 5 and 15 min. As a result of this experiment, it was observed that whereas 1, 3 and 15 min lead to an increase in the peak areas of 5.5, 7.2, and 4.4, respectively, the analysis of the sample after freezing/defrosting for 5 min resulted in filbertone areas 9 times higher than those obtained from the analysis of the sample without freezing. Thus, as mentioned in Section 2, a 5 min time was selected as the optimal defrosting time as it appeared to be the most advantageous to reach the semi-thawed state at which the release and subsequent retention of filbertone on the fibre is favoured. The repeatability of the procedure described, including the freezing/defrosting of the

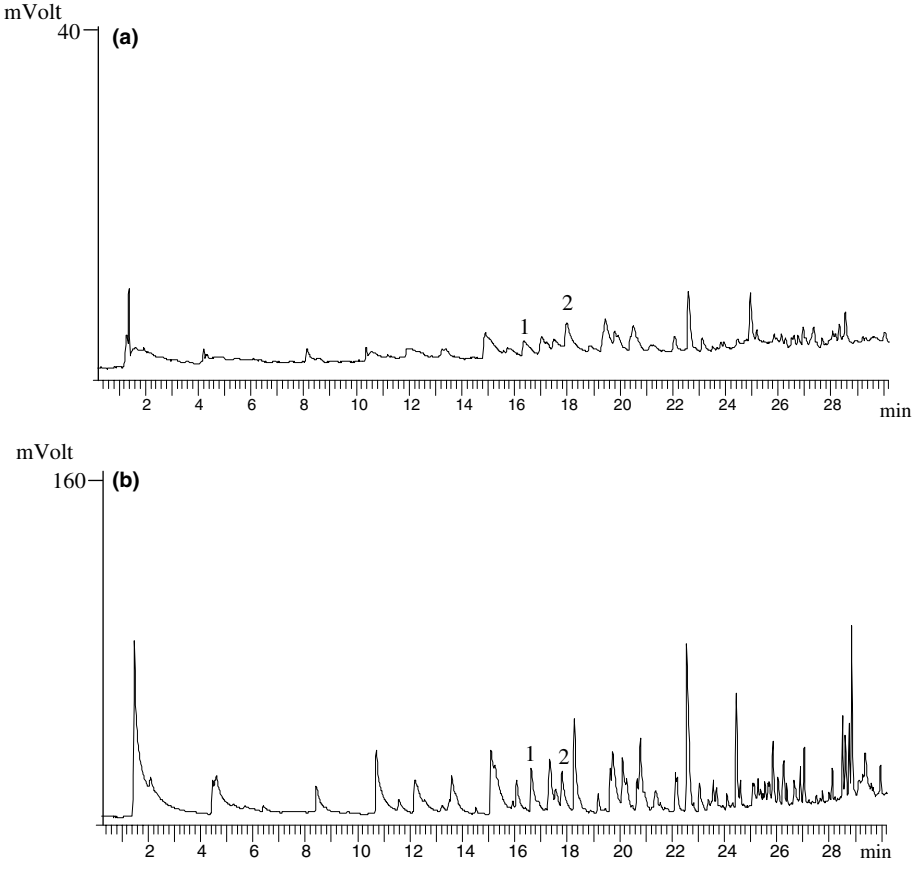

Fig. 1. SPME-GC-MS analysis of a virgin hazelnut oil (a) at room temperature and (b) after freezing/defrosting the sample for 5 min. Peak identification: (1) *R*-filbertone and (2) *S*-filbertone. Chromatogram b was recorded at a full range four times that for chromatogram a.

sample followed by the extraction by SPME and analysis of the extract by GC, was estimated by measuring the relative standard deviation (RSD) from a minimum of three replicates for all oil samples analysed. To that end, the peak areas obtained from the signal recorded by the FID were used. The values ranged from 1% to 12% in all instances. It is interesting to emphasize that despite equilibrium period was not applied, the semi-thawed state at which the sample was submitted to SPME could be adequately reproduced.

Fig. 1 shows the chromatogram resulting from analysing a virgin hazelnut oil by SPME/GC/MS at room temperature (a) and after freezing/defrosting the sample for 5 min (b). Taking into account the different full range at which both chromatograms were recorded, it is obvious that the previous freezing of the sample resulted in a clear increase in the sensitivity of the method for the extraction of volatile compounds from virgin hazelnut oil. This improvement in the sensitivity allowed us, in this specific case, to determine more reliably the enantiomeric composition of filbertone in virgin hazelnut oil.

The usefulness of the approach proposed in the present study was even more apparent in the case of refined hazelnut oils as the previous freezing of the sample enabled the eventual detection of filbertone to be accomplished in those oils in which its occurrence had not been established when analysing the sample without freezing. As an example, Fig. 2 illustrates the chromatogram obtained from the analysis of a refined hazelnut oil by SPME/GC/MS at room temperature (a) and after freezing/defrosting the sample for 5 min (b). Both chromatograms were recorded at the same full range. As can be observed, the analysis of the sample in a semi-thawed state allowed to detect the *S*-enantiomer of filbertone, although the *R*-enantiomer could not be surely identified due to its overlapping with other component of the oil.

The remarkable increase of sensitivity in the SPME extraction of the volatile compounds from hazelnut oil by means of the previous freezing of the sample might be owing to the fact that the loss of volatiles is minimised as a consequence of the crystalline structure that the oil exhibits when frozen.

The influence of freezing/thawing of the sample on the structure of membranes and tissues has been widely studied in various matrices (Ade-Omowaye, Taiwo, Eshtiaghi, Angersbach, & Knorr, 2003; Angersbach, Heinz, & Knorr, 2002; Torreggiani, Forni, Maestrelli, & Quadri, 1999). Most of these works are focused on the comparative study of the effect of freezing with that of other procedures, such as the application of an electric field or osmotic dehydration. Equally, quantitative changes in the composition of aromatic compounds in some foods during freezing and thawing have been observed by other authors (Larsen & Poll, 1995). However, none of these studies have been, to our knowledge, aimed to enhance the sensitivity of SPME.

To verify the results obtained from the freezing/thawing of the oil prior to the SPME extraction, a similar study based on the isolation of volatile terpenes, most of them of chiral nature, from diverse matrices other than oil (i.e., fruit juices and soft fruits) was additionally accomplished.

Regarding fruit juice, as a result of the optimisation of the defrosting time, an increase in peak areas of 2.5, 1.9, 1.6 and 1.2 times those found for the sample without freezing were obtained for defrosting times of 1, 3, 5 and 15 min, respectively. As a consequence, 1 min was selected as the optimal defrosting time. Fig. 3 represents the chromatogram obtained from the SPME-GC-MS analysis of a fruit juice when the sample was analysed (a) at room temperature and (b) after freezing/thawing the sample for 1 min. Both

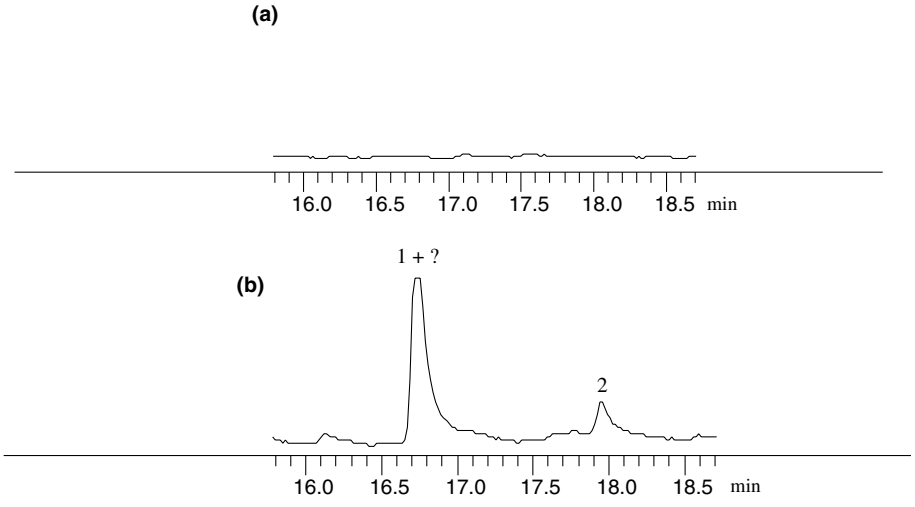

Fig. 2. SPME-GC-MS analysis of a refined hazelnut oil (a) at room temperature and (b) after freezing/defrosting the sample for 5 min. Peak identification: (1) *R*-filbertone and (2) *S*-filbertone. Both chromatograms were recorded at the same full range.

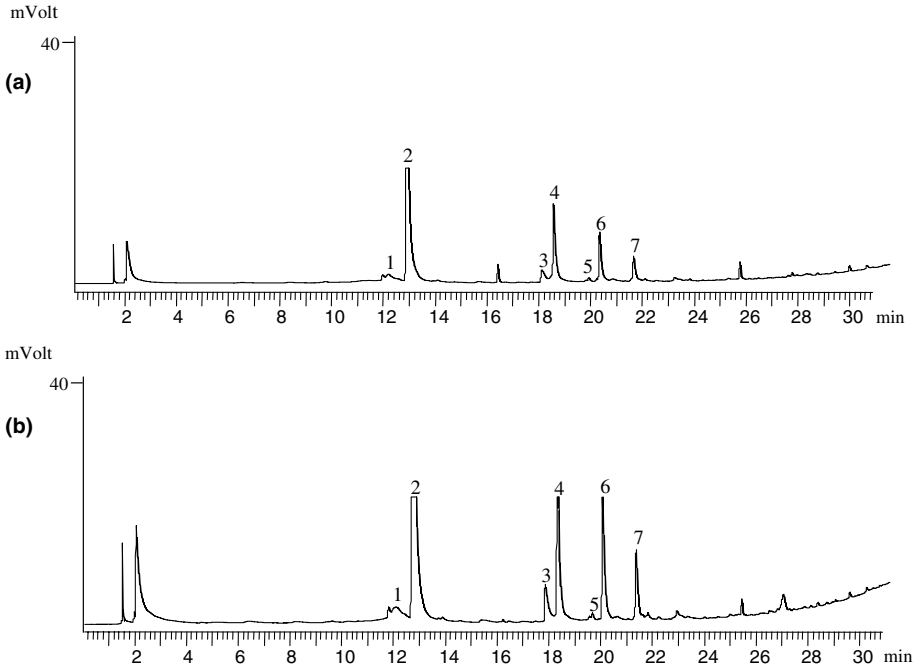

Fig. 3. SPME-GC-MS analysis of a commercial fruit juice (a) at room temperature and (b) after freezing/defrosting the sample for 1 min. Peak identification: 1. (−)-α-pinene, 2. (+)-α-pinene, 3. (−)-phellandrene, 4. (+)-phellandrene, 5. (−)-limonene, 6. (+)-limonene, 7. (+)-linalool. Both chromatograms were recorded at the same full range.

chromatograms were recorded at the same full range. As can be seen, both enantiomers of α-pinene, α-phellandrene and limonene together with the pure (+)-enantiomer of linalool could be detected in both cases, although it is clear from Fig. 3 that the terpene areas were greater when the sample was previously frozen/defrosted for 1 min. This sensitivity improvement allowed us, not only to confirm enantiomeric composition data found in previous studies (Ruiz del Castillo, Caja, Blanch et al., 2003; Ruiz del Castillo, Caja, & Herraiz, 2003; Ruiz del Castillo, Flores et al., 2004), but also to identify and quantify unambiguously the minor (−)-α-pinene in a real-life sample for the first time in our laboratory.

As far as the study on soft fruits is concerned, 15 min was chosen as the best defrosting time. On this occasion, peak areas twice those found when the sample was extracted without freezing were obtained. It is important to mention that defrosting times of 1, 3 and 5 min resulted in peak areas negligible since they did not allow the fruits to reach the semi-thawed state at which the other matrices were analysed and, therefore, the volatile terpenes were not given off. By applying the optimal defrosting time a number of terpenes making up the volatile fraction of the fruits were identified by mass spectrometry. Among them, it can be highlighted the occurrence of (±)-α-pinene, (+)-sabinene, (±)-β-pinene, (±)-terpinen-4-ol, (+)-α-terpineol and (−)-β-*trans*-caryophyllene as chiral components as well as *o*-cymene and γ-terpinene as non-chiral terpenes.

Likewise, RSDs values from three replicates lower than 10% were achieved for both juice and fruit samples. It is worth pointing out that the fact that the defrosting time varies with the matrix indicates that the semi-thawed state, which facilitates the release and subsequent retention of the volatile compounds on the fibre, is reached at distinct points according to the sample nature. This makes necessary the optimisation of the sample thawing period with each specific matrix to be considered. It is also interesting to note that the sensitivity of the method did not improve to the same extent with the studied matrices. In this respect, it is clear that further investigation concerning the effect of crystallization on the prevention of the volatile loss according to the matrix nature is still required.

All in all, the low efficiency of the SPME extraction for some minor volatile compounds from complex matrices may be in part solved by analysing the sample in a semi-thawed state. For that purpose, the freezing–thawing of the sample prior to the extraction might be, on the basis of the results shown in this work, a promising possibility to be considered to assay food quality because of its simplicity and accessibility.

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