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### SPME analytical method for the determination of Hexanal in hazelnuts as indicator of the interaction of active packaging materials with food aroma compounds

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<td>Pastorelli, Sarah; Joint Research Centre, IHCP, PCE Valzacchi, Sandro; Joint Research Centre, IHCP, PCE Rodriguez, Ana; Joint Research Centre, IHCP, PCE Simoneau, Catherine; Joint Research Centre, IHCP, PCE</td>
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Solid-phase Microextraction (SPME) analytical method for the determination of hexanal in hazelnuts as an indicator of the interaction of active packaging materials with food aroma compounds

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Abstract

Fatty foods are susceptible to lipid oxidation, which results in a deterioration of the quality of the product, since this process is responsible of generation of off-flavours. Hexanal has been reported to be a good indicator of the rancidity. A method based on solid-phase microextraction (SPME) coupled with gas chromatograph with flame ionization detection to determine hexanal formed in hazelnuts during storage was developed. The optimum conditions were the follow: carboxen-polydimethylsiloxane 75µm fibre, extraction time 10 min, equilibrium time 10 min and equilibrium temperature 60°C. A study to evaluate the effect of the oxygen scavengers on the oxidation process was also conducted by measuring the hexanal formed in hazelnuts stored with and without the oxygen absorbers sachets. Oxygen scavengers shown to reduce the oxidation, however the analysis of the sachet revealed that also other volatile compounds from the headspace were absorbed.

Keywords: SPME, hexanal, lipid oxidation, oxygen scavengers
Introduction

Food products with high fat content, such as hazelnuts and nuts in general, are susceptible to rancidity because of lipid oxidation, which involves the development of off-flavours that make the product undesirable for the consumers. As it is well known, the oxidation process is accelerated by different factors such as light and temperature. In order to delay the oxidation and to extend the product shelf-life, several approaches have been developed by the food industry. Special packaging techniques, such as modified atmosphere, oxygen-barrier packaging material and active packaging (oxygen scavengers) (Lee and Krochta 2002) as well as the addition of natural or synthetic antioxidants have been recently investigated (Stashenko et al. 2002).

Hexanal was found to be a good marker of the oxidative rancidity since it is one of the most abundant products of the oxidation of fats and its content increases during storage (Stashenko et al. 2002; Lee and Krochta 2002; Goodridge et al. 2003; Brunton et al. 2000; Shahidi 2001; Jensen et al. 2005). It is the main aldehyde formed during the oxidation of linoleic, gamma-linolenic and arachidonic acids (Shahidi 2001).

Different techniques for volatile isolation and pre-concentration, such as dynamic headspace, purge and trap (Cadwallader and Xu 1994), and static headspace (Romeu-Nadal et al. 2004) have been described in the literature. Although they are simple and fast, these methods present certain drawbacks. The first two of these, are not easy to automate and are time-consuming and on static headspace techniques can lack of sensitivity (Goodridge et al. 2003; Snow 2002). To overcome these disadvantages
during the last years solid phase micro extraction (SPME) has been proven to be an alternative, promising and innovative technique that combines the simultaneous extraction and pre-concentration steps. It is simple, fast, uses little solvent, does not require sample preparation and is inexpensive.

SPME has been successfully applied in food analysis to evaluate the volatile profiles in different foodstuffs: fruits (Augusto et al. 2000; De Lourdes-Cardeal et al. 2005), meat (Ramirez et al. 2004), vinegar (Natera-Marin et al. 2002), olive oil (Garcia-Gonzalez et al. 2004), soybean (Boué et al. 2003), truffle (Diaz et al. 2002) and also to analyse hexanal in several matrices: turkey (Brunton et al. 2000), powder infant milk formula (Fenaille et al. 2003) and freeze-dried chicken (Goodridge et al. 2003).

The purposes of the present paper were to develop a simple and fast SPME-GC-FID method to determine hexanal and to evaluate the effect of the oxygen scavengers on the oxidation of hazelnuts samples stored under controlled conditions.

**Materials and methods**

**Sampling**

Powdered hazelnuts packed with a transparent film were purchased from a local supermarket. A gas chromatography-flame ionization detector (GC-FID) analysis was performed immediately after being acquired. Then they were stored under controlled conditions of light and temperature in 20 mL hermetically closed flasks with and without oxygen absorbers sachets added to the packaging until analysis.
Oxygen-absorbing sachets based on iron powder and with capacities of 20 mL were used in the experiments.

Reagents and Analytical standards

Hexanal was purchased from Sigma-Aldrich (Steinheim, Germany) with purity of 98%. Two stock standard solutions of 500 mg/L of hexanal were prepared by using Milli-Q water and triacetin as solvents. Working solutions employed were prepared by diluting different amounts of the stock standard solution in Milli-Q water and triacetin respectively. Both solutions were stored at 4°C. Ultra pure water was obtained using a Milli-Q filter system (Millipore, Bedford, MA, USA) and triacetin was supplied from Sigma-Aldrich (Steinheim, Germany).

SPME procedure

The SPME holder for manual sampling and the commercial fibres tested in this study were purchased from SUPELCO (Bellefonte, PA, USA). Five fibres with different coating materials were examined: PDMS with 100 µm thickness; CAR/PDMS with 75 µm thickness, PDMS/DVB with 65 µm thickness, CAR/DVB with 65 µm thickness and DVB/PDMS/CAR with 50-30 µm thickness. Prior to their use they were conditioned by inserting them into the GC injector according the supplier’s instructions summarized in Table I.

One mL of the standard solution or 0.1 g in the case of the hazelnuts samples were placed in a 20 mL vial that was hermetically sealed with a PTFE-coated silicone septum and analysed by SPME-GC. A small magnetic stirring bar was added to the vial before it was capped. Then the vials were placed in an aluminium thermostated block at 60°C
for 10 min to reach the equilibrium with the headspace. After equilibrium time the SPME fibre was exposed to the headspace of the sample at 60°C for 10 min (extraction time). Then the fibres were directly inserted into the injector port of the GC for thermal desorption at the temperature reported in table I, during 15 min.

To ensure that all impurities were removed blank runs were conducted between sample analysis. Each sample extraction was carried out in triplicate.

Gas chromatography

Analyses were performed on a 5890 Hewlett-Packard gas chromatograph equipped with a flame ionization detector (FID). The GC system was operated using splitless injection mode. A 0.75 mm i.d. narrow-bore glass liner (Supelco, Bellefonte, USA) was used to prevent peak broadening (Vas and Vékey 2004) and consequently to improve the resolution of the analysis. A DB-wax fused capillary column (30 m 0.25 mm ID, 0.5 µm) was used for hexanal analysis. Operating conditions were as follows: injector temperature was set between 240-300°C depending on the fibre used and detector temperature was kept at 350°C. The ramp temperature was initially set at 50°C, then raised at the rate of 10°C/min to100°C, then increased at 20°C/min until 200°C and held for 7min. The carrier gas was helium with a flow rate of 1.2 mL/min.

A Hewlett Packard 6890 gas chromatograph equipped with a Hewlett Packard 5973 mass detector working in electronic impact ionization mode in full scan acquisition mode was used for hexanal identification. The chromatographic conditions were the same as those specified for the GC-FID system. Hexanal was identified by the library
search software of the instrument and confirmed by the correspondence of the retention
time obtained with the analysis of pure standard.

The software HP-Chem Station from Hewlett-Packard was used for data processing.

“[Insert Table I about here]”

Results and Discussion

SPME-GC method Optimisation

In developing gas chromatographic method several temperature ramps were studied, the
selected ramp was that provided the best resolution in a shorter time. Triacetin and
Milli-Q water were evaluated as solvents for standard solutions. Hexanal showed
considerable instability in water, however with triacetin good results in terms of
reproducibility were obtained, and for this reason triacetin was chosen as solvent.
Furthermore, as triacetin has a higher boiling point than hexanal, a good
chromatographic separation without interferences was achieved.

The SPME sampling procedure depends on several variables such as equilibrium and
extraction time and temperature, the amount of the sample, and the kind of the fibre
coating. In order to optimise all these parameters, several experiments were conducted.
Initially, a hexanal standard solution was used to fix time and temperature conditions,
then a series of assays with hazelnuts samples were conducted taking into account the
effect of the matrix. A PDMS 100 µm fibre was used to carry out the preliminary trials
since it is the most often used due to its physicochemical properties, it is also very
rugged and recommended for the extraction of volatile flavour compounds (Vas and
Vékey 2004; Kataoka et al. 2000). In a first series of assays the parameter studied was the equilibrium temperature. Different values were tested (50, 60, 70 and 80°C) keeping equilibrium time (10 min) and extraction time (10 min) fixed. Under these conditions the best results were found at 60 and 70°C, so that we selected the lower temperature. Once the temperature was optimised the equilibrium time was studied ranging from 5 to 60 min; it was found that 10 min was enough to reach the equilibrium, therefore an equilibration time of 10 min was selected for successive analyses. Three different extraction times, were also evaluated: 5, 10 and 20 min. Best results were achieved at 10 min. These results are in accordance with those reported by Ezquerro et al. 2003, where it is indicated that the extraction time depends on the compound, in general, for small compounds such as the hexanal after 10-20 min of exposure of the fibre to the headspace the signal (peak area) decreased.

In order to establish the optimal sample amount 0.1 g, 1 g, 10 g and 15 g of ground hazelnuts were assayed, and 0.1 g was considered a suitable sample amount to produce quantitative results. Finally, the effect of the dilution of the sample with Milli-Q water (1mL) and the addition of a saturated salt solution of NaCl were also evaluated. As it has been reported in the literature (Pino et al. 2002) the addition of the salt to the sample could modify the nature of the matrix and the extraction efficiency since this addition can affect the partition coefficient of the analytes, however in terms of improvement of analyte extraction from the sample no significant differences were observed so subsequent analyses were carried out with dry sample.
A small magnet bar was also added into the vial in order to decrease the time necessary for the equilibrium and to facilitate the transfer of the analyte from the matrix to the fibre coating material (Vas and Vékey 2004; Pillonel et al. 2002). Five commercial available fibres (PDMS, CAR/PDMS, PDMS/DVB, CAR/DVB and DVD/CAR/PDMS) were compared. The CAR/PDMS fibre had the highest sensitivity. This result is in agreement with those reported by Augusto et al. 2000 and Brunton et al. 2000. Natera-Marín et al. 2002 pointed out that the high efficiency of this coating could be attributed to the high porosity that provides a strong adsorption. Furthermore, Ezquerro et al., (2002) reported that for low molecular mass compounds the CAR/PDMS provides good results and Diaz-Maroto et al., (2004) obtained high recoveries when using a fibre containing a porous coating. According to the method proposed by Augusto et al. 2000, the inter fibres comparison (figure 1) was made calculating the normalized extraction efficiency (N\textsubscript{i,x}) defined as \( N = 100 \times \frac{A_{ix}}{A_{i \text{CAR/PDMS}}} \) where \( A_{ix} \) is the hexanal area analyzed with the fibre x and \( A_{i \text{CAR/PDMS}} \) is the corresponding area with the most efficient fibre (CAR/PDMS).

The experiments were performed in triplicate.

“[Insert Figure 1 about here]”

**Performance characteristics**

A calibration curve was carried out using a series of six hexanal standards of known concentration. Parameters of linearity were the follow: -388.05 (intercept); 974216 (slope); 0.999\((r^2)\); and 0.25-2.5 ng/mL (range). Another calibration line was constructed by spiking hazelnuts samples. 10 µl of a triacetin solution with different hexanal quantities were added to the samples. The equation was \( Y = 50632x - 120.94 \), the
determination coefficient and the linearity were 0.997 and 0.25-2.5 ng/mL respectively. Detection limit calculate in accordance with the Subcommittee on environmental analytical chemistry (ACS) guidelines (defined as a signal three times the height of the noise level) was 0.08 ng/mL. The value obtained is lower than the LOD obtained by Romeu-Nadal et al. 2004 in infant formulas.

Repeatability was estimated as relative standard deviation (RSD) for the determination of six extracts, from the same oxidized sample with an hexanal content of 0.25 ppb, each prepared separately, was 5.32%.

Evaluation of oxygen scavengers in the oxidation process.

In the first tests to evaluate the effect of the oxygen scavenger on the oxidation process the headspace of the vial containing the hazelnuts with and without the oxygen absorber sachets was analysed. The $O_2$ content was measured using a Chek Mate 9900 Dansensor (Milano, Italy) and it was found that in the flasks stored with oxygen scavenger practically all oxygen was removed while in the flasks without oxygen scavenger the content of oxygen was 18%.

A lower content in hexanal in the samples containing the absorber sachet were observed. The samples containing oxygen scavenger presented, after three days, a 12.25% less of hexanal than the samples stored without the oxygen absorber. The test results are summarized in Table II.

“[Insert Table II about here]”
In a second series of trials the content of the sachets were also analysed by SPME/GC-FID. The content of the sachets were place into 20 mL headspace vials and they were hermetically capped with a silicone septum. The SPME and chromatographic conditions were the same as those specified for the hazelnuts samples analysis. Different components were found and among these hexanal was also detected. For that reason other assays were conducted to investigate the possible absorption of volatile compounds by the oxygen scavenger.

Two vials (120mL) with 10mL of a hexanal solution in water (500 mg kg\(^{-1}\)) were prepared. In one of the vials, oxygen scavenger was suspended on the top of the container (not to be in contact with the liquid) and both vials were hermetically closed and stored at room temperature. After one week an SPME/GC-FID analysis of the headspace of the two vials were made.

As shown the figure 2, the hexanal content in the headspace of vials with the oxygen scavenger was considerably reduced comparing with the one without oxygen scavenger. The oxygen scavenger sachet was opened and the powder contained in the sachet was analysed. The quantity of hexanal found in the powder cannot explain the differences found in the 120mL headspace vials and this could be due to the fact that probably it’s difficult to extract again hexanal from the powder after the adsorption.

Briefly, although the oxygen scavengers shown to reduce the oxidation process, other volatile components among these hexanal were also absorbed. Since the hexanal is an indicator of the rancidity, this fact could mask the real state of the food product and
could mislead consumers. Further research is required in order to evaluate the interactions between food and oxygen scavenger.

“[Insert Figure 2 about here]”

References


volatile compounds of oak wood used for aging wines by headspace SPME-GC-MS (SIM). Journal of Agriculture and Food Chemistry 52:6857-6861.


Table I. Conditioning recommendations for different fibres

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<th>Fibre</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
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<tr>
<td>PDMS 100 μm</td>
<td>250°C</td>
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</tr>
<tr>
<td>CAR/PDMS 75 μm</td>
<td>300°C</td>
<td>1-2</td>
</tr>
<tr>
<td>PDMS/DVB 65 μm</td>
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<td>0.5</td>
</tr>
<tr>
<td>CAR/DVB 65 μm</td>
<td>220°C</td>
<td>0.5</td>
</tr>
<tr>
<td>DVB/PDMS/CAR 50-30 μm</td>
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Table II Evaluating oxygen scavengers: test results.

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<th>Samples stored without oxygen scavenger</th>
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<tr>
<td>(O_2(%))</td>
<td>0.001</td>
<td>18.0</td>
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<tr>
<td>Hexanal content in HS vial</td>
<td>0.55</td>
<td>0.63</td>
</tr>
<tr>
<td>(ng/mL)*</td>
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*Hexanal content analysed after 3 days of storage
Figure legends

Figure 1. - Normalized extraction efficiencies N_x (x= SPME fibre) measured for extraction with different fibres

Figure 2. - Chromatogram of hexanal content in headspace vial with and without oxygen scavenger
Figure 1
Figure 2