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Solid phase microextraction of volatile oxidation compounds in oil-in-water emulsions

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Abstract

Headspace solid phase microextraction (HS-SPME) has been used to isolate the headspace volatiles formed during oxidation of oil-in-water emulsions. Qualitative and quantitative analyses with an internal standard were performed by GC-FID. Four sample temperatures for adsorption (30, 40, 50 and 60 °C) and adsorption times in the range 10–25 min were tested to determine the conditions for the volatile concentration to reach equilibrium. The optimum conditions were at 50 °C for 20 min. The method was applied to monitor changes in volatile composition during oxidation of an o/w emulsion. SPME was a simple, reproducible and sensitive method for the analysis of volatile oxidation products in oil-in-water emulsions.

Keywords: Solid phase microextraction; Headspace; Oxidation; Volatiles; Emulsion; Oil-in-water

1. Introduction

Lipid oxidation is a major cause of quality deterioration in many natural and processed foods. It leads to the development of undesirable off-flavours and potentially toxic reactions (Halliwell, Murcia, Chirico, & Aruoma, 1995). The rate of formation and the products formed during lipid oxidation are affected by a variety of factors. In oil-in-water emulsions, solubility of reactants, catalysts, antioxidants and products in the two phases, and the migration of radicals across the interface, are among the factors that determine the rate of lipid oxidation and the product composition.

As a result of lipid oxidation, volatile compounds are formed and, because of the low flavour threshold of most of these compounds, they affect the sensory characteristics of oils and fat-containing products. The partitioning of lipid oxidation products between the oil phase, aqueous phase, and headspace can affect the sensory perception of food emulsions (McClements, 1999; Jo & Ahn, 1999). Hydrophobic flavour components can be perceived at lower concentrations in water than in oil. Many of the lipid oxidation products show higher solubilities in the oil phase. This means that, for a fixed concentration of volatile components, their concentration in the headspace of an emulsion decreases as the oil concentration increases.

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As a consequence, a low fat emulsion may be perceived as more oxidised than a high fat emulsion, even though both emulsions have the same concentration of volatile components.

The main methods used to analyse the volatile fraction of foods in recent years are: static headspace analysis and purge-and-trap dynamic headspace analysis (van Ruth, de Vries, Gearay, & Giannouli, 2002a, 2002b). Solid phase microextraction (SPME) is a rapid, sensitive and solvent-free sampling technique (Arthur & Pawliszyn, 1990) that has been applied to the analysis of volatile components in the environment and in a variety of foods including oils (Steenson, Lee, & Min, 2002; Vichi, Castellote, Pizzale, Conte, Buxaderas, & Lopez-Tamames, 2003; Jiménez, Beltrán, & Aguilera, 2004), orange juice (Steffen & Pawliszyn, 1996; Jia, Zhang, & Min, 1998), roasted coffee (Bicchi, Panero, Pellegrino, & Vanni, 1997) and cheese (Lee, Diono, Kim, & Min, 2003).

However, data in the literature on the application of SPME to analysis of volatiles formed by lipid oxidation in food emulsions are rare (Fabre, Aubry, & Guichard, 2002), except where the oil phase is separated prior to SPME analysis (Gómez-Alonso, Fregapane, Salvador, & Gordon, 2003; Keceli & Gordon, 2001).

The aim of this work is to optimise the SPME sampling method for analysis and characterisation of volatile lipid oxidation products present in oil-in-water emulsions.

2. Materials and methods

2.1. Materials

The lipid phase of the emulsions was composed of commercial sunflower oil purchased in a retail outlet. The oil was oxidised for 6 days at 60 °C in order to form a high content of volatile oxidation products. The aqueous phase comprised deionised water and Tween 20 (polyoxyethylene sorbitan monolaurate) purchased from Sigma–Aldrich (Spain). A manual SPME fibre holder unit and 50 and 30 μm DVB-CAR-PDMS fibres, purchased from Supelco (Bellefonte, PA, USA), were used.

2.2. Emulsion preparation

Oil-in-water emulsions (40 g) were prepared using 4 g of oil. The aqueous phase was water containing Tween 20 (0.1%). The emulsions were prepared by the dropwise addition of oil to the water phase cooled in an ice bath with continuous sonication with a Vibracell sonicator (Sonics and Materials, USA) for 6 min. The emulsions were left overnight to reach equilibrium at room temperature.

2.3. GC-FID analysis

GC analyses were performed with a Hewlett–Packard 5890 series II gas chromatograph equipped with FID detector and split/splitless injector. Chromatographic separation was carried out using a BPX5 fused silica column (25 m length, 0.22 mm i.d., and 0.25 μm film thickness; SGE, Milton Keynes, UK). The oven temperature was 40 °C for 10 min, followed by temperature programming to 200 °C at 3 °C/min. The FID temperature was 280 °C and the injection port was held at 260 °C. Helium was used as carrier gas.

2.4. SPME sampling conditions

A 2 g aliquot of emulsion was weighed in a 20 ml vial and 10 μl of internal standard solution (1.1 mg/l of 1,2-dichlorobenzene in methanol) were added. A magnetic follower was added and the vial was capped with a Teflon TM-faced rubber septum and aluminium cap (Fisher, Loughborough, UK). The vial was placed in a water bath on a magnetic stirrer and the sample was equilibrated for 15 min at the required temperature before SPME sampling. The septum was manually pierced with the SPME needle and the fibre was exposed to the emulsion headspace and transferred to the gas chromatograph where the volatiles were desorbed in the injection port. Each isolation and analysis was repeated three times.

Several parameters were tested to optimise the SPME sampling procedure. These included desorption time in the injection port (1, 3 and 5 min), volatile isolation time and emulsion temperature. The stirring time for isolation of volatiles was 10, 15, 20 or 25 min with the emulsion at 30, 40, 50 or 60 °C.

2.5. Reproducibility of the analysis of volatile compounds by headspace SPME

The reproducibility of the analysis of volatile compounds by headspace SPME (HS-SPME) was determined by analysing the concentration of each of the main volatile compounds and the total volatile content in the oil-in-water emulsion in six replicate determinations.

2.6. Identification of volatile compounds

The identification of compounds was made by comparison of retention time of GC peaks with those of the standard compounds.

2.7. Oxidation of oil-in-water emulsions

Two oxidation experiments were carried out. In each experiment, duplicate samples of oil-in-water emulsion (20 g) were stored for 10 h in 100-ml capped bottles at 60 °C, and aliquots (2 g) were withdrawn, each 2 h, for HS-SPME analysis.

2.8. Statistical analysis

Analysis of variance was applied to analyse the data. Tukey's test was applied to determine significant differences between the means ($p \leqslant 0.05$). The analyses were performed using SPSS statistical software.

3. Results and discussion

The effect of desorption time was evaluated. As the desorption time was reduced, the resolution of the chromatogram increased, since the overlapping of some peaks, observed for longer times was avoided, but the total peak area decreased (Table 1). However, higher desorption time is necessary to make sure that the fibre is clean. Therefore, in order to obtain the maximum peak area with adequate resolution, the desorption time of 5 min was selected for the experiments.

The effect of volatile adsorption time and emulsion temperature on the total peak area of the volatile compounds in the emulsion is shown in Fig. 1. Generally, with an increase in the emulsion temperature, the concentration of the volatiles in the headspace increased for each adsorption time. There was no significant difference between 30 and 40 °C, but at 50 and 60 °C this effect was clear.

The effect of adsorption time on the total volatile peak area is less clear. Between 10 and 15 min there was no significant increase in the total volatile peak area, but at 50 °C there appeared to be a significant increase between 15 and 20 min. However, there was no further significant increase between 20 and 25 min. At 60 °C, the total volatile peak area continued to increase between 15 and 25 min. The reasons for the changes in volatile peak area with time are not completely clear. It may be that there is competition between water vapour and hydrophobic oxidation products for active sites on the fibre, and this may take time to reach equilibrium. Also, the continued increase in volatiles at 60 °C suggests that further lipid oxidation is occurring during the adsorption period, with increasing levels of volatiles being formed.

Table 1
Effect of the desorption time of the fibre on volatile total peak area in oil-in-water emulsions. Data are means ± SD ($n = 3$)

Desorption time (min)	Area units
1	329.7 ± 13.3[a]
3	331.2 ± 11[a]
5	388.6 ± 29[b]

[a,b] Means with different letter show significant differences at $p \leqslant 0.05$.

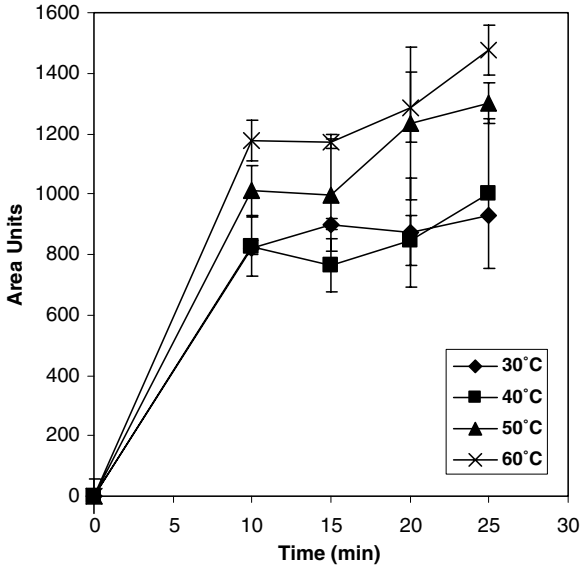

Fig. 1. Effect of temperature and time on the equilibrium of oxidation compounds between SPME coating and the headspace of oil-in-water emulsion.

The concentration of volatile compounds in the headspace of the emulsions increased with increasing temperature. This rise in concentration with temperature is similar to that described for oils (Steenson et al., 2002) and is presumably due to the increase of vapour pressure with temperature. However, in orange juice, a decrease in the concentration of headspace volatiles with an increase in temperature has been described, and this has been explained by greater increase of the water vapour in the headspace than the increase in organic compounds (Steenson et al., 2002; Jia et al., 1998). The emulsions studied in this investigation contained 10% oil, which is much higher that the organic components in the orange juice, and consequently the emulsions behaved similarly to an oil system. The increase in emulsion temperature during adsorption did not produce any new compounds. At 60 °C, the increase in the concentration of volatiles in the headspace of the emulsion may be due to oxidation proceeding during the extraction process. Therefore, the optimal SPME sampling conditions selected were 50 °C for 20 min since, under these conditions, there was little further increase in volatile concentration after this time, but the volatile concentration was significantly higher than that at lower temperatures.

The volatile components of emulsions prepared from oxidised sunflower oil were analysed (Fig. 2) to develop the assay to determine the reproducibility of the SPME analysis.

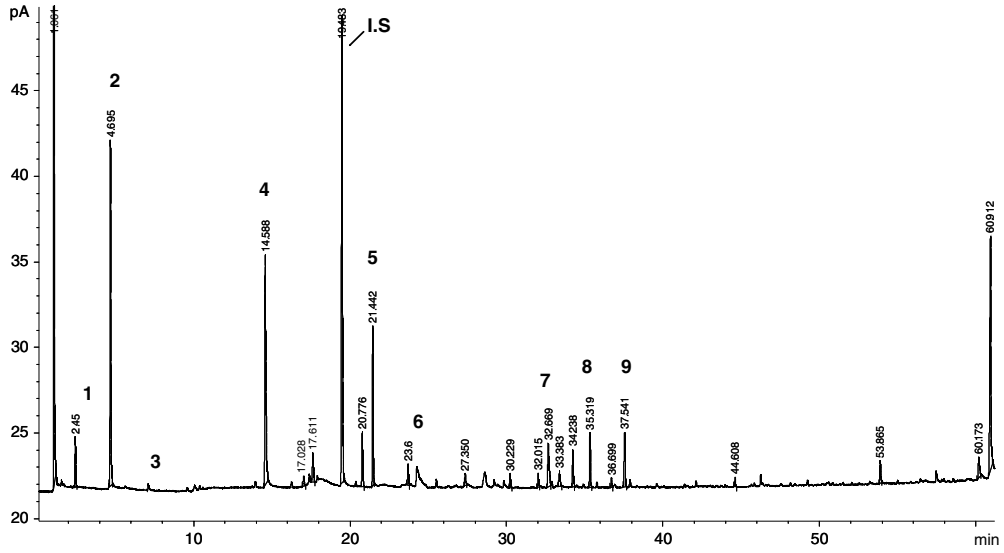

Fig. 2. GC-chromatogram of oil-in-water emulsion prepared with oxidised sunflower oil for equilibrium assays. Volatile compounds: 1, pentanal; 2, hexanal; 3, *t*-2-hexenal; 4, non-identified; I.S., internal standard; 5, nonanal; 6, *t*-2-nonenal; 7, 2,4-decadienal; 8, *t*-2-undecenal.

Table 2
Reproducibility for the determination of the major volatile oxidation compounds in a oil-in-water emulsion prepared with a oxidised sunflower oil

Replicates	Pentanal (ppm)	Hexanal (ppm)	*t*-2-Hexenal (ppm)	tr. 14.5[a] (ppm)	I.S.[b] (ppm)	Nonanal (ppm)	*t*-2-Nonenal (ppm)	*t*-2-Decenal (ppm)	2,4-Decadienal (ppm)	*t*-2-Undecenal (ppm)	Total volatiles (ppm)
1	0.37	3.57	0.06	3.10	5.43	1.73	0.56	0.59	0.48	0.56	63.2
2	0.36	3.28	0.06	2.83	5.47	1.62	0.63	0.70	0.46	0.63	61.3
3	0.39	3.28	0.06	3.04	5.42	1.71	0.67	0.77	0.50	0.69	67.7
4	0.36	3.36	0.07	3.00	5.44	1.72	0.55	0.63	0.47	0.59	69.6
5	0.36	3.22	0.07	2.86	5.40	1.62	0.37	0.51	0.46	0.45	70.0
6	0.36	3.36	0.05	2.99	5.41	1.68	0.43	0.57	0.43	0.50	61.6
Mean	0.37	3.35	0.06	2.97	5.43	1.68	0.54	0.63	0.46	0.57	65.6
SD	0.01	0.12	0.01	0.10	0.02	0.05	0.12	0.10	0.02	0.09	4.00
RSD (%)	3.21	3.62	9.76	3.51	0.45	2.74	21.54	15.22	5.12	15.01	6.10

[a] Non-identified compound.
[b] Internal standard.

Six replicates were analysed and the relative standard deviation was calculated (%RSD). The concentrations and RSD values for the main oxidation compounds are shown in Table 2. The uptake of internal standard showed a RSD of 0.45%. For the major oxidation compounds and total volatile concentration RSD values ranged between 2.7% and 21.5%, but for most of the compounds the RSD values were below 10%.

Volatile aldehydes have great importance as oxidation products because of their contribution to the aroma of oxidised oils and emulsions, and hexanal is commonly monitored to assess the formation of secondary oxidation products during lipid oxidation. Accelerated oxidation experiments were performed with samples of emulsions removed after 0, 2, 4, 6, 8 and 10 h of forced oxidation at 60 °C for SPME analysis. The variation of the volatile profile during these experiments is illustrated in Fig. 3. Pentanal, hexanal, *t*-2-hexenal, nonanal, *t*-2-nonenal, 2,4-decadienal and *t*-2-undecenal were detected in the headspace. Total volatile content increased with oxidation time with a sharp increase in hexanal production after 4 h. (Fig. 4). Hexanal is the main volatile formed during the oxidation of lipids via linoleic acid 13-hydroperoxide. Nonanal is another major volatile formed during oxidation of an emulsion containing oil rich in oleic acid, and the nonanal concentration increased more rapidly than that of hexanal (Fig. 4), as described for olive oil oxidation by Vichi, Pizzale, Conte, Buxaderas, and Lopez-Tamames (2003).

A method for the isolation and analysis of volatile oxidation products from oil-in-water emulsions has been developed. Headspace solid phase microextraction analysis conditions, including fibre desorption time, volatile isolation time and emulsion temperature, were studied. The reproducibility of peak areas was found to

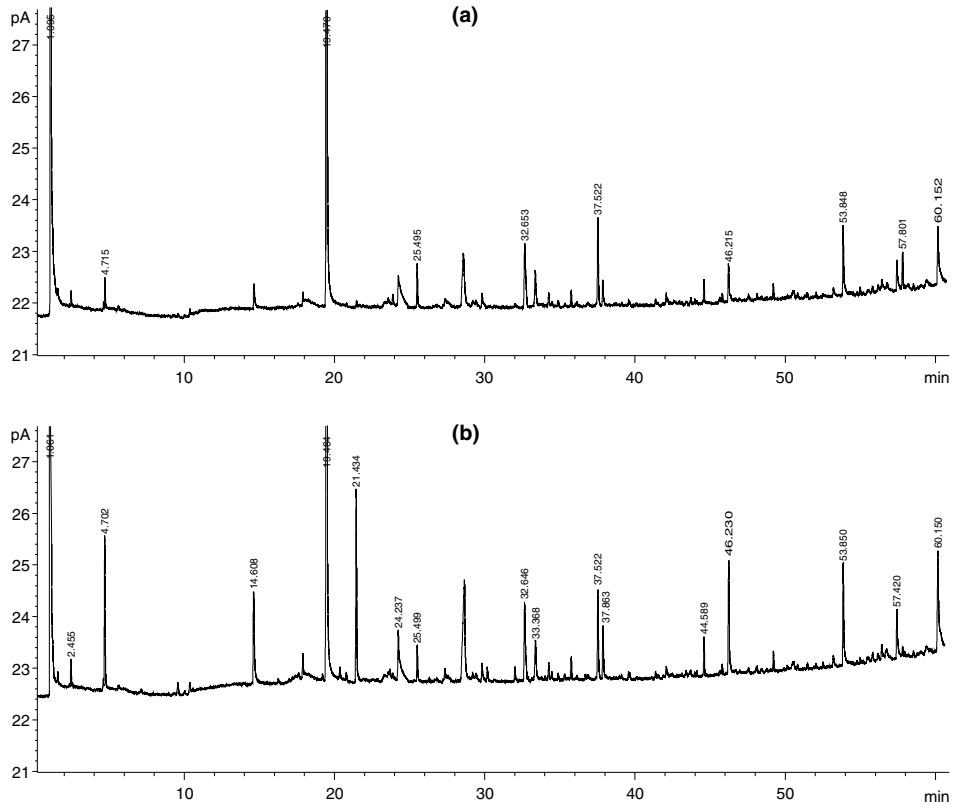

Fig. 3. Changes in GC-chromatograms of oil-in-water emulsion during forced oxidation at 60 °C for 0 h (a) and 10 h (b).

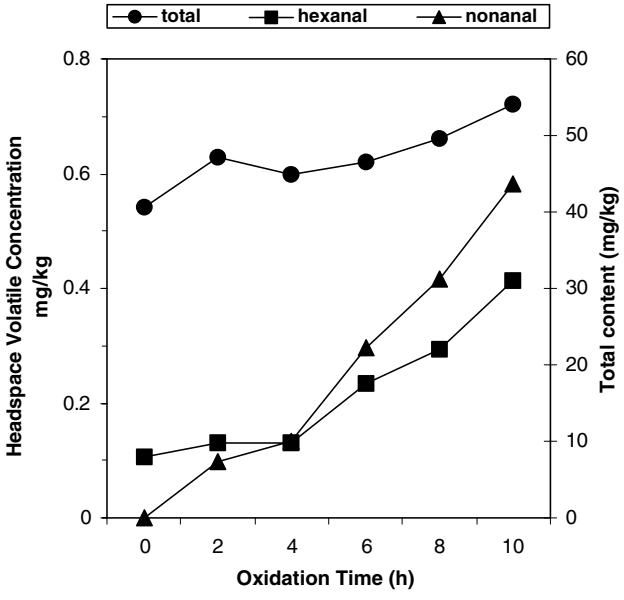

Fig. 4. Variation of hexanal, nonanal and total volatile contents during forced oxidation of oil-in-water emulsion at 60 °C.

range between 0.5% and 22% RSD, although, for the major oxidation products, the variation was below 10% RSD. The concentration of volatiles was determined by an internal standard method and the SPME method was used to monitor the oxidation of an oil-in-water emulsion. The HS-SPME method was shown to be a reliable and useful technique for measuring the concentration of volatile oxidation products in oil-in-water emulsions.

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