ELSEVIER

Journal of Chromatography A, 886 (2000) 175–181

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Solid-phase microextraction with on-fibre derivatisation applied to the analysis of volatile carbonyl compounds

Elena E. Stashenko*, Miguel A. Puertas, William Salgar, Wilman Delgado, Jairo René Martínez

Chromatography Laboratory, School of Chemistry, Industrial University of Santander, A.A. 678, Bucaramanga, Colombia

Received 9 February 2000; received in revised form 11 April 2000; accepted 18 April 2000

Abstract

We have used a fast, sensitive and efficient method for the analysis of volatile carbonyl compounds (saturated aliphatic and unsaturated aldehydes) based on solid-phase microextraction with on-fibre derivatisation. Pentafluorophenylhydrazine was absorbed onto a poly(dimethylsiloxane)/divinylbenzene-coated fibre and exposed to the vapours of aldehyde-containing matrices. The hydrazones formed on the fibre were desorbed into the gas chromatograph injection port and quantified by means of electron-capture detection with high sensitivity (10–90 fmol) and good reproducibility (RSD<10%). The method was applied to the headspace-sampling of volatile carbonyl compounds released during the thermally-induced degradation of sunflower oil.

Keywords: Derivatization, GC; Vegetable oils; Food analysis; Carbonyl compounds

1. Introduction

The oxidative degradation of polyunsaturated fatty acids involves a complex group of chain-propagation reactions that lead to the formation of low-molecular-mass aldehydes and ketones. The determination of these products can be used to monitor the progress of lipid peroxidation and to study antioxidant activity [1–3]. However, the high reactivity and volatility of low-molecular-mass carbonyl compounds impose the need for their derivatisation prior to their detection by a spectroscopic or chromatographic technique. The reaction of aldehydes with dinitrophenylhydrazine has been used to obtain hydrazone derivatives that can be analysed by HPLC after their extraction with dichloromethane [4]. Various other techniques have been applied to the determination of carbonyl compounds, with different levels of reproducibility, sensitivity and selectivity [5]. We have reported the use of 2-hydrazinobenzothiazole [6] and pentafluorophenylhydrazine (PFPH) [7] as derivatisation agents for the gas chromatographic analysis of carbonyl compounds present in lipid matrices. Although these methods provide good reproducibility, they involve an extensive work-up and consume materials and solvents for the derivatisation and isolation steps using liquid–liquid or solid-phase extraction techniques [6,7]. Martos and Pawliszyn developed an original method consisting of the on-fibre derivatisation with *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) of gaseous formaldehyde using gas chromatography with flame ionisation detection [8]. In the present work we

*Corresponding author.

E-mail address: elena@tucan.uis.edu.co (E.E. Stashenko)

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00479-9

employed a method for the analysis of volatile carbonyl compounds using solid-phase microextraction with on-fibre derivatisation with PFPH. The method does not consume solvents or other supplies and constitutes a one-step sample preparation procedure which affords high sensitivity and good reproducibility. We applied this method to the determination of volatile carbonyl compounds formed during the thermally-induced lipid peroxidation of vegetable oils.

2. Experimental

2.1. Reagents and materials

The standards of 14 carbonyl compounds tested, namely, ethanal, propanal, butanal, pentanal, hexanal, heptanal, octanal, nonanal, decanal, acrolein, crotonaldehyde, *trans*-2-pentenal, *trans*-2-hexenal, and *trans*-2-heptenal, were purchased from Aldrich (Milwaukee, WI, USA) and used to obtain hydrazones with PFPH (97%, Aldrich). For headspace solid-phase microextraction (HS-SPME) fused-silica fibres were used, coated with poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB) (65 μm) or poly(dimethylsiloxane) (PDMS) (100 μm) (Supelco, Bellefonte, PA, USA). Sunflower oil was obtained from the local market.

2.2. Preparation of hydrazones

The procedure described elsewhere [7,9–11] was used to obtain individual pentafluorophenylhydrazones from reactions between PFPH and a 50% stoichiometric excess of each one of the pure carbonyl compounds. Product characterisation and purity determination were based on high-resolution gas chromatography–mass spectrometry. The resulting compounds were used to prepare standard solutions of each hydrazone at 0.1, 0.3, 1.0, 2.5, 5, 10, 20, and 40 μ*M*, which were employed in chromatographic calibration.

2.3. Headspace solid-phase microextraction and on-fibre derivatisation

In order to select the coating material to use, PDMS- and PDMS–DVB-coated silica fibres were exposed for 5 min to the vapours of an aqueous 2.5 m*M* PFPH solution at room temperature. Aldehyde-free water (HPLC grade, J.T. Baker, Phillipsburg, PA, USA) was used in all experiments. The derivatisation agent was desorbed into the gas chromatograph injection port, in which a 0.75-mm internal diameter narrow-bore insert (Supelco) was used. Chromatographic peak areas and calibration curves were used for PFPH quantification. The equilibration time required for saturation with PFPH was determined by exposing the fibre for 10, 20 30, 45, 60 and 80 min to the vapours of a 0.1 m*M* PFPH aqueous solution at room temperature. During this time the solution was magnetically stirred at 900 rpm (Nuova II stirring hot plate, Thermolyne, Dubuque, IA, USA). The progress of the on-fibre derivatisation of the carbonyl compounds was determined by exposing a PDMS–DVB SPME fibre saturated with PFPH, for 5, 10, 20, 40, 60 and 70 min, to the vapours of a 2 μ*M* aqueous solution of each carbonyl compound at room temperature. All experiments were done in triplicate.

2.4. Headspace sampling of volatile carbonyl compounds from heated sunflower oil.

The HS-SPME derivatisation method was applied to the determination of the carbonyl compounds released by the thermally-induced lipid degradation of sunflower oil. In household use, vegetable oil degradation takes place during cooking, particularly when frying food. Oil temperatures between 180 and 220°C can be achieved during these processes [12,13]. In our previous works on the determination of saturated aliphatic and unsaturated carbonyl compounds dissolved in lipid matrices, we heated the oils to 180–250°C [7,14]. 2.0 g of sunflower oil placed in a 4.5 ml screw-cap vial provided with a poly(tetrafluoroethylene) septum and a thermocouple fixed to the outside wall, were heated to 220°C for 30, 60 and 120 min. In preparation for the sampling, a PDMS–DVB-coated SPME fibre was doped by exposure to the vapours of an aqueous 0.1 m*M* PFPH solution for 60 min at room temperature. Chromatographic quantification, based on a calibration curve, of the amount of PFPH absorbed, showed that under these conditions 4.1 ± 0.38 nmol of PFPH absorb on the

PDMS–DVB SPME fibre. The volatiles released by the heated sunflower oil were sampled in the vapour phase by direct insertion of the PFPH-doped fibre through the septum. However, initial data showed that at 220°C there was considerable desorption of PFPH from the fibre. In order to avoid this loss, the sampling was performed at a lower temperature, 170°C, at which no desorption of PFPH was observed. After the oil heating period at 220°C was completed, the sealed vial with sunflower oil was immediately transferred to a sand bath maintained at 170°C and allowed to equilibrate for 5 min. The SPME fibre was then inserted and exposed to the vapour phase. The fibre exposure time at 170°C was set to 5 min, based on previous experiments which used exposure times between 2 and 7 min and showed maximum hydrazone absorption at 5 min. The hydrazones formed on the SPME fibre were desorbed by direct insertion of the SPME fibre into a gas chromatograph injection port at 260°C for 5 min.

2.5. Chromatographic analysis

High-resolution gas chromatographic analysis of the samples was performed on a Hewlett-Packard (HP) 5890A Series II gas chromatograph equipped with split/splitless injector (260°C) and an electron-capture detection system, operated at 280°C. Chromatographic data were processed with HP GC Chemstation A.06.03 software (Hewlett-Packard). The column used was a DB-1 (J&W Scientific, Folsom, CA, USA) 60 m×0.25 mm, 0.25 μm. The oven temperature was programmed from 100°C (5 min hold) to 250°C (5 min) at 10°C min^{-1}. Helium (99.995%) was used as a carrier gas (inlet pressure 200 kPa) with linear velocity 26 cm s^{-1}. An argon–methane mixture (9:1, v/v) at a 60 ml/min flow was used as auxiliary gas in the electron-capture detection system.

3. Results and discussion

The sampling method used in this work was based on the ability of PFPH to react almost quantitatively with carbonyl compounds to form hydrazones [7,9–11], which can be analysed with high selectivity and sensitivity by means of the electron-capture detector, thanks to the five fluorine atoms on the PFPH moiety. The derivatisation reaction was carried out on the SPME silica fibre, on which PFPH had been previously absorbed. Comparison of the PFPH amounts absorbed on PDMS–DVB- and PDMS-coated fibres showed that PDMS–DVB allowed the absorption of 2.2 times more PFPH than PDMS did, under the same conditions. Addition of potassium chloride to the PFPH aqueous solution allowed higher PFPH loading levels onto the fibre (up to 20% higher, at 30% KCl), but also increased the absorption of interfering impurities present in the derivatisation agent. A similar case was reported by Bao et al. in the SPME of PFBHA derivatives of carbonyl compounds [15]. The progress of the equilibration of the PDMS–DVB-coated SPME fibre with the vapour phase above a 0.1 m*M* PFPH aqueous solution at room temperature is shown in Table 1, where equilibration after 60 min is apparent. Based on these results, a 60 min total exposure time to KCl-free 0.1 m*M* PFPH aqueous solutions at room temperature was used in the loading of the SPME fibres with PFPH prior to their use in the sampling of carbonyl compounds.

Fig. 1 illustrates the change in the chromatographic areas corresponding to the hydrazones formed on SPME fibres exposed for different times to the vapours of 2 μ*M* aqueous solutions of individual carbonyl compounds at room temperature. These plots showed that the equilibration time was similar for most of the carbonyl compounds tested. Unsaturated aldehydes, with the exception of crotonaldehyde and acrolein, exhibited equilibration times higher than 70 min. However, complete equilibrium is not an indispensable requisite for the SPME method, as long as the sampling conditions

Table 1
Progress of PFPH loading on a PDMS–DVB (65 μm) SPME fibre

Fibre exposure time (min)	Detector response to PFPH desorbed from SPME fibre
10	5283.0
20	7752.2
30	10 411.3
45	14 628.7
60	19 008.7
80	19 536.3

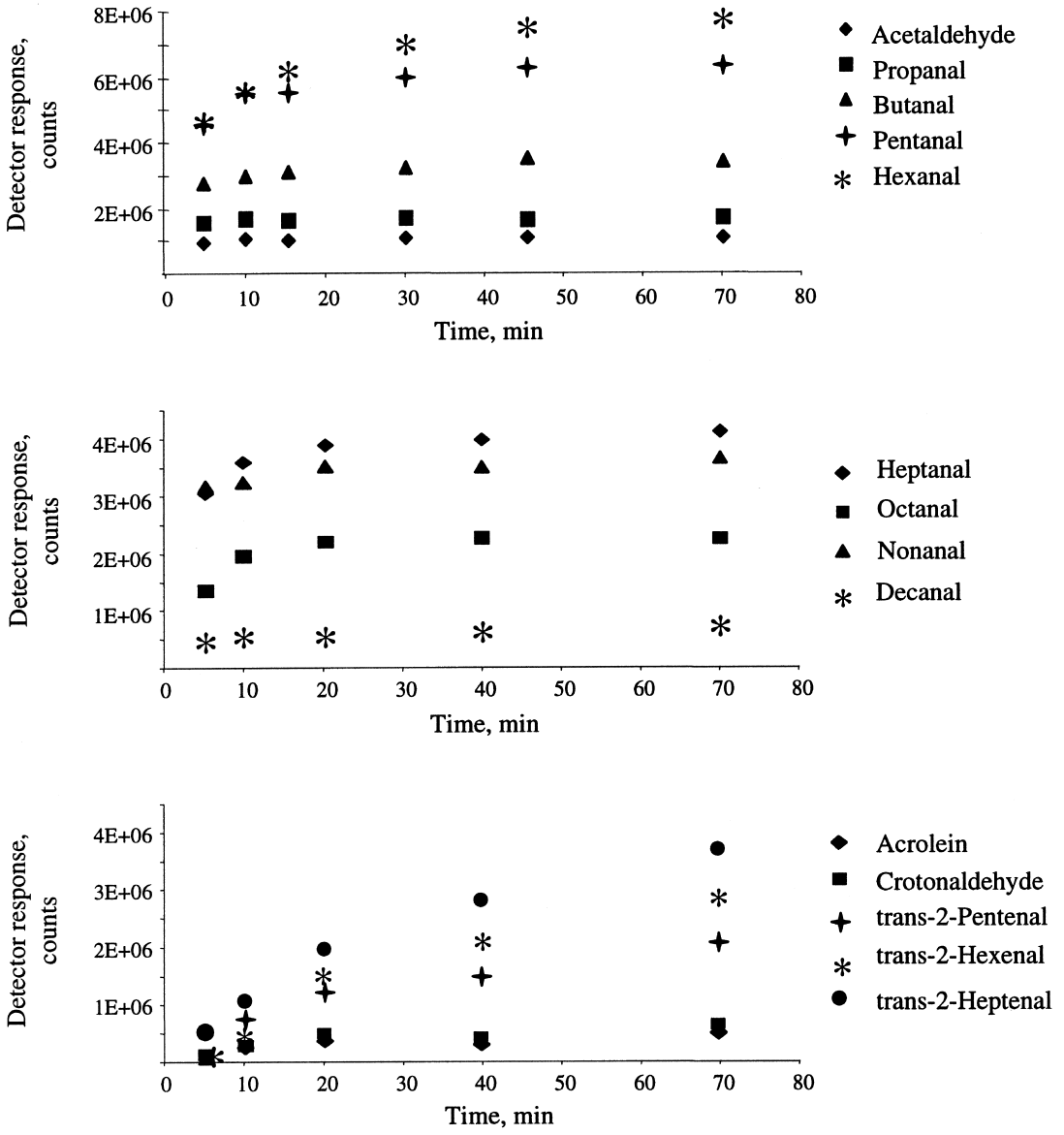

Fig. 1. Absorption time profiles for PFPH derivatives of carbonyl compounds in water using HS-SPME. Sample volume was 2 ml. Spiking level was 2 μ*M*. The sample was magnetically stirred.

used for calibration are reproduced during the method application to standard solutions and unknown samples [16].

Calibration curves and chromatographic response factors of individual hydrazones were calculated from the peak areas in the chromatograms obtained from direct injection of their calibration solutions. The minimum detection level (at $S/N=5$) of the HS-SPME method employed was between 10 and 90 fmol for the various carbonyls under study (Table 2), with an acceptable reproducibility (RSD<10%). The calibration curves obtained showed a good linear response in the concentration interval examined (Table 2). Typical chromatograms obtained from exposure of the SPME fibre loaded with PFPH to 2 μ*M* aqueous solutions of the individual aldehydes

Table 2
Minimum detection levels obtained by the HS-SPME with on-fibre derivatisation and electron-capture detection of volatile carbonyl compounds measured as their pentafluorophenylhydrazone derivatives

Carbonyl compound	MDL[a] (fmol)	Linear regression coefficient[b]
Acetaldehyde	90.0±9.7[c]	0.998
Propanal	45.0±8.1	0.992
Acrolein	11.0±8.7	0.998
Butanal	43.0±7.1	0.992
Crotonaldehyde	16.0±5.5	0.999
Pentanal	41.0±10.0	0.984
Hexanal	43.0±4.5	0.996
Heptanal	34.0±6.3	0.998
Octanal	40.0±8.7	0.999
Nonanal	39.0±4.9	0.983
Decanal	41.0±3.8	0.999
trans-2-Pentenal	11.0±4.5	0.993
trans-2-Hexenal	10.0±9.9	0.999
trans-2-Heptenal	12.0±7.7	0.999

[a] MDL: Minimum detection level.
[b] For the 0.1–40 μmol/l interval.
[c] Mean of three determinations±RSD (%).

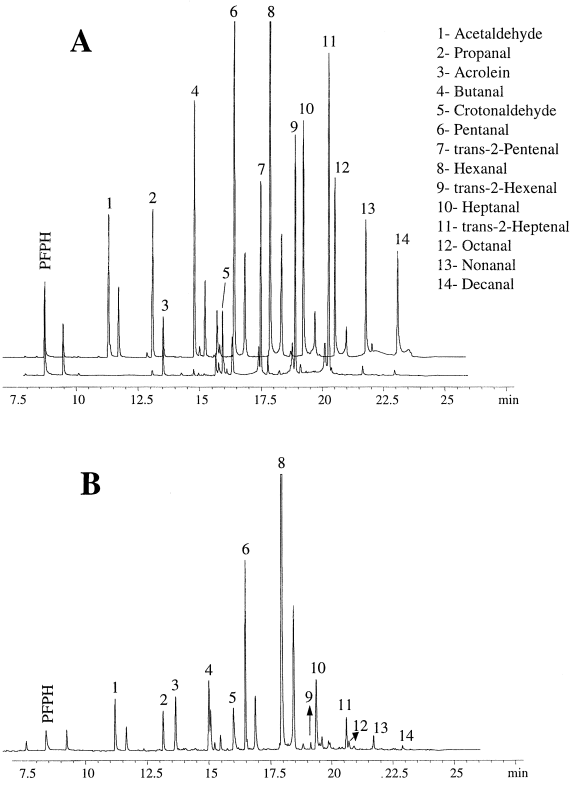

Fig. 2. Typical gas chromatographic profiles obtained after on-fibre derivatisation with PFPH of carbonyl compounds (A) from a standard aqueous solution (2 μ*M* of each carbonyl compound) and (B) from heated (120 min, 220°C) sunflower oil. Column DB-1 60 m×0.25 mm.×0.25 μm. Electron–capture detection. 1 – acetaldehyde, 2 – propanal, 3 – acrolein, 4 – butanal, 5 – crotonaldehyde, 6 – pentanal, 7 – *trans*-2-pentenal, 8 – hexanal, 9 – *trans*-2-hexenal, 10 – heptanal, 11 – *trans*-2-heptenal, 12 – octanal, 13 – nonanal, 14 – decanal.

under study, are shown in Fig. 2. The well-resolved peak with retention time around 8 min corresponded to the formaldehyde derivative. Persistent background contamination problems caused rather large fluctuations in formaldehyde peak areas and made us exclude formaldehyde data from all the tables in this paper. Each asymmetric carbonyl compound gave rise to two peaks due to the formation of two geometric isomers of the corresponding hydrazone. For quantitative purposes, we obtained a reduction of about 40% in RSD when we worked exclusively with the area under the bigger peak. This improvement resulted mainly from smaller integration errors in the case of partially overlapping chromatographic peaks [7]. The appearance of unreacted PFPH in the chromatograms confirmed that the complete derivatisation of the carbonyl compounds diffused into the fibre. A second thermodesorption of the SPME fibre in the injection port afforded a peak-free chromatogram, showing that the conditions used in the first desorption yield an essentially clean fibre, which can be utilised in other analyses. In their related on-fibre derivatisation method, Martos and Pawliszyn showed that the reproducibility of the derivatisation agent absorption is maintained even after 20 repeated injections with the same fibre [8]. This on-fibre derivatisation method was compared with the derivatisation in solution followed by HS-SPME. A 2 μM standard hexanal solution was used in both cases. For the derivatisation in solution, 40 μl of this solution was added to 2 ml of an aqueous 0.1 mM PFPH solution and allowed to react at room temperature for 1 h. A PFPH-free PDMS/DVB fibre was exposed for 40 minutes to the vapours of the magnetically stirred solution and then transferred to the injection port (260°C, 5 min). The recovery of the hexanal PFPH derivative by on-fibre derivatisation/desorption was 87±2%, while that of the derivatisation in solution followed by HS-SPME was

61±5.2%. Thus, the method presented here has the advantages of higher precision and recovery, and shorter total analysis time.

Sunflower oil is rich in linoleic (64%) and oleic (24%) acids [7,14], which constitute the fatty acid components susceptible to lipid peroxidation. This degradation occurs through the formation of hydroperoxide intermediates which suffer bond scissions and secondary oxidations that lead to low-molecular-mass aldehydes [17–19]. In order to illustrate an application of the HS-SPME method, it was used in the analysis of the carbonyl compounds released by commercial sunflower oil heated to 220°C. Table 3 presents the on-fibre derivatisation yields at room temperature for all the carbonyl compounds available, calculated with the amount of PFPH derivatives found on the SPME fibre exposed for 5 min at room temperature to the vapours of 2 g of sunflower oil, before and after addition of standard carbonyl compounds to the level of 5 nmol/g of oil. The concentration of aldehydes in the oil was determined by direct derivatisation with PFPH in solution followed by solid-phase extraction and chromatographic analysis [7]. Although the equilibration times were similar for the carbonyl compounds tested (Fig. 1), the yields of PFPH derivatives on the fibre varied markedly due to differences in molecular structure, reactivity, and vapour pressure. Table 4 contains the amounts of aldehydes determined for heating periods of 0, 30, 60 and 120 min after 5 min SPME fibre exposure. The presence of low-molecular-mass aldehydes in the unheated sunflower oil (0 min) revealed some degree of autoxidation in the original oil. The total amount of carbonyl compounds found as PFPH derivatives on the SPME fibre increased with the heating time. However, not all carbonyl compounds behaved in the same manner. Thermally-induced oxidation caused sharp increases in heavier aldehydes ($C_n>5$), particularly hexanal, which is the main degradation product from linoleic and oleic acids [20]. We did not observe 2,4-decadienal, which, together with hexanal, results from bond scission at the opposite ends of the pentadiene moiety in linoleic acid after hydroperoxide formation at carbons 9 and 13 [21]. The progressive decrease in the amount of acrolein detected may be explained by its high reactivity and the oxidation and polymerization reactions that can take place at these high temperatures. Thus, in order to avoid the compounding effects of the different behaviour exhibited by the various carbonyl compounds, studies aimed at evaluating antioxidant activity could simply monitor the concentration changes for one particular carbonyl compound. Lehmpuhl and Birks [22] developed a gas chromatographic method for the determination of carbonyl compounds based on their derivatisation with 2,4,6-trichlorophenylhydrazine (TCPH) and subsequent

Table 3
Derivatisation yields for on-fibre HS-SPME derivatisation of carbonyl compounds present in sunflower oil

Carbonyl compound	Carbonyl compound concentration in oil (nmol/g)	Amount of PFPH derivative on fibre (pmol)		Derivatisation yield (%)
		Before spiking[a]	After spiking[a]	
Acetaldehyde	102.4	760.3	908	1.477
Propanal	25.7	72.9	325.1	2.522
Acrolein	12.8	11.3	38.5	0.272
Butanal	11.2	11.3	336.6	3.253
Crotonaldehyde	8.6	3.5	17.6	0.141
Pentanal	35.0	20.7	93.2	0.725
Hexanal	43.3	54.6	107	0.524
Heptanal	128.0	11.1	19.5	0.084
Octanal	3.8	1.3	1.52	0.002
Nonanal	–	–	0.87	0.009
trans-2-Pentenal	9.1	–	4.1	0.041
trans-2-Hexenal	–	–	1.24	0.012
trans-2-Heptenal	2.9	–	1.1	0.011

[a] Sunflower oil (2 g) were spiked at 5 nmol/g with each carbonyl compound.

Table 4
Carbonyl compounds from heated sunflower oil (220°C) sampled by HS-SPME with on-fibre derivatisation

Carbonyl compound	Amount (pmol)[a]			
	Heating time (min)			
	0	30	60	120
Acetaldehyde	130.2±0.3	48.6±3.6	60.8±4.2	62.9±4.9
Propanal	22.5±1.4	46.3±5.2	41.1±3.5	47.5±4.3
Acrolein	7.3±0.08	23.6±0.5	16.5±2.6	16.0±1.8
Butanal	1.7±0.03	98.6±5.6	86.7±6.4	82.6±7.3
Crotonaldehyde	2.4±0.03	24.7±4.7	30.9±3.1	25.3±4.7
Pentanal	17.3±5.6	107.4±9.5	116.9±19.4	150.3±14.0
Hexanal	17.5±0.8	567.7±16.7	596.5±18.2	787.5±38.4
Heptanal	6.19±0.2	143.0±14.5	150.6±9.7	273.6±20.5
Octanal	0.1±0.05	1.2±1.0	2.4±2.6	4.1±1.8
Nonanal	ND[b]	7.7±0.4	11.2±0.9	11.3±1.2
Decanal	ND	10.5±3.1	11.4±2.6	19.1±3.5
trans-2-Hexenal	0.3±0.02	3.5±0.1	3.4±0.4	4.5±0.4
trans-2-Heptenal	ND	9.8±0.9	11.8±0.9	16.1±1.5
Total	205.49±5.8	1092.6±26.2	1140.2±30.1	1500.8±47.2

[a] Mean of three independent measurements±RSD (%).
[b] ND: Not detected.

electron capture detection. The derivatization takes place in small cartridges that contain TCPH-impregnated octadecyl silica, a setup that was applied to the analysis of carbonyl compounds in air. A detection limit in the fmol range was also obtained and the reproducibility was similar to that reported here. The HS-SPME method is applicable to the analysis of carbonyl compounds in air, with the advantages that it does not require the use of a heating step to carry out the derivatisation reaction after sampling, nor a solvent extraction of the formed hydrazones is necessary in order to obtain the sample that is injected into the gas chromatograph.

4. Conclusions

Saturated aliphatic (C_2–C_{10}) and unsaturated (C_3–C_7) aldehydes, final products of lipid peroxidation, were sampled from the vapour phase by derivatisation with PFPH absorbed onto PDMS–DVB SPME fibres. Loading of PFPH onto the fibre was carried out at room temperature from a 0.1 m*M* PFPH standard aqueous solution. The derivatisation products, hydrazones, were desorbed into the injection port and analysed by high-resolution gas chromatography using electron-capture detection. Very high sensitivity (10–90 fmol) and good reproducibility (RSD<10%) were obtained. The method was applied to the headspace-sampling of volatile aldehydes generated during thermally-induced lipoxidation in vegetable (sunflower) oils. This relatively simple, sensitive and reproducible technique could be successfully applied to other lipid matrices subjected to heating, irradiation or other type of chemical modification. The in vitro antioxidant activity of natural or synthetic substances could be measured using this method, by monitoring the amount of low-molecular-mass carbonyl compounds released in lipid systems under oxidative conditions in the presence or absence of the possible antioxidant agent. This technique could also be used in quality control operations to evaluate the deterioration level of lipid-containing products.

Acknowledgements

Financial support from COLCIENCIAS (Grant 1102-05-267-96) is gratefully acknowledged.

References

[1] H. Esterbauer, R.D. Schaur, H. Zollner, Free Radical in Biology & Medicine 11 (1991) 81.
[2] H. Zollner, R.J. Schaur, H. Esterbauer, Oxidative Stress: Oxidants and Antioxidants, Academic Press, New York, 1991, 337.
[3] E. Frankel, Chem. Phys. Lipids 44 (1987) 73.
[4] H. Esterbauer, H. Zollner, Free Rad. Biol. Med. 7 (1989) 197.
[5] K. Moore, J. Roberts, Free Rad. Res. 28 (1998) 659.
[6] E.E. Stashenko, J.W. Wong, J.R. Martínez, A. Mateus, T. Shibamoto, J. Chromatogr. A 752 (1996) 209.
[7] E.E. Stashenko, M.C. Ferreira, L.G. Sequeda, J.R. Martínez, J.W. Wong, J. Chromatogr. A 779 (1997) 360.
[8] P.A. Martos, J. Pawliszyn, Anal. Chem. 70 (1998) 2311.
[9] Y. Hoshika, Y. Takata, J. Chromatogr. 120 (1976) 379.
[10] Y. Hoshika, G. Muto, J. Chromatogr. 152 (1978) 224.
[11] M. Tomita, T. Okuyama, J. Chromatogr. 526 (1990) 174.
[12] P. Fellows, Tecnología Del Procesado De Alimentos — Principios Y Prácticas, Acribia, Zaragoza, 1994, p. 503.
[13] J.-C. Cheftel, H. Cheftel (Eds.), Introducción A La Bioquímica Y Tecnología De Los Alimentos, Vol. 1, Acribia, Zaragoza, 1992, p. 229.
[14] E. Stashenko, M. Cervantes, Y. Combariza, H. Fuentes, J.R. Martínez, J. High Resolut. Chromatogr. 22 (1999) 343.
[15] M.-L. Bao, F. Pantani, O. Griffini, D. Burrini, D. Santiani, K. Barbieri, J. Chromatogr. A 809 (1998) 75.
[16] J. Ai, Anal. Chem. 69 (1997) 3260.
[17] E.N. Frankel, W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood, B.C. Weedon, Lipids 12 (1977) 901.
[18] E.N. Frankel, W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood, B.C. Weedon, Lipids 12 (1977) 908.
[19] E.N. Frankel, W.E. Neff, W.K. Rohwedder, B.P.S. Khambay, R.F. Garwood, B.C. Weedon, Lipids 12 (1977) 1055.
[20] E.N. Frankel, J. Am. Oil Chem. Soc. 61 (1984) 1908.
[21] M.C. Dobarganes, J.J. Ríos, M.C. Pérez-Camino, Grasas Aceites 37 (1986) 61.
[22] D.W. Lehmpuhl, J.W. Birks, J. Chromatogr. A 740 (1996) 71.