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Determination of hexanal as indicator of the lipidic oxidation state in potato crisps using gas chromatography and high-performance liquid chromatography

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Abstract

Hexanal (an oxidative state indicator) formed in the headspace of potato crisps during storage was evaluated using two different procedures. First, solid-phase microextraction, an innovative sampling preparation methodology was used. It consisted on the absorption of analytes directly from samples and subsequent thermal desorption on the gas chromatograph (GC) injector. Then, a reversed-phase high-performance liquid chromatographic technique (HPLC) was employed to quantify hexanal in the form of 2,4-dinitrophenylhydrazone derivative. Methods were evaluated in what concerns to validation parameters such as linearity, repeatibility and detection limit. GC (LOD = 1 ng/ml) method resulted in more sensitive method than HPLC (LOD = 9 ng/ml). The most suitable technique for hexanal measurement was selected.

Keywords: Food analysis; Oxidation; Hexanal; Lipids

1. Introduction

Deterioration of high fat content food generally occurs as result of lipid oxidation. This process is decisive on the reduction of food products shelf-life, once it is responsible for the production of undesirable odours, texture deterioration, decrease of nutritional value and formation of potentially toxic substances [1,2].

Food industry has great interest in preventing, as well as on the early detection of lipid oxidation due to the economical profits that can achieve directly from the increase of sells number and consumer acceptance because, this way, products will preserve all their qualities during a longer period and any change that might adversely affect the taste of the product is early detected [3]. Therefore, a substance (indicator) that could evaluate the state of lipid oxidation, advising or not the products' consumer is of great interest. Hexanal has become a known indicator thanks to be a major product of oxidation of fats and to increase with storage [4]. It is formed during the oxidation of linoleic acid via the 13-hydroperoxide and it has an odour described as "grassy" which contributes to off-flavours, and it is easily detected once have a low odour threshold (in water: 4.5 μg/kg) [1,2,5,6].

In the last few years hexanal has been determined in food matrixes, using many different sample preparation methods and detection techniques (e.g. gas chromatography, liquid chromatography, spectrophotometry) [7].

Hexanal has been evaluated in: cooked turkey [4], freeze-dried chicken myofibrils [2]; fish [8]; vegetable oils [9]; etc.

In this work, a study using two chromatographic techniques, HPLC and GC, is made, trying to find out which is the most suitable for the determination of hexanal in potato crisps.

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In the first part of the paper, an innovative sample preparation technique, solid-phase microextraction (SPME) followed by gas chromatography (GC), was used. SPME was introduced in the 1990s by Arthur and Pawliszyn and has successfully been applied to the study of the volatile profile of foodstuffs [10].

SPME technique utilises a fiber (coated fused silica) which adsorb the analytes from samples. The fiber may be directly immersed in liquid samples (DI-SPME) or in the headspace of a gaseous, liquid or solid samples (HS-SPME). In spite of DI-SPME efficiency, it can not be performed in solid samples because the fiber is too weak to be pushed in solid material [3]. HS-SPME is more recommended because it allows shorter extraction times, a longer fiber lifetime (once the fiber it is not in contact with the sample) [11,12]. Finally, the fiber is inserted in the GC injection port for thermal desorption and subsequent gas chromatography.

In the second part of the work, an HPLC method is used. As previous authors have reported, HPLC presents advantages over GC because of the superior separation efficiency and due its major sensitivity. However, these methods require a pre- or post-column derivatization step. Numerous derivatization agents have been used [13]: dansylhydrazine [14], 4-dimethylaminobenzene-4′-sulphonylhydrazine and more frequently 2,4-dinitrophenylhydrazine [15], which is well suited for the detection of traces [15]. Moreover, the use of HPLC with reversed-phase columns (RP-HPLC) results in a better resolution [15]. In the present paper hexanal is quantified as its 1,4-dinitrophenylhydrazine derivative by RP-HPLC.

The paper's main purpose is to study the progress of hexanal during the storage of potato crisps, using two different methods, GC and HPLC. The most suitable method for hexanal determination in crisps is selected regarding all outcomes and drawbacks.

2. Experimental

2.1. Sampling

Potato crisps were purchased from a local supermarket. According to the package information, they were fried in olive oil. Sample preparation procedures were performed at selected days. Meanwhile, they were stored in two different conditions. One group was stored in the darkness at room temperature, simulating the normal storage conditions. The other group was stored in presence of natural light at room temperature. This way, potato crisps stability was evaluated by means of an accelerated storage test in order to evaluate the suitability of hexanal as an early oxidation marker [1].

Potato crisps' brand used was selected regarding the type of package. A transparent packaging film was chosen in order to allow the study of the influence of light on the hexanal formation.

2.2. Analytical standards and reagents

Hexanal [CAS 66-25-1] was supplied from Sigma–Aldrich (Madrid, Spain) and had a purity above 99%. For SPME, an hexanal standard solution of 100 mg/l was prepared in water. Ultrapure water was obtained with a Milli-Q filter system (Millipore, Bedford, MA, USA).

All chemicals were of analytical grade. Acetonitrile, methanol, ethanol and *n*-hexane were from Merck (Darmstadt, Germany). Hexanal and 2,4-dinitrophenylhydrazine were purchased from Sigma (Steinheim, Germany). Sep-Pak DNPH-silica cartridges were from Waters (Milford, MA, USA).

2.3. SPME experimental procedure

2.3.1. Sample preparation

Potato crisps samples were ground with a commercial grinder, and homogenised. Approximately 0.1 g of sample was mixed with ultrapure water (1 ml) in 20 ml vials (Sun International Trading, USA). The vials were hermetically capped with PTFE-faced silicone septum. Then, samples were mixed for 3 min in a vortex (Autovortex SA6, Stuart Scientific, Redhill, UK).

2.3.2. Headspace SPME (HS-SPME)

Following homogenisation, the vials were heated in a module (Stuart Scientific, UK) at 70 °C for 5 min to reach the equilibrium between the sample and above headspace [16].

The SPME holder (Supelco, Bellefonte, PA, USA) was used to perform the experiments. A fused silica fiber coated with a 50/30 μm layer of divinylbenzene–carboxen–polydimethylsiloxane (DVD–CAR–PDMS; II Supelco) was used to extract hexanal from potato crisps.

Prior to first use, fibers were conditioned, by inserting them for 4 h at 270 °C in the GC injection port. After equilibrium time, the holder needle was exposed to the headspace during 20 min (extraction time) at 70 °C. Then, the fiber was withdrawn and removed from the vial. Fibers were immediately thermally desorbed in the GC injector for 5 min at 250 °C to prevent contamination. A scheme of the SPME extraction procedure is presented in Fig. 1.

2.3.3. Gas chromatography–mass spectrometry system and conditions

A Fisons GC 8000 series gas chromatograph (Manchester, UK) equipped with a programmed split/splitless injector and a mass-selective detector MD800 was used to perform all GC analysis.

A narrow-bore glass liner (SGE, USA) of 0.80 mm i.d. was used to substitute common liners in order to improve peaks resolution [11,17]. A cross-linked 5% phenyl–95% methylsiloxane (30 m × 0.25 mm i.d. × 1 μm film thickness) DB-5 column (DB J&W Scientific, CA, USA) was employed. Helium was used as carrier gas at a head pressure of 70 kPa.

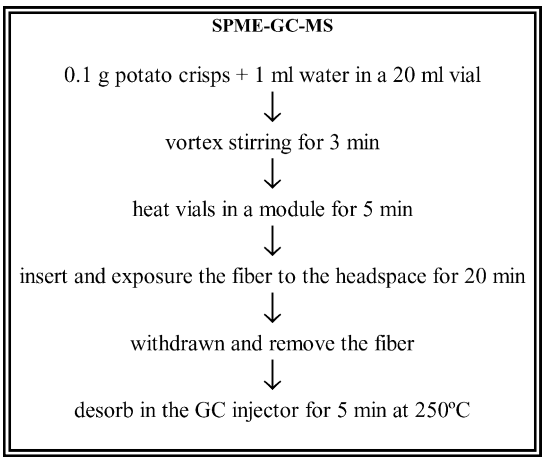

Fig. 1. SPME–GC–MS extraction procedure.

The oven temperature was programmed as follows: 40 °C for 1 min, then to 120 °C at 20 °C/min, held for 8 min, then to 260 °C at 20 °C/min and finally held for 2 min. The split valve was opened 2 min after injection. The injector temperature was 260 °C.

The mass spectras were obtained using a mass selective detector (MD 800, Manchester, UK) under electron impact ionization at a voltage of 70 eV and data acquisition was done at a scan rate of 0.45 s^{-1} over an *m*/*z* range of 35–300. The software used to process peak areas was Masslab (version 1.4). The confirmation of hexanal was performed by comparing the observed mass spectra with that recorded in Wiley spectrometry Library and by identical retention time of a standard injected in the same conditions.

2.4. LC experimental procedure

2.4.1. Preparation of 2,4-DNPHi solution

The 2,4-DNPHi solution was prepared by mixing 90 ml of ethanol, 2 ml of hydrochloric acid, 50 mg of DNPH and 8 ml of water.

2.4.2. 2,4-DNPH standard

DHPH derivative of hexanal was prepared by dissolving 1.8 mg 2,4-DNPH standard in 100 ml of ethanol in a volumetric flask (18 mg/L).

2.4.3. Extraction

Hexanal was extracted from potato crisps, previously homogenized by magnetic stirring for 5 min, adding 5 ml of ethanol to 1 g of sample. Then, 1 ml of the supernatant was removed and mixed with 1 ml of the 2,4-dinitrophenylhydrazine solution. The mixture was incubated at 40 °C for 45 min (these mixtures were stable for 2 h stored at 4 °C). All samples were filtered through a 0.5 μm Millipore filter (Bedford MA, USA) and afterwards analysed by HPLC–UV detection.

Table 1
Trials for extraction procedures optimisation of HPLC method

Magnetic stirring	
Extraction solvent	EtOH
Extraction time	5–10–15–30 min
Reaction time	30–45 min
Reaction temperature	Room temperature, 40 °C
Ultrasounds	
Extraction solvent	EtOH
Extraction time	15–30–60 min
Reaction time	30–45 min
Reaction temperature	Room temperature, 40 °C
DNPH-silica cartridges	
Elution solvent	EtOH–hexane
Elution volume	2–3 ml
Sample volume	0.5–1 ml

Several trials were carried out to optimise extraction of hexanal. Parameters studied were summarised in Table 1. Two other extraction procedures were studied (Table 1). At first, a method which homogenisation step consisted in ultrasounds stirring, then a method using DNPH-silica cartridges. The optimised procedure using DNPH-silica cartridges involve the solubilisation of 1 g of sample to 5 ml of ethanol. Then, homogenization was performed for 5 min with constant magnetic stirring. The mixture was centrifuged at 5500 rpm for 15 min and then the supernatant was removed. An aliquot (1 ml) of the supernatant was passed through the DNPH-Silica cartridge, then it was dried for 10 min under vacuum and finally the hexanal was eluted with 2 ml of ethanol. This solution was filtered (0.22 μm Millipore), then analysed by GC–MS. Schemes of the optimised extraction procedures with derivatization step are presented in Fig. 2.

2.4.4. Equipment and chromatographic conditions

2.4.4.1. LC–MS—just for hexanal identification. A SpectraPhysics series P200 liquid chromatograph equipped with a Rheodyne loop (50 μl) and a mass detector (Fisons VG Platform) (VG Biotech, Altrincham, UK) was used to

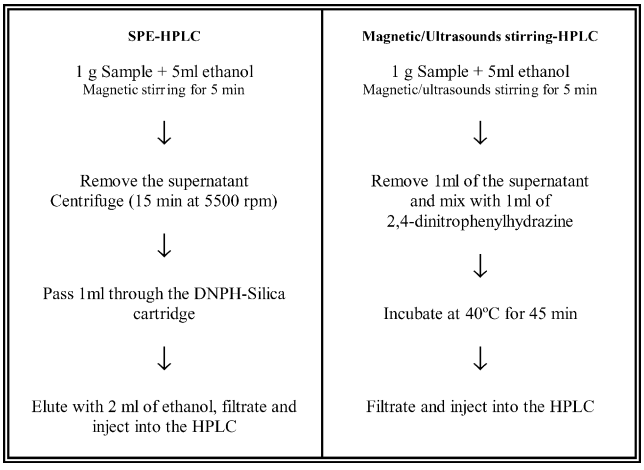

Fig. 2. Derivatization–HPLC–UV extraction procedures.

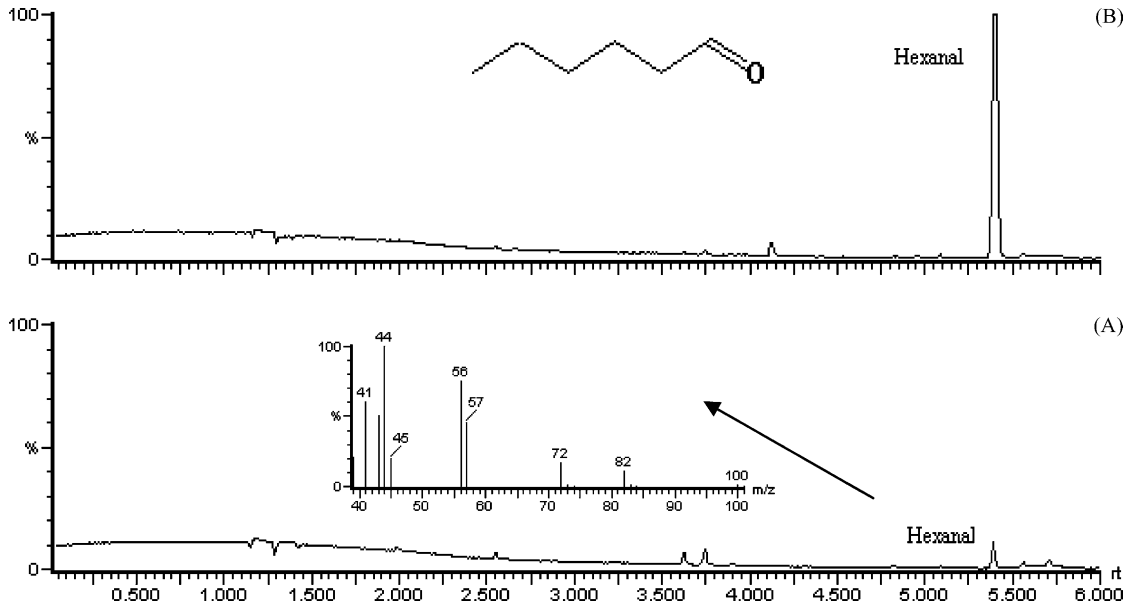

Fig. 3. GC–MS chromatograms of potato crisps stored for 1 month: (A) darkness and (B) lightness and hexanal GC–MS spectra.

identify hexanal. Full scan and single-ion recording (SIR) were obtained every second with a scan delay time of 0.1 s. The column used was a Kromasil 100 C_{18} 5 μm 15 cm × 0.4 cm and the mobile phase was acetonitrile–water (75:25) at a flow rate of 1 ml/min performing in isocratic mode. Detector operated under the following conditions: negative atmospheric pressure chemical ionization (APCI) mode; probe temperature 400 °C; source temperature 130 °C, cone voltage −20 V, electron multiplier voltage 700 V, drying gas nitrogen at 425 l/h, APCI sheet gas nitrogen at 175 l/h.

2.4.4.2. HPLC–UV—for hexanal quantification. The HPLC system (Hewlett–Packard, CA, USA) consisted of an HP1100 quaternary pump, an HP1100 degassing device, a 20 μl injection loop (Rheodyne, Cotati, CA), an HP1100 UV detector, a column heater SP8792 (San José, CA, USA). The HPLC system was controlled by a personal computer running HP Chem Station Software. Operating conditions were as follows: the mobile phase was methanol–water (75:25, v/v) at a flow rate of 1.5 ml/min, column Tracer Extrasil ODS2 (25 cm × 0.4 cm i.d., 5 μm particle size) set at 25 °C. The absorbance was monitored with an UV detector and set at 365 nm.

3. Results and discussion

3.1. Methods optimisation

3.1.1. SPME–GC–MS

The formation of hexanal from potato crisps oxidation was evaluated using a DVB–CAR–PDMS fiber. Díaz et al. had also selected this fiber of medium polarity to extract the

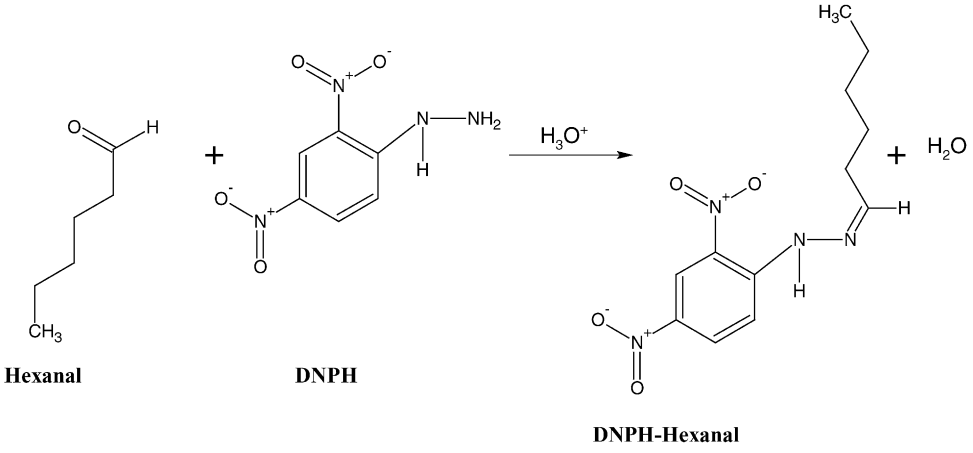

Fig. 4. Derivatization reaction of hexanal with dinitrophenylhydrazine (DNPH).

volatile components from truffles because reduce discrimination toward very nonpolar and polar volatile compounds [18].

In the first approach, potato crisps samples were used in the dry state. Further experiments were performed with samples homogenised with water. Two factors contributed for the use of a slurry instead of a dry sample. In one hand, according to literature, the extraction of volatiles is much more efficient from the liquid state [1]. In the other hand, in order to achieve a reliable hexanal quantification, both standard solutions (used to construct the calibration curve as described in Section 2) and samples were in the liquid state and were subject to the same extraction procedure.

With the aim of avoiding artifacts, samples were extracted just after its preparation. For all experiments, the adsorption time and temperature, were set at 20 min and 70 °C, respectively (Table 1). These conditions provided reproducible and sensitive results and avoided fiber saturation even for the more oxidised potato crisps. Regarding the desorption conditions, optimal temperature and time were 260 °C and 3 min, respectively. These were optimised in order to ensure total desorption from fibre and, this way, avoid carryover effects. Running blank samples were regularly made to check possible carryover or fiber ageing (which originate new peaks). Fig. 3 shows a GC–MS chromatogram of the sample after a 1 month storage stored in the lightness and in the darkness.

3.1.2. Derivatization–RP-HPLC

Preliminary assays were performed in order to establish optimal extraction conditions (extraction volume, solvent extraction, reaction time, etc). Table 1 summarises the trials. Three extraction procedures were optimised: (1) with DNPH cartridges, (2) with magnetic stirring, (3) with ultrasounds stirring. The first and second extraction procedures differ only in the homogenisation step. Due to the importance of this step in the final result, assays were performed with magnetic stirring and ultrasounds. Magnetic stirring resulted in a more effective method. Regarding the method which used cartridges, although its short analysis time, it resulted in a much more expensive procedure. For this reason, magnetic stirring method was selected. The derivatization reaction is presented in Fig. 4. Fig. 5 corresponds to HPLC–UV chromatograms of potato crisps samples.

Solutions stability was studied at regular intervals of 30 min at 4 °C for 5.5 h. Low temperatures allowed to decrease the reaction speed. Results showed that solutions were stable for 2 h at 4 °C, after this period they should not be injected once false results could be achieved.

With respect to hexanal identification, this was done by comparing of the retention time (t_r) with the standard and confirmed by LC–MS.

In order to achieve the best conditions in LC–MS, several assays were performed. SIR and full scan modes were used (Fig. 6). Several probe temperatures (350, 400 and 500 °C) and cone voltages (−20 and −30 V) were evaluated. Best response was achieved with 400 °C and −20 V. Characteristic

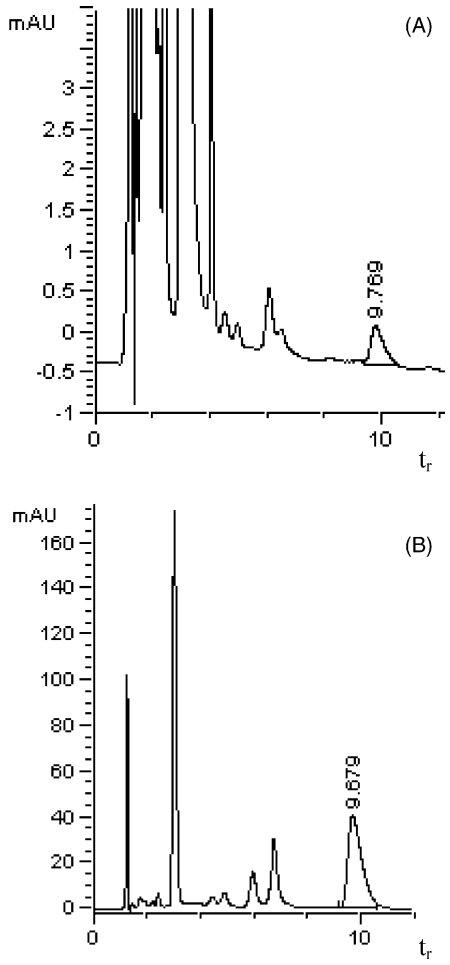

Fig. 5. HPLC–UV chromatograms of potato crisps stored for 3 months: (A) darkness and (B) lightness.

masses (*m*/*z*) were 182, 279. The first corresponded to the fragment $[M–(N{=}CH–(CH_2)_4–CH_3)]^-$ and the second to the ionization form $[M - H]^-$ of the molecule.

3.2. Performance of analytical methods

Both methods were calibrated using a series of hexanal standards of known concentration. Calibration lines were constructed using five concentrations of standard, so that concentration in the sample was at the middle of the range. The equation obtained was $y = 159.95 \times -18.658$, the determination coefficient and the linearity range were 0.999 and 0.11–11.00 μg/ml respectively, for HPLC–UV method. For the GC–MS method, parameters of linearity were the follow: -3×10^6 (interception); 54 609 (slope); 0.9894 (r^2) and 10–2000 μg/L (range).

The detection limit, estimated in accordance with ACS guidelines (defined as signal three times the height of the noise level) [19] was 9 ng/ml for HPLC–UV, whereas for GC–MS was 1 ng/ml. Yasuhara and Shibarnoto [20] has reported similar detection limits using a gas chromatographic method for quantitative of volatile aldehydes in

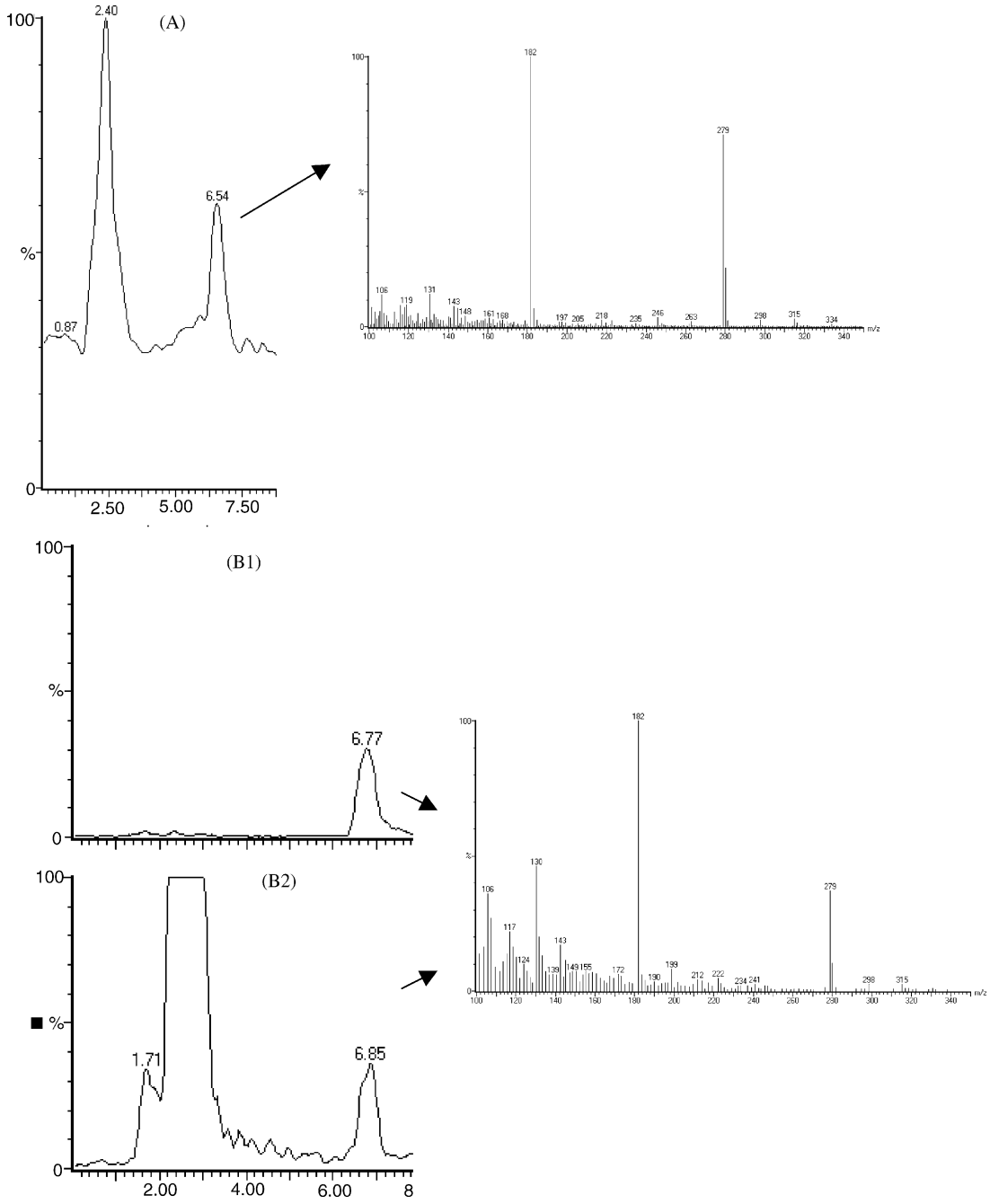

Fig. 6. LC chromatograms with MS detection: (A) full scan mode for a hexanal standard solution and full scan mass spectra and (B) SIR mode for the *m*/*z* 279 (1), 182 (2) and full scan mass spectra of correspondent potato crisps sample.

fish flesh. Repeatibility, was estimated as RSD for the determination of six extracts (each prepared separately from the same homogenised sample). The result obtained for the HPLC method was 2.40% and for the GC method it was 7.56%.

Due to the best linearity and repeatibility of the HPLC method the assays of recovery were carried out just for the liquid chromatographic method. Recovery achieved after spiking samples, with about the same amount of hexanal present in oxidised samples, was 102 ± 10%.

3.3. Hexanal profile and content during potato crisps storage

The GC–MS method was used to evaluate the suitability of hexanal as indicator of the lipid oxidation process. During a month (at 1, 4, 8, 15 and 30 days) analysis were performed in order to study the evolution of hexanal in both storage conditions.

Results (see Fig. 7) showed that in potato crisps stored in the darkness there was no hexanal change, disregarding

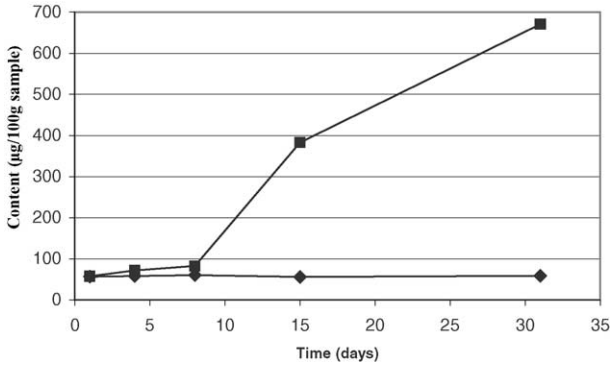

Fig. 7. Hexanal content during a 1-month storage (SPME–GC).

minor fluctuations, while in potato crisps stored in presence of natural light there was a relevant increase, specially after the eighth day. Grosso and Resurreccion [21] has also reported a similar hexanal evolution during a 110 days storage of peanuts.

On the other hand, an HPLC–UV method was developed in order to quantify hexanal, in samples stored at lightness and darkness. Regarding the results obtained with the GC method, which indicate there is no significative change when crisps are stored in the darkness, analysis were performed at day 1 and day 90 (just before validity expiration). The hexanal content has not changed significantly (Fig. 8), although a slight increase have been observed (from 20.05 to 21.40 μg/100 g sample).

With respect to samples stored in presence of light, analysis were performed at days 1, 10, and 31, once it was expected an increase similar to the one found with the GC method. Indeed, with the HPLC method, it was also observed an amazing increased from 23.20 μg/100 g sample at day 1 to 689.03 μg/100 g sample at day 10 and 949.25 μg/100 g at

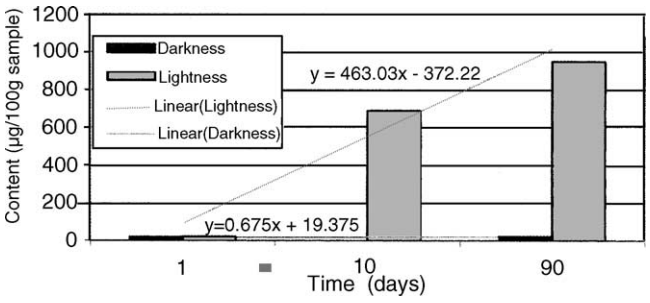

Fig. 8. Hexanal content during a 3-month storage (derivatization–HPLC).

day 90. Therefore, although crisps were in the validity period, they were not acceptable for consumer because they presented a rancid odour and taste.

4. Conclusions

The SPME method optimised in the present paper, is suitable for the identification and quantification of hexanal once presents a low detection limit. For the quantitative determination of hexanal in potato crisps, HPLC method represents a good alternative due to display an excellent repeatability and linearity.

In brief, our results indicated that hexanal is a good indicator of the lipid oxidation state of potato crisps because increases during storage and indicates deterioration of the sensorial proprieties.

References

[1] F. Fenaille, P. Visani, R. Fumeaux, C. Milo, P.A. Guy, J. Agric. Food Chem. 51 (2003) 2796.
[2] C.F. Goodridge, R.M. Beaudry, J.J. Pestka, D.M. Smith, J. Agric. Food Chem. 51 (2003) 4185.
[3] J. Ruiz, J. Ventanas, R. Cava, J. Agric. Food Chem. 49 (2001) 5115.
[4] N.P. Brunton, D.A. Cronin, J. Frank, R. Monahan, Durcan, Food Chem. 68 (2000) 339.
[5] S.-Y. Lee, J.M. Krochta, J. Agric. Food Chem. 50 (2002) 2022.
[6] F.L. Martin, J.M. Ames, J. Am. Oil Chem. Soc. 78 (2001) 863.
[7] R.F. Nascimento, J.C. Marques, B.S. Lima Neto, D. De Keukeleire, D.W. Franco, J. Chromatogr. A 782 (1997) 13.
[8] M.C. Da Cunha Veloso, V.M. Da Silva, G.V. Santos, J.B. De Andrade, J. Chromatogr. Sci. 39 (2001) 173.
[9] C.M. Seppanen, A. Casallany, A. Sari, J. Am. Oil Chem. Soc. 78 (2001) 1253.
[10] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[11] J. Pino, M.P. Martí, M. Mestres, J. Pérez, O. Busto, J. Guasch, J. Chromatogr. A 954 (2002) 51.
[12] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[13] H. Kaneda, M. Takashio, T. Osawa, S. Kawakishi, S. Koshino, J. Food Sci. 61 (1996) 105.
[14] J. Zhang, L. Zhang, Z. Fana, V. Llacqua, Environ. Sci. Technol. 34 (2000) 2601.
[15] B. Reindl, H.J. Stan, J. Chromatogr. 235 (1982) 481.
[16] D. Jung, S.E. Ebeler, J. Agric. Food Chem. 51 (2003) 200.
[17] O. Pinho, I.M.P.L.V.O. Ferreira, S. Casal, J.O. Fernandes, M.B.P.P. Oliveira, M.A. Ferreira, Chromatographia 53 (2001) S-390.
[18] P. Díaz, F.J. Señoráns, G. Reglero, E. Ibañez, J. Agric. Food Chem. 50 (2002) 6468.
[19] American Chemical Society (ACS), Subcommitee of Environmental Analytical Chemistry, Anal. Chem. 52 (1980) 2242.
[20] A. Yasuhara, T. Shibamoto, J. Agric. Food Chem. 43 (1995) 94.
[21] N.R. Grosso, A.V.A. Resurreccion, J. Food Sci. 67 (2002) 1530.