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Comparison between high-performance liquid chromatography and gas chromatography methods for fatty acid identification and quantification in potato crisps

A. Sanches-Silva, A. Rodríguez-Bernaldo de Quirós, J. López-Hernández*, P. Paseiro-Losada

Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Pharmacy, University of Santiago de Compostela, 15782 Santiago de Compostela, Spain

Abstract

A reversed-phase high performance liquid chromatographic (RP-HLPC) method was compared with a gas chromatography–flame ionization detection (GC–FID) method for determining fatty acids in potato crisps. Different extraction procedures were used. Fatty acids were quantified by linear regression. Both methods presented good precision (R.S.D. $\leq$ 5.88%) and recovery ($\geq$82.31%). The precision using HPLC method was slightly better than for GC–FID method. There was good agreement between the fatty acid composition of potato crisps analysed by both methods. For most purposes the HPLC method would be better. However, when more fatty acids need to be analysed, GC is a more suitable method.
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1. Introduction

Snack food such as potato crisps are an important example of the remarkable industry and consumer demand for more stable food products with increased shelf life. This is because food products with high fat content are highly susceptible to oxidation [1].

A good starting point for evaluating this phenomenon is the analysis of the most critical factors involved in the process. Light is known to be an important factor in the oxidation mechanism as it is closely involved in the formation of free radicals [2]. This paper will evaluate the changes in fatty acid (FA) content after a 3-month storage period in the presence of light.

A considerable amount of research has been devoted to the development of methods for fatty acids analysis. In the past few decades they have advanced considerably. Conventional methods (e.g. gravimetric, spectrophotometric, volumetric, colorimetric) are gradually falling out of use as efficient methods are being introduced.

The greater complexity of food samples has prompted newer technical procedures such as supercritical fluid chromatography (SFC), chiral chromatography, silver ion chromatography; stable carbon isotope ration analysis (SCIRA), nuclear resonance spectrometry (NMR), near-infrared spectroscopy (NIR), Fourier transform infrared spectroscopy (FT-IR), FT-Raman spectroscopy and capillary electrophoresis [3,4]. Nevertheless, GC and HPLC chromatographic procedures are still the most extensively used [5].

The major advantages of HPLC over GC are lower temperatures during analysis, which reduces the risk of isomerization of double bonds, and the possibility of collecting fractions for further investigations [3,6].

HPLC analysis of fatty acids can be carried out with refractive index (RI), ultraviolet absorption (UV), fluorescence (FL), electrochemical and evaporative light-scattering (ELSD) detection.

RI detection is a universal technique, very suitable for quantitative analysis when thermostable cells and isocratic elution are used, once it is affected by temperature and flow programming. This detector is used in the IUPAC method, despite its poor sensitivity and inadequacy for analysis of complex mixtures [4,7,8].

ELSD is stable, sensitive, easy to use, does not present baseline drifts and is not affected by changes in mobile

* Corresponding author. Tel.: +34-981598450; fax: +34-981594912.
E-mail address: qnlhjul@usc.es (J. López-Hernández).

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phase composition or temperature. However, it is not common equipment in a control lab [4,7,8].

FL or UV detection offer the advantage of sensitivity. They are able to analyse at nanogram levels. Due to the lack of a chromophore in FA molecules, chromatographic derivatization step is essential when FL detection is employed. Nevertheless, with UV detection, this step can be avoided as we describe in the HPLC method developed in this paper [4,9].

Although the application of HPLC to fatty acid analysis has increased over the last decade, GC is still the most widely used technique [10]. This well-established procedure coupled with flame ionization detection (GC–FID) is very efficient and rapid when complex mixtures with broad molecular ranges are analysed [7].

The purpose of this paper was to compare a RP-HPLC–UV without derivatization step and a GC–FID method for fatty acids separation and quantification in potato crisps. Moreover, the proposed methodologies were discussed for routine use in terms of sample preparation, time analysis, drawbacks and validation parameters.

2. Experimental

2.1. Sampling

Potato crisps fried in olive oil, according to the package information, were bought from a supermarket.

GC and HPLC analysis were performed on the same day: immediately after being purchased (fresh potato crisps) and following a 3-month storage period exposed to natural light (oxidized potato crisps).

Packages were sealed under a nitrogen atmosphere after each analysis to avoid the oxidative effect of air.

2.2. Reagents and analytical standards

All reagents were of analytical quality. Methanol, ethanol, HCl, *n*-hexane, acetonitrile and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Toluene and sodium sulfate were from Sigma-Aldrich (Madrid, Spain). Ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA).

The analytical standards: oleic acid (18:1, $n = 9$); linoleic acid (18:2, $n = 9, 12$); linolenic acid (18:3, $n = 9, 12, 15$) and their methyl esters were from Sigma-Aldrich. Stock solutions for HPLC analysis were prepared in methanol, while for GC analysis were diluted in hexane. All were stored at 4 °C and remained stable for at least 2 weeks.

Methanolic HCl was prepared by slow addition of HCl to methanol (5:95 (v/v)), with constant stirring. NaOH (0.5 M) in ethanol–water (94:4 (v/v)) was obtained by dissolving the NaOH in water and then diluting with ethanol until the desired concentration was achieved.

2.3. Calibration curves

A standard mixture of the fatty acids was prepared in methanol for HPLC analysis and in hexane for GC analysis. The concentration of the standard solution for the HPLC method was 5.7 (18:1, $n = 9$), 3.2 (18:2, $n = 9, 12$), 0.116 (18:3, $n = 9, 12, 15$) mg/100 ml while for the GC analysis (expressed as mg/50 ml) was 250.0, 150.0 and 10.0 for 18:1 ($n = 9$), 18:2 ($n = 9, 12$) and 18:3 ($n = 9, 12, 15$), respectively. Both standard mixtures had the sample fatty acid profile.

Calibration curves were constructed using diluted solutions with a range concentration such that the concentration of fatty acids in the sample lay in the middle of the range. The curves consisted of a plot of peak area versus concentration.

2.4. Sample preparation

2.4.1. HPLC

Samples were prepared according to a modification of the method described by Li et al. [6].

In a 15 ml glass tube, about 0.1 g of potato crisps was added to 1 ml 0.5 M NaOH. The mixture was centrifuged (Eba 12 centrifuge, Hettich, Kirchlengern, Germany) at 5500 rpm for 5 min after vortex mixing (Autovortex SA6, Stuart Scientific, Redhill, UK) and one night at room temperature. The supernatant was transferred to another tube and 1 ml 0.6 M HCl was added. After 1 min on vortex, the solution was allowed to settle for 30 min.

Afterwards, samples were evaporated to dryness under a N_2 stream. Residue was re-dissolved with 10 ml of methanol and 20 μl of the solution were analysed by HPLC. Samples were stored at 4 °C in the dark until analysed.

2.4.2. GC

The method was similar to the one reported previously by Sukhija and Palmquist [11]. It presented a single step that combines lipid extraction and transesterification. Toluene (2 ml) and freshly prepared methanolic HCl (3 ml) was added to 0.1 g of sample.

After 2 h in a water bath (70 °C) under a nitrogen atmosphere, 5 ml of a 6% K_2CO_3 solution and 1 ml of toluene were added to the tubes and it content mixed in a vortex. Following centrifugation at 1100 rpm (5 min), the organic phase was dried with sodium sulfate and filtered by a Millipore 0.45 μm.

A 1 μl aliquot was injected into the GC. Prior to injection, the samples were maintained at 4 °C in the dark. Both sample preparation procedures were performed in a light protected laboratory with the temperature maintained under control.

2.5. Apparatus and chromatographic conditions

2.5.1. HPLC–UV

The HPLC system (Hewlett-Packard, CA, USA) equipped with a HP1100 quaternary pump, an HP1100 degassing de-

vice, a 20 μl injection loop (Rheodyne, Cotati, CA, USA), a column thermostatting system (Spectra-Physics 8792, San Jose, CA, USA), a UV HP1100 detector set at 195 nm and HP ChemStation chromatography software.

Chromatographic separation was performed with a Tracer Extrasil ODS2 column (25 cm × 0.4 cm i.d., 5 μm particle size) at 60 °C.

Mobile phases were (A) acetonitrile–water (25:75 (v/v)); (B) acetonitrile. Acetic acid (0.12%) was added to both mobile phases. Mobile phases were filtered prior to use. Gradient program started with 50% of each mobile phase and changed linearly to A–B (15:85) within 6 min, then returned to initial proportion until 20 min.

Flow rate was 1.2 ml/min until 6 min and then increased to 2.0 ml/min to allow a correct column cleaning. Fatty acid identification was carried out not only on basis of retention time but also with respect to mass spectra (acquired by LC–MS) and UV spectra (acquired by spectrophotometry).

2.5.2. LC–MS

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 identify fatty acids. Single-ion recording (SIR) was obtained every second with a scan delay time of 0.01 s. The column and mobile phase was the same as in HPLC–UV analysis (flow rate 1 ml/min). Detector operated under the following conditions: negative atmospheric pressure chemical ionization (APCI) mode; probe temperature 500 °C; source temperature 130 °C, cone voltage −30 V, electron multiplier voltage 700 V, drying gas nitrogen at 425 l/h, APCI sheet gas nitrogen at 175 l/h.

2.5.3. UV-Vis spectrometry

A UV scan from 190 to 400 nm was performed with a Cary 3E UV-Vis double-beam spectrophotometer fitted. The software Cary Win UV was used for data acquisition.

2.5.4. GC–FID

A Fisons-8000 series gas chromatograph comprised with a flame ionization detector (FID 80) and an AS 800 autosampler (Manchester, UK) was used.

The GC system was fitted with a fused silica capillary column with polyethyleneglycol phase Supelcowax 10 (30 m × 0.32 mm i.d. × 0.25 μm film thickness) (Supelco, Madrid, Spain). A 50 cm deactivated fused silica column (0.32 mm i.d.) was used to protect the analytical column. Helium was used as carrier gas with a flow rate of 1.2 ml/min.

The injection port and detector temperatures were set at 240 and 260 °C, respectively. The column temperature was programmed as follows: 1 min at 160 °C, ramp at 3.5 °C/min to 230 °C, isothermal at 230 °C for 14 min. Injection was performed in the split mode with a split ratio of 1:30.

The software Chrom-Card for Windows (version 1.18) was used for data processing.

2.5.5. Statistical analysis

Data were statistically analysed using the SPSS software package (version 11.0). Differences between HPLC and GC and among potato crisps were determined using *t*-test for related and independent samples ($P < 0.05$).

3. Results and discussion

3.1. Sample preparation

The method developed to prepare samples for RP-HPLC analysis avoids derivatization, this way it is possible to use free fatty acids for further analysis or purposes. The first step uses 0.5 M sodium hydroxide in a solution of ethanol–water (94:4 (v/v)) as saponification reagent. Ethanol dissolves the sodium salts of fatty acids. The second step, extraction by 0.6 M HCl, separates fatty acids from salts and other water-soluble impurities, avoiding HPLC column contamination. Finally, FAs are transferred from their sodium salt to their free acid forms (dissolved in methanol) [6]. Czauderna and Kowalczyk [3] used a similar sample preparation procedure, which employed 2 M NaOH and 4 M HCl. However, this method requires subsequent derivatization with dibromacetophenone in the presence of triethylamine.

In the past, the preparation of food samples prior to GC–FID analysis consisted of two steps: lipid extraction and transesterification. Lipid extraction was usually carried out with mixtures of organic solvents like chloroform–methanol or automatically with Soxhlet or Goldfish apparatus [12]. These approaches are laborious, time consuming, expensive and require large sample amounts and large reagent volumes [13,14]. In order of overcome these main drawbacks, new methods have been developed such as “in situ” procedures [15–17]. These consist of one single step for extraction and methylation (which reduces analysis time, cost and work) as described in Section 2.

Methyl esters can be prepared with alkaline, acid or alkaline and acid catalysis as in the American Oil Chemists’ Society (AOCS) official method [4,18]. Diazomethane can also be used for free fatty acid although its short shelf life and the handling care required are a major limitation [13].

Despite advantages of alkaline catalysts (e.g. short reaction time and room temperature) an acid catalyst has been selected (methanolic HCl) since this reagent derivatizes free and linked acids with little risk of saponification [13].

Ulberth and Henninger [17] studied the influence of water in the transesterification reaction and concluded that a moisture content of 40.7% did not interfere with the process. Thus, potato crisp samples do not represent a problem.

Toluene was used as solvent because of its effectiveness and toxicity when compared to other solvents (benzene, hexane, tetrahydrofuran and chloroform) [13].

The amount of sample used is a critical factor during the extraction [14]. If the sample exceeds the capacity of the solvent, false contents may be determined. Moreover, a

sample too small in size may yield a considerable variation coefficient. In order to avoid these problems distinct quantities of sample were tested (0.1–0.5 g). 0.1 g was considered to be the most suitable amount to produce quantitative results.

3.2. Chromatographic analysis

During method development several chromatographic conditions were assayed in order to optimise the mobile phase, flow rate and column temperature.

Various mobile phases initial proportions (A, acetonitrile–water; B, acetonitrile) were tested: 100% A; A–B (75:25); A–B (50:50); 100% B. The best results were obtained starting the mobile phase gradient with A–B (50:50). Acetonitrile was used instead of methanol because several authors have reported that acetonitrile reduces retention time of unsaturated components and seems to be more effective on account of its lower viscosity [19].

Glacial acetic acid was added to both mobiles phases to suppress the ionization of fatty acids [19]. Moreover, it is easily removed by evaporation when fatty acid fractions are further used. Carboxylic group absorbance of acetic acid does not affect the chromatogram [6].

Several flow rates were tried between 0.8 and 2 ml/min. Column was thermostatted in a range of temperature from 25 to 65 °C at 10 °C intervals. The optimal conditions for achieving a good chromatographic resolution were 1.2 ml/min and 60 °C.

As been reported by Li et al. the selected wavelength for HPLC–UV was 195 nm [6]. The highest absorbance found when an UV scanning was carried out using a FA standard was 205 nm. Nevertheless, 195 nm was selected in order to minimize possible interferences.

In preliminary studies carried out in this laboratory, the GC method was optimised assaying several split ratios (1:30; 1:50 and 1:100), and the effect of a pre-column in the peaks resolution was also evaluated.

With respect to peaks identification, in the GC method fatty acid methyl esters (FAMEs) were identified only by comparing their retention time (t_r) with standards, whereas in the HPLC method a confirmation by LC–MS (Fig. 2) and UV spectrophotometry was necessary because different FAs may be eluted simultaneously [6].

In order to achieve the best conditions in LC–MS, several assays were performed. SIR mode was selected due to a higher sensitivity in relation to full scan mode (Fig. 1A–C). Several probe temperatures (200, 350 and 500 °C) and cone voltages (10, 30 and 60 V) were evaluated. Best response was achieved with 500 °C and 30 V. Characteristic masses (*m/z*) were 281, 279 and 277 for oleic, linoleic and linolenic acids, respectively (Fig. 1D–F). They corresponded to the ionization form $[M–H]^-$ of each molecule.

According to Baty et al. [9], one of the difficulties of FAs analysis by HPLC is the separation of FA with the same effective carbon number (like palmitoleic, linoleic and myristic acids). However, this does not affect current analysis once the three FAs analysed belong to the same carbon series. Thus, peaks were fully resolved, sharp and without shoulders.

Regarding the elution order of FAs, both methods are based on chain length and degree of unsaturation. FA retention times increase with carbon number for saturated and unsaturated FAs. Within the same series, in HPLC, FAs are eluted from the highest degree of unsaturation to the lowest (t_r (18:1, $n = 9$): 3.96 min; t_r (18:2, $n = 9, 12$): 2.87 min; t_r (18:3, $n = 9, 12, 15$): 2.25 min) and in GC, using a polar column like Supelcowax 10, FAs are eluted in the opposite order (t_r (18:1, $n = 9$): 16.91 min; t_r (18:2, $n = 9, 12$): 17.82 min; t_r (18:3, $n = 9, 12, 15$): 19.08 min) [8,20].

Regarding analysis time, sample preparation is much more time consuming in HPLC than in GC method. Neverthe-

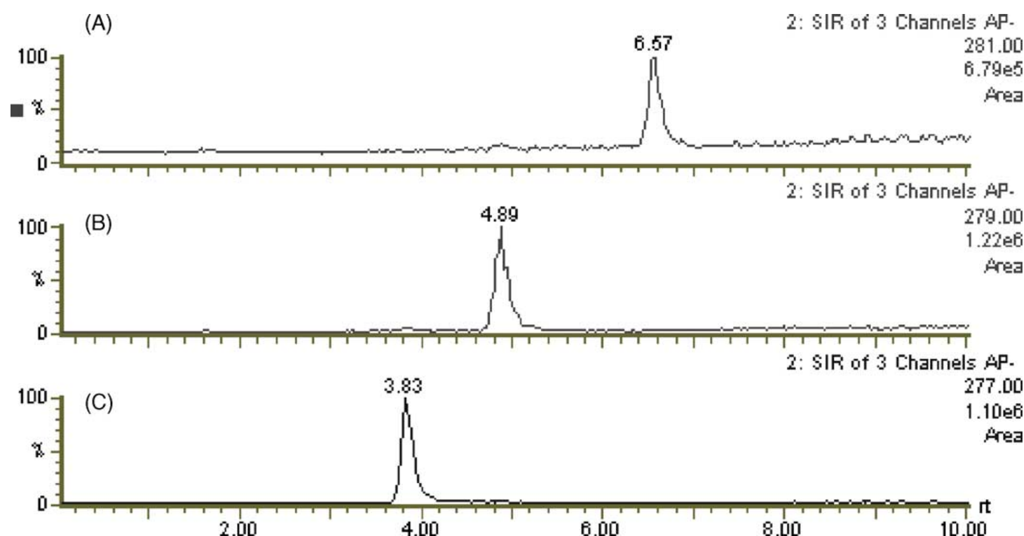

Fig. 1. LC chromatogram with MS detection (SIR mode) for the *m/z* 281 (A), 279 (B) and 277 (C). Full scan mass spectra of linolenic (D), linoleic (E) and oleic (F) acids.

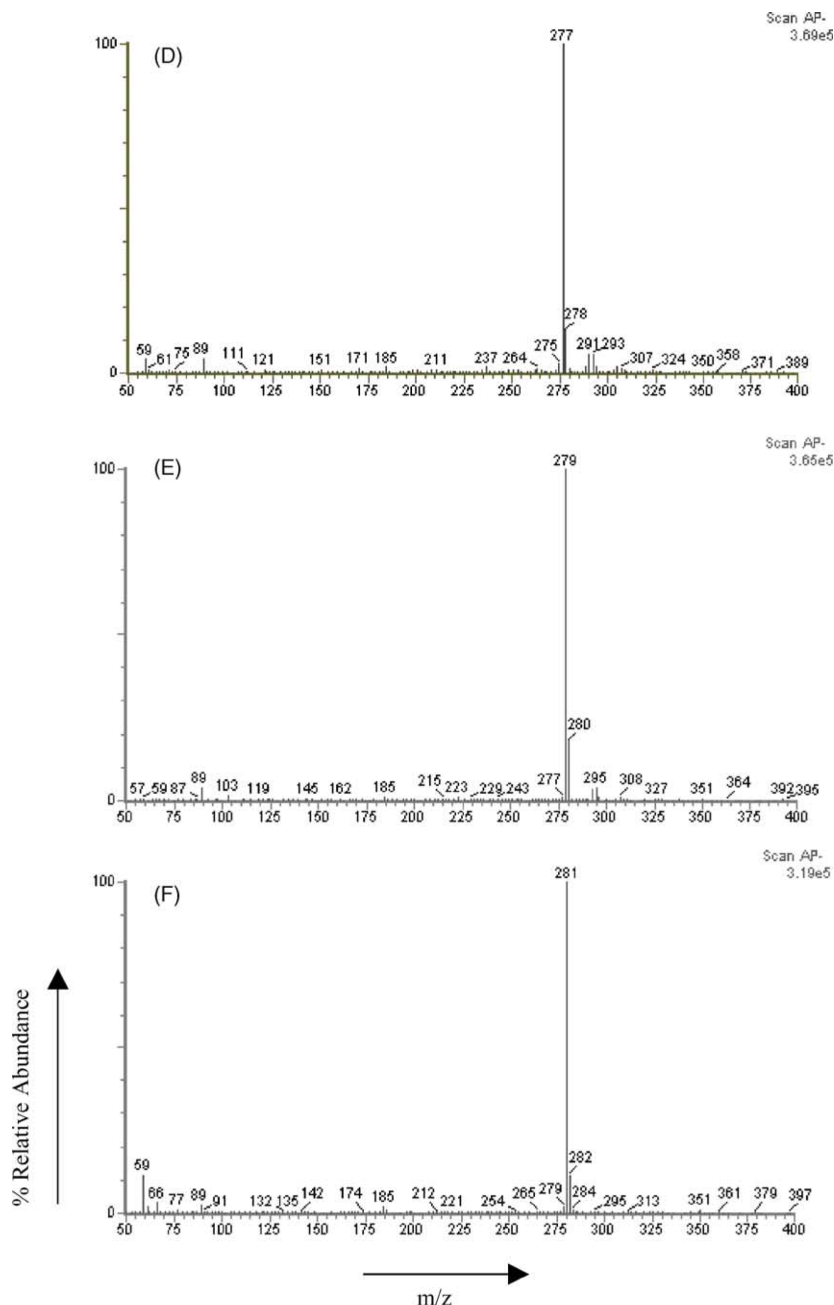

Fig. 1. (*Continued*).

less this disadvantage was largely compensated by run time (20 min in HPLC compared with 35 min in GC), precision and sensitivity, as further described, for the HPLC method.

3.3. Method validation

Calibration curves were linear over the concentration range of 0.85–259.9 μg/ml using HPLC and 2.0–5000.0 μg/ml using GC (Table 1). The calibration data for both methods also presented high correlation coefficients (≥0.9980), indicating suitability for FA quantification.

Six determinations of the same sample were performed using the same reagents and apparatus to evaluate method precision on the basis of the relative standard deviation (R.S.D.) of potato crisps.

Both methods presented good precision (Table 1). Method precision for HPLC (≤4.93%) was slightly better than for GC–FID (≤5.60%), except for 18:3 ($n = 9$, 12, 15).

The Guidelines of the American Chemical Society (ACS) [21] were used to determine detection limits (Table 1) (defined as the signal three times the height of the noise level). HPLC method (LOD ≤ 0.74 μg/ml) was more sensitive than GC method (LOD ≤ 5.00 μg/ml) for all compounds

studied. Limit detection values using HPLC method are lower than those obtained by Li et al. [6], whose work has used a similar extraction procedure. Regarding the method proposed by Czauderna and Kowalczyk [3], it presented better LODs than the HPLC method proposed here, however, on the other hand it requires a derivatization step and has a long analysis time. Recovery was tested for both methods using standard addition procedure. Six samples of potato crisps were spiked before extraction (with the same amount as the expected in the sample). Mean recoveries, listed in Table 1, were always satisfactory and higher than 82.31%.

3.4. Correlation between results obtained using both methods

Two independent groups (fresh potato crisps and potato crisps stored for 3 months) were compared with respect to the fatty acids contents obtained by HPLC and GC methods (Table 2).

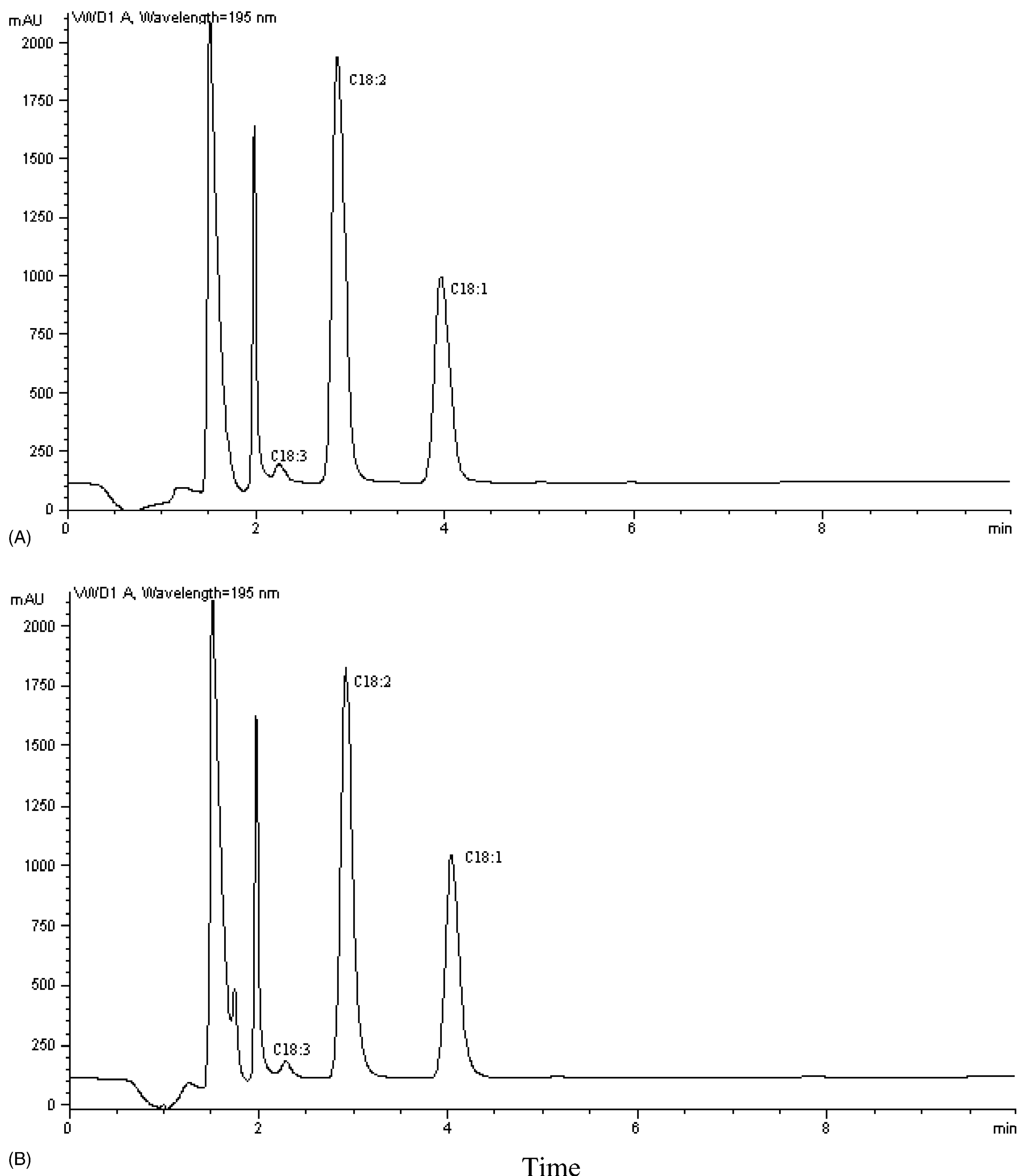

Fig. 2. HPLC chromatograms of fatty acids: (A) fresh potato crisps; (B) oxidized potato crisps.

The comparison of these two methods depends not only on the sample preparation but also on chromatographic analysis. A factor calculated as $M_{r(FA)}/M_{r(FAME)}$ was used to convert a FAME in its FA. FA contents determined by HPLC showed a remarkably good correlation with the corresponding calculated values from GC method, since results were not significantly different ($P > 0.05$).

3.5. Fatty acids content of potato crisps

According to the fatty acids profile of potato crisps (Figs. 2 and 3), 18:1 ($n = 9$) was the predominating FA detected in the potato crisps fried in olive oil. This is in agreement with the results obtained by Pantzaris [22]. Fatty acids (18:1, $n = 9$; 18:2, $n = 9$, 12; 18:3, $n = 9$, 12, 15) were chosen due to their nutritional value [23] as well as for the particular

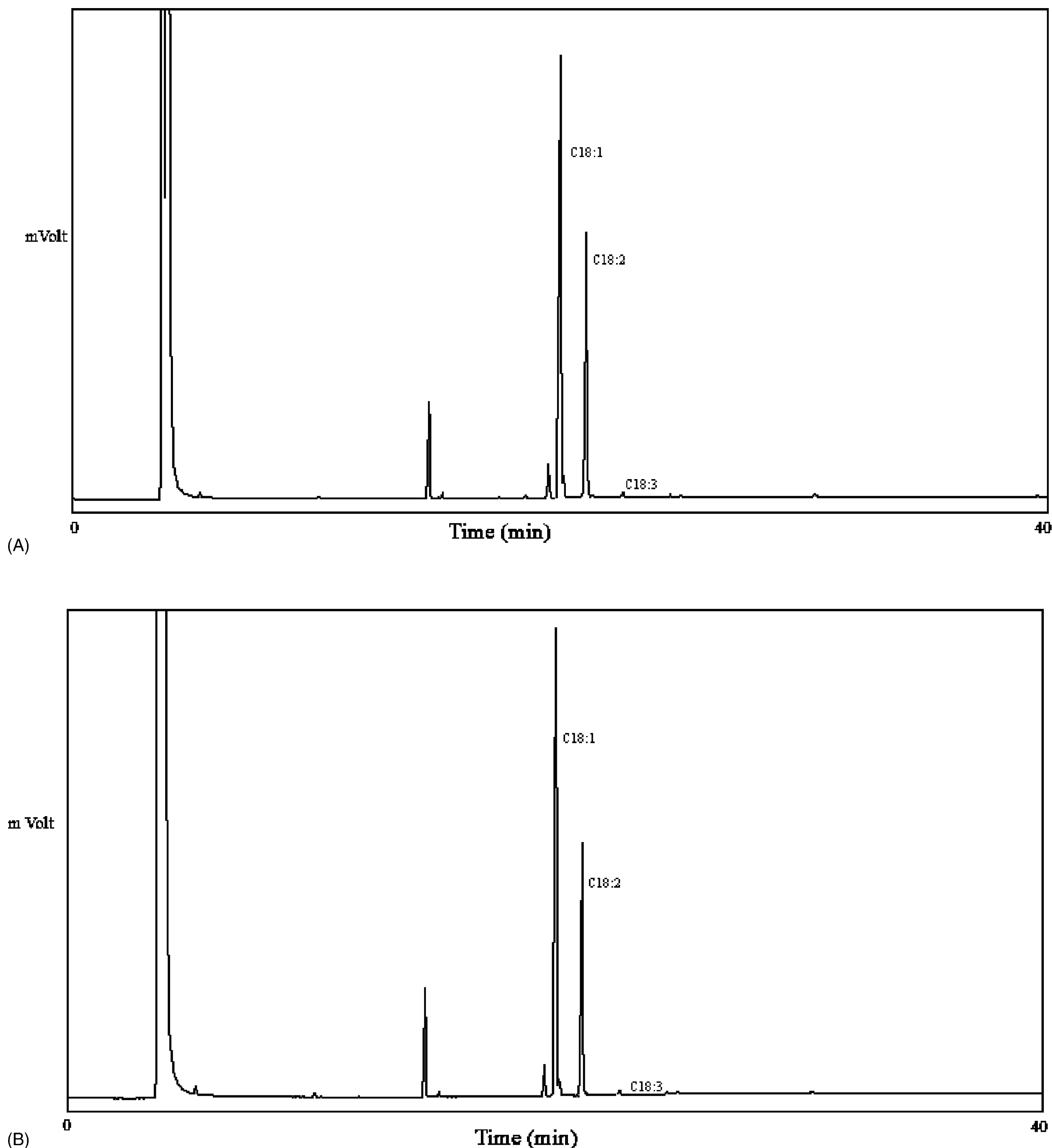

Fig. 3. GC chromatograms of fatty acids: (A) fresh potato crisps; (B) oxidized potato crisps.

Table 1
Comparison of methods validation parameters

	Parameter					
	Precision (%)	Recovery (%)	LOD (μg/ml)	Linearity	r^2	Range (μg/ml)
18:1 ($n = 9$)						
GC	5.60	82.31	5.00	$y = 1082x + 40671$	0.9980	50.0–5000.0
HPLC	4.93	108.92	0.01	$y = 14.375x + 8.526$	0.9999	1.3–259.9
18:2 ($n = 9$, 12)						
GC	5.16	83.80	3.00	$y = 1053.8x + 23920$	0.9980	30.0–3000.0
HPLC	3.87	97.09	0.23	$y = 39.767x + 8.6848$	0.9999	1.17–235.0
18:3 ($n = 9$, 12, 15)						
GC	5.58	95.30	1.00	$y = 677.4x + 381.82$	0.9999	2.0–200.0
HPLC	5.88	101.30	0.74	$y = 52.36x + 36.958$	0.9990	0.85–29.6

Table 2
Fatty acid content of potato crisps (g/100 g ± S.D.; $n = 4$) determined by HPLC and GC methods

Method	Potato crisps	18:1 ($n = 9$)	18:2 ($n = 9$, 12)	18:3 ($n = 9$, 12, 15)
GC	Fresh	10.564 ± 0.640	5.478 ± 0.323	0.163 ± 0.007
	Oxidized	5.022 ± 0.803	2.698 ± 0.405	0.079 ± 0.010
HPLC	Fresh	10.080 ± 0.591	5.353 ± 0.194	0.148 ± 0.015
	Oxidized	5.457 ± 0.248	2.820 ± 0.269	0.0783 ± 0.147

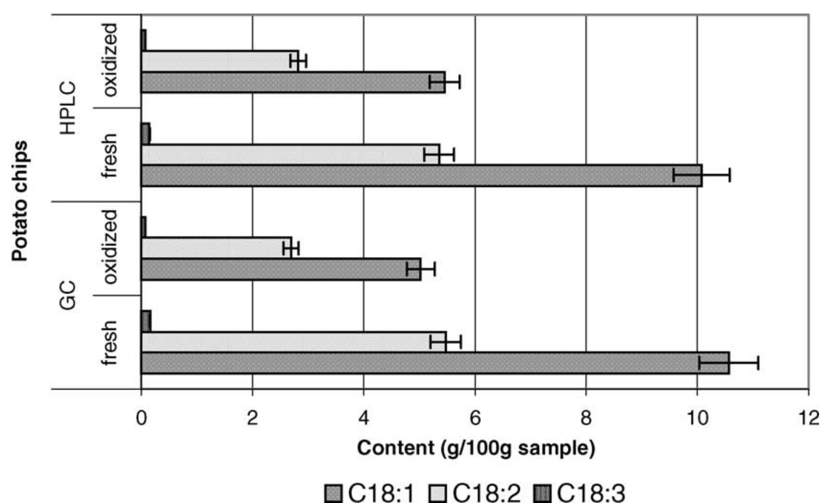

Fig. 4. Comparison of FA content (g/100 g) of fresh and oxidized potato crisps.

interest they contribute to this work. Unsaturated fatty acids are more prone to attack by free radicals [24], thus their content allows (to some extent) the evaluation of the lipid oxidation state. In fact, the content of the three FAs evaluated decreased significantly ($P < 0.05$) after a 3-month period storage in the presence of light (Fig. 4). However, the most affected was linolenic acid because it has three double bonds.

The HPLC method is sensitive and precise and may be considered as a good alternative analytical tool for the routine determination of 18:1 ($n = 9$), 18:2 ($n = 9$, 12) and 18:3 ($n = 9$, 12, 15) in potato crisps. However, when a more complete study of FA profile is required, GC is a more suitable method, because HPLC may present co-elution of peaks.

References

[1] L. Masson, C. Urra, M. Izaaurieta, J. Ortiz, P. Robert, N. Romero, E. Witting, Grasas Aceites 52 (2001) 175.
[2] J.B. Gutierrez, Ciencia Bromatologica, Diaz de Santos, Madrid, 2000, p. 300.
[3] M. Czauderna, J. Kowalczyk, J. Chromatogr. B 760 (2001) 165.
[4] R. Aparicio, R. Aparicio-Ruiz, J. Chromatogr. A 881 (2000) 93.
[5] E.W. Hammond, Trends Anal. Chem. 8 (1989) 308.
[6] Z. Li, T. Gu, B. Kelder, J.J. Kopchick, Chromatographia 54 (2001) 463.
[7] A.A. Carelli, A. Cert, J. Chromatogr. 630 (1993) 213.
[8] S.M. Pons, A.I.C. Bargalló, M.C.L. Sabater, J. Chromatogr. A 823 (1998) 475.
[9] J.D. Baty, R.G. Willis, R. Tavendale, J. Chromatogr. 353 (1986) 319.
[10] N.C. Shantha, G.E. Napolitano, J. Chromatogr. 624 (1992) 37.

[11] P.S. Sukhija, D.L. Palmquist, J. Agric. Food Chem. 36 (1988) 1202.
[12] M.J. Gonzalez-Castro, M.J. Oruña-Concha, J. Lopez-Hernandez, J. Simal-Lozano, A. Ganza-Gonzalez, Z. Lebensm. Unters. Forsch. 203 (1996) 370.
[13] A.I. Carrapiso, C. García, Lipids 55 (2000) 1167.
[14] A.I. Carrapiso, M.L. Timon, M.J. Petron, J.F. Tejeda, C. Garcia, Meat Sci. 56 (2000) 159.
[15] F. Ulberth, M. Henninger, Fat Sci. Technol. 97 (1995) 77.
[16] C. de la Cruz, J.L. Hernández, J.S. Lozano, J. Chromatogr. A 891 (2000) 367.
[17] F. Ulberth, M. Henninger, J. Am. Oil Chem. Soc. 69 (1992) 174.
[18] N.C. Shantha, E.A. Decker, B. Henning, J. AOAC Int. 76 (1993) 644–649.
[19] M.S. Rao, K. Hidajat, C.B. Ching, J. Chromatogr. Sci. 33 (1995) 9.
[20] M.S. Manku, J. Chromatogr. Sci. 21 (1983) 367.
[21] American Chemical Society (ACS), Anal. Chem. 52 (1980) 2242.
[22] T.P. Pantzaris, Grasas Aceites 49 (1998) 319.
[23] J.M. Verdú, Nutrición y Alimentación Humana, Ergon, Madrid, 2002, pp. 74–6.
[24] T. Richardson, J.W. Finley, Chemical Changes in Food During Processing, AVI Publishing Company, Westport, CT, 1985, p. 95.