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Application of high-temperature gas chromatography to the analysis of used frying fats

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RESUMEN

Aplicación de la cromatografía de gases a elevada temperatura en el análisis de aceites y grasas de fritura.

La determinación de compuestos polares es el método analítico más utilizado en el análisis de los aceites y grasas de fritura. En este estudio se aprovechan las posibilidades actuales de la cromatografía de gases a elevada temperatura que permite cuantificar los dos grupos mayoritarios de ácidos grasos polares como ésteres metílicos: los monómeros oxidados y los dímeros. Con tal fin, la fracción de compuestos polares se tranesterifica y los ésteres metílicos obtenidos se separan en una columna de VF-5ht Ultimetal, usando tricosanoato de metilo como estándar interno, en las siguientes condiciones: 150 °C durante 5 minutos, 5 °C/min hasta 370 °C y 5 minutos a 370 °C. Los resultados se comparan con los obtenidos mediante técnica alternativa más compleja basada en la cromatografía de exclusión por tamaño molecular.

PALABRAS CLAVE: Aceites de fritura – Compuestos polares – Cromatografía de gases a elevada temperatura – Dímeros – Monómeros oxidados.

SUMMARY

Application of high-temperature gas chromatography to the analysis of used frying oils.

The determination of polar compounds is the most commonly applied technique in the analysis of used frying fats and oils. High-temperature gas chromatography allows for a quantitative determination of oxidized monomeric FAME and dimeric FAME thus giving extra information on oil degradation starting from the fraction of polar compounds. Polar compounds are transesterified and methyl esters are separated in a VF-5ht Ultimetal column (150 °C -held for 5 min- rising at 5 °C min^{-1} to 370 °C and held for 5 min) using methyl tricosanoate as internal standard. Results are compared with those obtained by more complex alternative methodology using high-performance size-exclusion chromatography.

KEY-WORDS: Dimeric FAME – High-temperature gas chromatography – Oxidized monomeric FAME – Polar compounds – Used frying oils.

1. INTRODUCTION

The quality of used frying fats can be analyzed by means of a variety of analytical techniques depending on the type of frying process, food fried and laboratory facilities (Choe and Min, 2007; Navas *et al*., 2007; Orthoefer and List, 2007; Sánchez-Gimeno *et al*., 2008; Berdeaux *et al*., 2009). Polar compound determination stands out as the most commonly used methodology to evaluate frying fat alteration and has been included in the regulations of different countries to establish limits of alteration for human consumption (Dobarganes and Márquez Ruiz, 1998). Increased knowledge on fat degradation under different conditions can be achieved through the application of high-performance size-exclusion chromatography (HPSEC) to the complex mixture of compounds included in the polar compound fraction (Dobarganes *et al*., 2000). Thus, significant groups of compounds, including oxidation, polymerization and hydrolytic compounds, can be quantitated.

Methodologies based on the simplest fatty acid derivatives, the fatty acid methyl esters, permit a direct evaluation of the altered fatty acyls included in triglyceride molecules by gravimetric determination (Dobarganes *et al*., 1984). Further, polar fatty acid methyl esters can be analyzed by HPSEC to provide an overall profile of the oxidized and polymerized groups of fatty acids (Márquez-Ruiz *et al*., 1990).

High-temperature gas chromatography (HTGC) allows for the separation and analysis of molecules of low volatility and is applied routinely in the analysis of biodiesels for controlling the unreacted material (triacylglycerols, partial glycerides, fatty acids) as well as the reaction products, i.e. fatty acid methyl esters (FAME) and glycerols. The method has been adopted due to its high accuracy in quantifying minor components and also because all the compounds of interest are obtained in the same analysis (European Standard, 2003).

The possibility of using frying oils as raw material in the preparation of biodiesel has led to the need for identifying minor compounds formed at frying temperatures (Ruiz Méndez et al., 2008a; 2008b). As biodiesels are obtained by oil transesterification, the identification of any specific compound from used frying oils is present as fatty acid methyl esters (FAME).

Under the conditions of high temperature applied for separation in the standard method,

oxidized monomeric FAME and dimeric FAME have sufficient volatility to elute and, consequently, their detection is possible. In a previous paper, dimeric fatty acid methyl esters were considered the most specific group of compounds to detect the presence of used frying oil as raw material in biodiesel production (Ruiz Méndez *et al*., 2008b). However, to our knowledge, HTGC has not yet been applied for the quantitation of new compounds formed during frying.

In this paper, the application of HTGC for the determination of oxidized monomeric FAME and dimeric FAME is proposed not only as a possibility for the evaluation of the two major groups of modified fatty acids formed at frying temperatures but also as a complementary technique to be easily combined with polar compound determination, taking into account that gas chromatography is, at present, a technique available in any laboratory where the determination of polar compounds is carried out.

2. MATERIALS AND METHODS

2.1. Chemicals

Methyl tricosanoate (C23:0) and methyl 12-hydroxystearate were supplied by Nu-Check-Prep (Elysian, MN, USA). Methyl *trans*-9,10-epoxystearate and methyl 12-oxostearate were purchased from Sigma-Aldrich (Steinheim, Germany). Silica gel 60 for column chromatography (particle size 0.063-0.200 mm) and sodium were acquired from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade and obtained from local suppliers.

2.2. Samples

Pure fatty acid methyl esters

Methyl *trans*-9,10-epoxystearate, methyl 12-oxostearate and methyl 12-hydroxystearate were used as representative of oxidized monomeric FAME.

Non-polar dimeric FAME were prepared as representative of dimeric FAME. Methyl linoleate was heated at 220 °C for six days under nitrogen atmosphere to avoid the formation of oxygenated compounds. The purification of dimers was carried out in a silica column by eliminating the unreacted methyl linoleate eluting with hexane: diethylether (95:5, v/v) (Dobarganes *et al*., 1984). A second fraction eluted with hexane: diethylether (88:12, v/v) comprises non-polar FAME dimers.

A solution of methyl tricosanoate in *tert*-butyl methyl ether (1 mg/mL) was used as internal standard.

Used frying oils

Ten samples of used frying oils discarded from restaurants were used. Also, refined olive oil was heated at 180 °C in the absence of foods for 25 and 40 h following the standard procedure developed for oil termoxidation (Barrera Arellano *et al*., 1997).

2.3. Analytical Procedures

Determination of polar compounds.

Total polar compounds were determined in oil samples by silica column chromatography following the method proposed by the IUPAC (IUPAC, 1987).

Quantitation of polar FAME and their distribution in oligomeric (i.e. trimeric and higher oligomeric), dimeric and oxidized monomeric FAME by high-performance size-exclusion chromatography.

FAME were obtained by transesterification of oil samples with sodium methoxide and hydrochloric acid-methanol and subsequent recovery of methyl esters (Dobarganes *et al*., 1984). Methyl esters were separated by silica column chromatography using hexane/diethyl ether (95:5) to elute a non-polar fraction and diethyl ether to obtain the polar fraction. Quantitation of non-polar FAME was based on the gravimetric determination of the non-polar fraction. Oxidized monomeric FAME, dimeric and oligomeric FAME were likewise quantified in the polar fraction by HPSEC. The separation was performed on two 100 and 500 Å Ultrastyragel columns (25 cm $\times$ 0.77 cm i.d.) packed with porous, highly cross linked styrene-divinylbenzene copolymers (film thickness: 10 µm) (Hewlett-Packard, Avondale, PA, USA) connected in series, with tetrahydrofuran (1 mL/min) as the mobile phase. The methodology was described in detail, including calibration and reproducibility data, in an earlier publication (Márquez-Ruiz *et al*. 1990).

Quantitation of dimeric and oxidized FAME monomers by high-temperature gas chromatography (HTGC) starting from polar compounds

Transesterification of polar compounds. FAME were obtained by transmethylation with sodium methoxide at room temperature according to Berdeaux *et al* (1999). A 100 mg oil sample of polar compounds obtained by the standard method stated above (IUPAC. 1987) was accurately weighed into a screw-capped centrifuge tube (13 cm $\times$ 10 mm I.D.) and a volume of 1 mL of *tert*-butyl methyl ether (TBME) containing 1 µg/mL of C23:0 used as internal standard was added. Then, 0.5 mL of 0.2 M NaOMe solution in methanol was added, the tube was closed, shaken for 1 min. and allowed to stand at room temperature for 2 min. For neutralization purposes, 0.1 mL of 0.5 M H_2SO_4 was added and the mixture was shaken for a few seconds. Finally, 1 mL of water was added, shaken for 10 s and centrifuged at 5000 rpm for 1 min. One µL of the organic layer was injected into the chromatographic system.

High-temperature gas chromatography. FAME were analyzed by gas-liquid chromatography using an HP 6890 Series chromatograph (Hewlett-Packard. Avondale. PA. USA) equipped with a split-splitless injector operating in the split mode with a 40:1 split ratio at 350 °C. A VF-5ht Ultimetal (15 m × 0.25 mm, 01¦ µm) (Varian Inc., Middleburg, The Netherland) and a flame ionization detector at 360 °C were used. The analyses were run using hydrogen as carrier gas (1mL/min) and with the following oven temperatures: 150 °C (held for 5 min) rising at 5 °C min^{-1} to 370 °C (held for 5 min).

3. RESULTS AND DISCUSSION

Table 1 summarizes the polar compounds and the composition of polar FAME in the used frying oils selected when analyzed by the combination of adsorption and size-exclusion chromatography. As can be observed, polar compounds ranged from 14.5 to 39.2% representing most of the used frying oils in fast food outlets. This range in polar compounds corresponds to levels of polar FAME between 5.5 and 15.1% and most of them were oxidized monomeric and dimeric FAME.

Under the selected HTGC conditions, Figure 1 shows the profiles obtained after the transesterification of polar compounds (A) and for polar FAME (B). It is clear that the elimination of non-polar FAME, shown in profile B, is not essential as a significant response for the two groups of compounds of interest, i.e. oxidized monomer and dimers, was also obtained when starting from polar compounds (A). Between both peaks, at around 26 min, the group of sterols eluting in the polar fraction is also observed.

The interest in the determination starting from the polar compounds is three-fold. First, polar compound determination is carried out in many laboratories; thereby the polar fractions obtained and containing all the modified fatty acyl groups, are ready for any other useful determination. Second, gas chromatography is available in any laboratory and the technique can be applied at a low cost, avoiding the need for an HPLC system and the expensive size-exclusion columns necessary to achieve the results given in Table 1. Third, in the analysis by HPSEC equal response factors are assumed for the different groups of compounds due to the difficulties in finding an adequate internal standard. However, in the gas-chromatographic analysis, an internal standard can be used to improve quantitative results. In this respect, methyl tricosanoate (C23:0) was selected.

Figure 2 shows the procedure proposed for the quantification of oxidized monomeric and dimeric FAME. For the calculation of the response

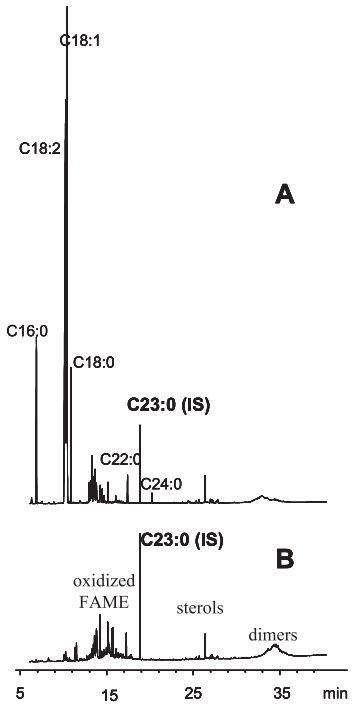

Figure 1
Significant part of the chromatograms corresponding to (A) FAME obtained after transesterification of polar compounds and (B) polar FAME.

Table 1
Quantitative determination of total polar fatty acid methyl esters and their distribution in used frying oils by adsorption and high-performance size-exclusion chromatography

Sample	Polar compounds (%)	Non-polar FAME (%)	Polar FAME and their distribution (%)			
			Total	Oligomeric FAME	Dimeric FAME	Oxidized monomeric FAME
1	14.5	94.5	5.5	0.7	2.0	2.9
2	17.4	93.2	6.8	0.9	3.3	2.6
3	22.8	91.3	8.7	1.2	4.0	3.6
4	23.0	91.0	9.0	0.6	5.1	3.3
5	25.5	89.6	10.4	1.1	5.9	3.4
6	25.7	89.5	10.5	1.3	5.7	3.5
7	27.5	89.3	10.7	1.1	6.2	3.4
8	27.6	90.3	9.7	0.5	5.5	3.8
9	33.1	85.1	14.9	3.5	7.4	4.0
10	39.2	84.9	15.1	2.9	8.4	3.8

factors relative to the C23:0, solutions of the internal standard with an increasing concentration of oxidized monomeric FAME, i.e. epoxy, keto and hydroxy FAME**,** and pure non-polar dimeric FAME were used. For oxidized fatty acids, the response factor found was between 0.82 and 0.87 and for non polar dimers between 0.63 and 0.70. According to these values, the correction factors applied were 1.18 and 1.53 for monomers and dimers, respectively. Figure 3 shows the chromatograms for standards of the major oxidized monomeric FAME in used frying oils (Marmesat *et al.*, 2008) and for purified non-polar dimeric FAME under the conditions applied. Oxidized monomeric FAME eluted at around 200 °C while dimers eluted later at around 300 °C. Two pure FAME, methyl heneicosanate (C21:0) and methyl tricosanoate (C23:0), were initially tested to find the adequate internal standard (see Figure 1A) and C23:0 was selected because it did not overlap with any other compound in real samples. As can be observed, non polar dimeric FAME elute as a complex group of peaks due to the formation of a high number of individual compounds differing in the number of double bonds, in the position of the dimeric C-C bond in the molecule, and in the distinct geometrical isomeric forms. For quantitative purposes, both groups of compounds were quantified by summing up all their corresponding individual peaks.

One additional advantage of the quantitative determination starting from polar compounds is that, even if the weight of the polar fractions vary greatly, the percentage composition of the fraction after derivatization to FAME is similar regardless of the level of polar compounds in the samples. Thus, in Table 2, starting from the percentages of polar compounds and total polar FAME, total non-polar FAME have been calculated by difference. In the last column, the ratio between non polar and polar FAME has also been calculated. As can be observed, the ratio ranges from 0.54 to 0.82 although most of the values were around 0.66 indicating, as expected, that polar triglycerides possess two non-polar and one altered acyls group per molecule. This is clearly shown in Figure 4 corresponding to the analysis of 3 samples of polar compounds from olive oil thermoxidized for 0, 25 and 40 h. As can be observed, the profile for samples corresponding to 25 and 40 hours of heating were similar in spite of the different percentage of polar compounds (around 20 and 40 % respectively). In the initial oil, the major peaks correspond to β-sitosterol and oxidized monomeric FAME while dimeric FAME were absent. As the degradation is greater, the increase in polar compounds is only reflected by the lower contribution of sterol peaks to the HTGC profiles obtained.

Table 3 summarizes the results obtained by size-exclusion chromatography and by high-temperature gas chromatography for the 10 samples selected. It is worthy to note that, in spite of the high degradation of samples 9 and 10, trimeric FAME were not detected

Table 2
Relationship between non-polar and polar fatty acid methyl esters in polar compounds

Sample	Polar compounds (%)	Polar FAME (%)	Non-polar FAME (%)	Non-polar FAME /Polar FAME
1	14.5	5.5	9.0	0.61
2	17.4	6.8	10.6	0.64
3	22.8	8.7	14.1	0.62
4	23.0	9.0	14.0	0.64
5	25.5	10.4	15.1	0.69
6	25.7	10.5	15.2	0.69
7	27.5	10.7	16.8	0.64
8	27.6	9.7	17.9	0.54
9	33.1	14.9	18.2	0.82
10	39.2	15.1	24.1	0.63

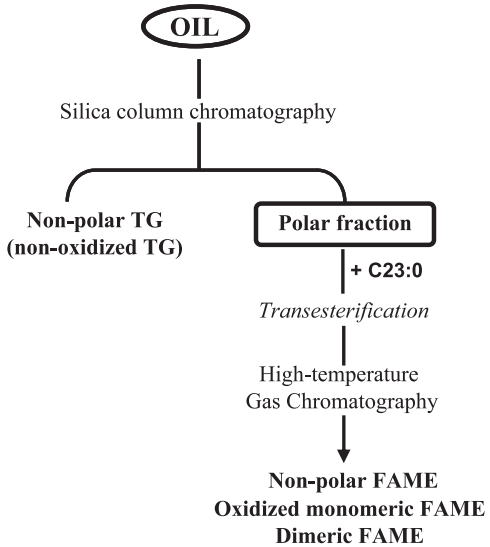

Figure 2
Combination of adsorption chromatography and high-temperature gas chromatography for the analysis of used frying fats.

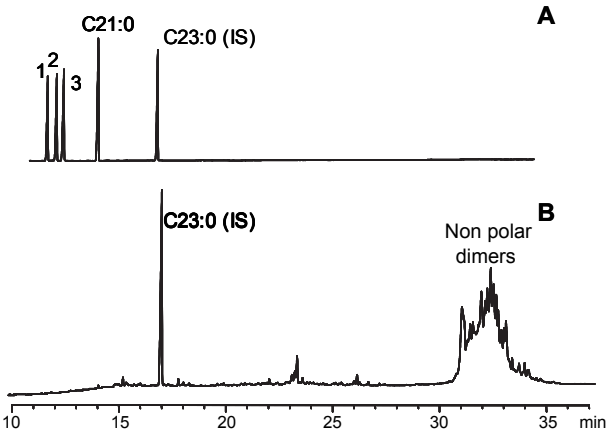

Figure 3.
Significant part of the chromatogram corresponding to (A) pure oxidized monomeric FAME and (B) isolated non-polar dimeric FAME. 1, methyl 9,10 epoxystearate; 2, methyl 10-oxostearate; 3, methyl 10-hydroxystearate.

Table 3
Comparison of the results obtained for dimeric fatty acid methyl esters and oxidized monomeric fatty acid methyl esters by size-exclusion chromatography and high-temperature gas chromatography

	Size-exclusion chromatography (%)		High-temperature gas chromatography (%)	
Sample	Dimeric FAME	Oxidized monomeric FAME	Dimeric FAME	Oxidized monomeric FAME
1	2.0	2.9	2.2	3.3
2	3.3	2.6	3.0	2.9
3	4.0	3.6	4.2	4.2
4	5.1	3.3	5.1	3.8
5	5.9	3.4	5.4	4.0
6	5.7	3.5	5.0	4.3
7	6.2	3.4	5.6	4.1
8	5.5	3.8	5.0	4.9
9	7.4	4.0	6.4	5.0
10	8.4	3.8	7.7	4.7

by HTGC under the conditions used, although they could be quantified as oligomeric FAME (trimeric and higher oligomeric FAME) by size-exclusion chromatography (Table 1). This is probably due to two concurrent reasons. On one hand, the exponential increase of individual compounds as compared to dimeric FAME which in turn would elute in a very wide zone of the chromatogram due to their different volatility and, on the other hand, to their low concentration as compared to dimeric FAME.

In general, the results obtained by HTGC were higher for oxidized monomeric FAME and lower for dimeric FAME. However, as can be observed in Figure 5, a high correlation between both sets of data was found for both groups of compounds. As mentioned above, response factors were assumed equal for both groups of compounds in the analysis by size-exclusion chromatography. In this study, the internal standard applied in HTGC for quantitative purposes is expected to have contributed to more exact results.

To sum up, the results presented here show that complementary information to that obtained from polar compounds can be easily and rapidly obtained by quantifying the major groups of altered fatty acids formed during frying due to the action of temperature and oxygen.

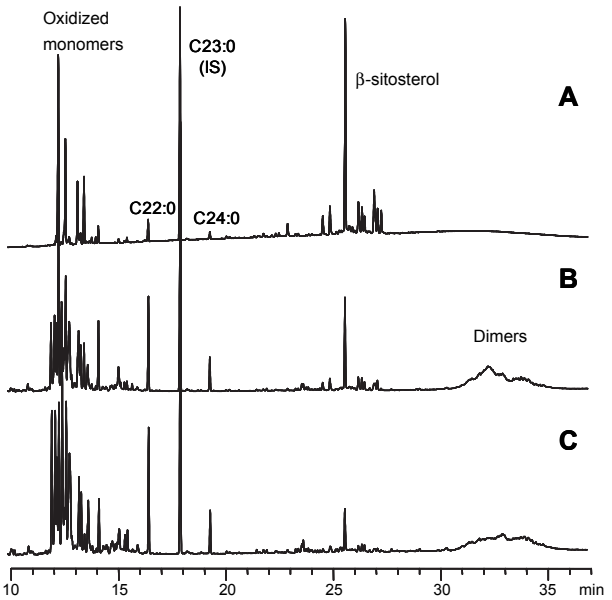

Figure 4
Significant part of the chromatograms corresponding to FAME from polar compounds: (A) initial sample; (B) sample with 20% polar compounds; (C) sample with 40 % polar compounds.

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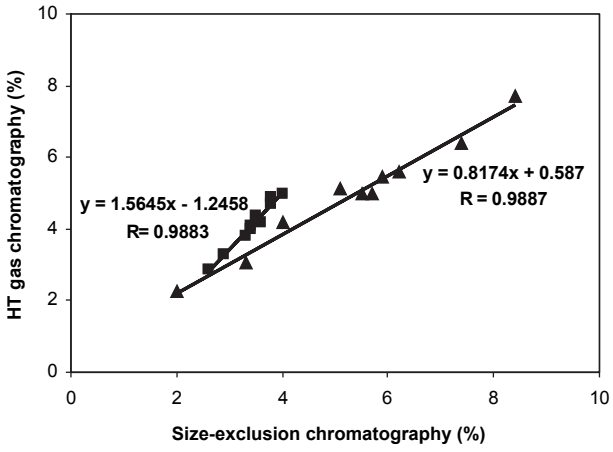

Figure 5
Relationships between the measurements obtained by high-performance size-exclusion chromatography and high-temperature gas chromatography.for oxidized monomeric FAME (■) and for dimeric FAME (▲).

REFERENCES

Barrera-Arellano D, Márquez-Ruiz G, Dobarganes MC. 1997. A simple procedure to evaluate the performance of fats and oils at frying temperatures *Grasas y Aceites* **48**, 231-235.

Berdeaux O, Márquez-Ruiz G and Dobarganes MC. 1999. Selection of methylation procedures for quantitation of short-chain glycerol-bound compounds formed during thermoxidation. *J Chromatog A* **863,** 171-181.

Berdeaux O, Dutta PC, Dobarganes MC, Sébédio JL. 2009**.** Analytical methods for quantification of modified fatty acids and sterols formed as a result of processing. *Food Anal Methods* **2,** 30-40.

Choe E, Min, DB. 2007 Chemistry of deep-fat frying oils: *J Food Sci.* **72**, R77-R86.

Dobarganes MC, Márquez Ruiz G. 1998**.** Regulation of used frying fats and validity of quick tests for discarding the fats. *Grasas Aceites***, 49**, 331-335**.**

Dobarganes MC, Pérez-Camino MC, Gutiérrez González-Quijano R. 1984. Métodos analíticos de aplicación en grasas calentadas. I. Determinación de ésteres metílicos no alterados. *Grasas y Aceites* **35**, 172-177.

Dobarganes MC, Velasco J, Dieffenbacher A. 2000. The determination of polar compounds, polymerised triacylglycerols, oxidized triacylglycerols and diacylglycerols in fats and oils. *Pure Appl. Chem.* **72,** 1563-1575.

European Standard EN14105. 2003. Fat and oil derivatives - Fatty Acid Methyl Esters (FAME) - Determination of free and total glycerol and mono-, di-, triglyceride contents. European Committee for Standardization, Brussels, Belgium.

IUPAC. 1987. Standard methods for the analysis of oils, fats and derivatives. Standard Method 2.507-International Union of Pure and Applied Chemistry. Pergamon Press, Oxford

Marmesat S, Velasco J, Dobarganes MC. 2008. Quantitative determination of epoxyacids, ketoacids and hydroxyacids formed in fats and oils at frying temperatures**.** *J Chromatog. A* **1211,** 129-134.

Márquez Ruiz G, Pérez-Camino MC, Dobarganes MC. 1990. Combination of adsorption and size-exclusion chromatography for the determination of fatty acid monomers, dimers and polymers. *J. Chromatog.* A **514**, 37-44.

Navas JA, Tres A, Codony R, Guardiola F. 2007. Optimización de métodos analíticos para la evaluación de la calidad de grasas y aceites utilizados en el proceso de fritura en continuo. *Grasas y Aceites* **58**, 148-153.

Orthoefer FT, List GR. 2007. Evaluation of used frying oil. In *Deep frying: chemistry, nutrition and practical applications (2nd edition),* ed. M.D. Erickson, 329-342. Champaign, IL: AOCS Press.

Ruiz Méndez MV, Marmesat S, Liotta A, Dobarganes MC. 2008a**.** Analysis of used frying fats for biodiesel production. *Grasas y Aceites* **59,** 45-50**.**

Ruiz-Méndez MV, Liotta A, Marmesat S, Dobarganes MC. 2008b. Detection of the presence of used frying oil as raw material in biodiesel production. *Grasas y Aceites **59**, 383-388.*

Sánchez-Gimeno AC, Benito M, Vercet A, Oria R. 2008. Aceite de oliva virgen extra del Somontano: evaluación de las modificaciones físico- químicas tras la fritura doméstica de patatas prefritas congeladas *Grasas y Aceites* **59**, 57-61.

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