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Food Chemistry 103 (2007) 1502–1507
Food Chemistry
www.elsevier.com/locate/foodchem

Combination of chromatographic techniques for the analysis of complex deodoriser distillates from an edible oil refining process

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Received 2 June 2006; received in revised form 30 June 2006; accepted 22 August 2006

Abstract

An analytical procedure, combining adsorption and size-exclusion chromatography, for the accurate quantification of the main groups of compounds present in complex deodoriser distillates, has been developed. Silica columns were used to quantify two fractions differing in polarity. The nonpolar fraction contains hydrocarbons, alkyl esters and triglycerides. The polar fraction includes partial glycerides, fatty acids and sterols as the main groups. Further separation of both fractions by size-exclusion chromatography, allowed separation and quantification of the main groups of compounds. Additionally, a detailed quantification of the specific compounds of interest present in the fractions could be further achieved by gas chromatography after adding adequate internal standards. The need for application of the procedure to complex deodoriser distillates is justified.

Keywords: Adsorption chromatography; Deodoriser distillates; Edible oils; Gas chromatography; Size-exclusion chromatography

1. Introduction

The most valuable by-product from edible oil refining is the distillate obtained in the deodorisation stage. In this step, undesirable substances affecting the taste and flavour of the final products are eliminated, but also fatty acids, fatty esters, fatty alcohols, triglycerides and bioactive compounds, such as tocopherols, phytosterols or squalene, are lost in significant amounts. Therefore, distillation residues are particularly suitable as starting materials for the preparation of natural compounds of high-added value (Chu, Baharin, Che Man, & Quek, 2004; Moreira & Baltanás, 2004). An accurate quantification of the main components present in such a complex mixture is of great interest as the economic value of deodoriser distillates depends on their composition (Clark & Frandsen, 1998).

Several analytical methodologies have been proposed for a rapid evaluation of the global composition of distillates. Direct analysis of the sample dissolved in hexane, using thin-layer chromatography coupled to a flame ionisation detector (TLC-FID), allows quantitation of the major groups differing in polarity (Ruiz-Méndez, Márquez-Ruiz, & Dobarganes, 1995). Most applied is the analysis by gas chromatography after sample silylation (Durant, Dumont, & Narine, 2006; Verleyen et al., 2001) but, in our experience, depending on the crude oil composition before refining and on the type of refining process applied, the distillates can be highly variable and the one-step analyses might be inaccurate, due to the overlapping of different groups of compounds, e.g., free fatty acids and alkyl fatty esters.

A general advantage of HPLC compared to GC is that derivatization is not necessary, which reduces analysis times. Recently, two HPLC methods for the analysis of lipid classes have been described. Schaefer, Kuchler, Simat, and Steinhart (2003) have successfully separated wax

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doi:10.1016/j.foodchem.2006.08.015

esters, sterol esters, fatty acid methyl esters on a diol column, although peak splitting of lipid classes was observed, mostly due to the different degrees of saturation of the fatty acyl groups in lipid molecules. The method proposed by Torres, Vázquez, Señoráns, and Reglero (2005) utilises a silica column and a gradient elution of isooctane, methyl *tert*-butyl ether and 2-propanol in different proportions. Separation of neutral lipids has been achieved in less than 9 min. These methods utilise evaporative light scattering detectors (ELSD) which have brought a major advance in the detection of lipid classes by HPLC; no lipid derivatization is required and a quantitative response is claimed. Nevertheless, GC is the most applied technique in the analysis of deodoriser distillates.

All the methods mentioned above give information on the distillate composition in a one-step analysis. However, at present, there is a special interest in the distillates from olive and pomace oil refining due to their high contents of squalene. The established analytical methods are inaccurate due both to the complexity of the distillates, characterised by high concentrations of fatty esters, and the overlapping of minor compounds with the standards added for quantitative purposes.

In this study, an analytical procedure, combining adsorption and size-exclusion chromatography, for the accurate quantification of the main groups of compounds in complex deodoriser distillates, is proposed. First, adsorption chromatography on a silica column allows quantitation of two well-defined fractions of different polarity. Analysis of both fractions, by means of high-performance size-exclusion chromatography, allows quantitation of specific groups of compounds. Also, when required, the analysis of specific compounds, by gas chromatography of both fractions, allows their quantitation in less complex samples.

2. Materials and methods

2.1. Chemicals

Triolein, squalene, ethyl pentadecanoate, pentadecanoic acid, 5-α-cholestane-3β-ol, hexamethyldisilazane, trimethylchlorosilane and pyridine, were supplied by Sigma–Aldrich Chemie Gmbh (Steinheim, Germany). Silica gel 60 for column chromatography (particle size 0.063–0.100 mm) was obtained from Merck (Darmstadt, Germany). All other chemicals and reagents were of analytical grade and were obtained from local suppliers.

2.2. Samples

Deodoriser distillates from industrial refining of olive-pomace, olive, soybean, and sunflower oils were obtained from OLEOTEJAR (Benamejí, Córdoba, Spain). All samples were stored at −20 °C prior to analysis. Before analyses, distillates were dissolved in hexane at a concentration of 0.1 g/ml.

2.3. Analysis of deodoriser distillates

2.3.1. Quantitation of nonpolar and polar fractions by adsorption chromatography

Nonpolar and polar fractions were separated from 5 ml of sample solution (0.5 g of distillates) by silica column chromatography (8 g silica-H_2O, 95:5 (w/w)). The nonpolar fraction containing triglycerides (TG), hydrocarbons (H) and alkyl esters (AE) as major constituents, was eluted with 60 ml of *n*-hexane/diethyl ether (90:10, v/v). A second fraction, comprised of fatty acids (FA) diglycerides (DG), monoglycerides (MG), as well as sterols and tocopherols was eluted with 60 ml of diethyl ether. Both fractions were determined gravimetrically. Details of the technique can be found in a previous publication, including the standardised technique (Dobarganes, Velasco, & Dieffenbacher, 2000).

Efficiency of the separation of nonpolar and polar fractions has to be checked to achieve accurate quantitative results. Thus, separation of fractions was routinely monitored by TLC, using small plates of Silica Gel 60 (5×10 cm aluminium plates, 0.25 mm thickness). Plates were deliberately overloaded, eluted with light petroleum-diethyl ether-acetic acid (80:20:1) and visualised with iodine vapour. A neat separation between the two fractions is normally found.

2.3.2. Separation by high-performance size-exclusion chromatography

Conditions applied for HPSEC of total sample and both fractions were as follows: sample solutions of 10–15 mg /ml in tetrahydrofuran were used for the analysis. A HP-1050 Automatic injector and a HP-1050 pump (Hewlett-Packard, CA, USA) with a 10 μl sample loop, and three 50, 100 and 500 Å Ultrastyragel columns (25 cm × 0.77 cm I.D.), packed with a porous, highly cross-linked styreneivinylbenzene copolymer (<10 μm) (Waters Associates, Milford, MA, USA) connected in series, and a refractive index detector (Hewlett-Packard, CA, USA) were used.

External standard calibration was used for quantification of the main compounds present in the nonpolar fraction. Triolein, squalene and ethyl pentadecanoate were used as reference materials for hydrocarbons, alkyl esters and triglycerides, respectively. Five different concentrations, covering the range from 1 to 10 mg/ml for each standard, were selected. The parameters obtained for the linear calibration curves and for the response factors referred to triolein are shown in Table 1. For the polar fraction, the major groups of compounds, namely DG, MG and FA, have similar responses and, consequently, the amount of each group of compounds, was calculated assuming equal response factors (Dobarganes et al., 2000).

2.3.3. Separation by gas chromatography

For a detailed analysis of specific compounds, nonpolar and polar fractions of deodoriser distillate can be finally analysed by means of GC. A standard solution of squalene and ethyl pentadecanoate in hexane (1 mg ml^{-1} each) was

Table 1
Linear calibration curve parameters and response factors of the main groups of compounds present in the nonpolar fraction of deodoriser distillates

$y = ax + b$	n	a	b	R^2	RF
Triolein	10	24.848	1.809	0.9998	1
Squalene	10	16.777	0.822	0.9938	0.67
Ethyl pentadecanoate	10	12.459	0.116	0.9984	0.50

x, analyte concentration (mg/ml), y, area; a, slope; b, intersection; R^2 squared correlation coefficient; n = number of data.

used for the analysis of the nonpolar fraction. For the polar fraction, a standard solution of pentadecanoic acid and 5-α-cholestane-3β-ol in hexane (1 mg ml^{-1} each) was used.

The polar fraction was analysed after silylation to increase the volatility of the components. Samples of 50 ± 0.1 mg were accurately weighed and 1 ml of internal standard solution was added. The mixture was then diluted with hexane up to a volume of 10 ml. An aliquot (1 ml) was put into screw-capped tubes, the solvent eliminated and the residue redissolved in 0.5 ml of a mixture of anhydrous pyridine, hexamethyldisilazane and trimethylchlorosilane (9:3:1, v/v/v). The tubes were placed in an oven at 70 °C for 20 min. Samples were analysed within 6 h of preparation.

Separations were performed on a Hewlett-Packard 5890 series gas chromatograph with cold on-column injection on a TBR 5 CB Low bleed/MS fused silica capillary column, 15 m × 0.25 mm, 0.1 μm (Teknokroma, Barcelona, Spain) and with flame ionisation detection. The analyses were run using hydrogen as carrier gas at a pressure of 41.3 kPa and with the following oven temperature programme: 50 °C rising at 15 °C min^{-1} to 155 °C held for 10 min, and further heating at 7 °C min^{-1} to 235 °C, held for 7 min and a final heating to 340 °C, held for 15 min. A flame ionisation detector was used at 360 °C.

2.4. Precision and accuracy

Precision was determined for separation on the silica column and in HPSEC by replicate analyses ($n = 3$) and was expressed as the relative standard deviation. Accurate results were obtained for nonpolar constituents in HPSEC by external calibration using a standard solution of triolein and applying the previously calculated response factors for hydrocarbon and alkyl ester quantitation.

3. Results and discussion

Fig. 1 summarises the procedure applied for the quantitation of the main groups of compounds present in deodoriser distillates. The first separation by silica column allowed separation and gravimetric determination of two fractions of different polarity. Further evaluation by HPSEC allowed separation and quantitation of the main groups of compounds in each fraction on the basis of differences

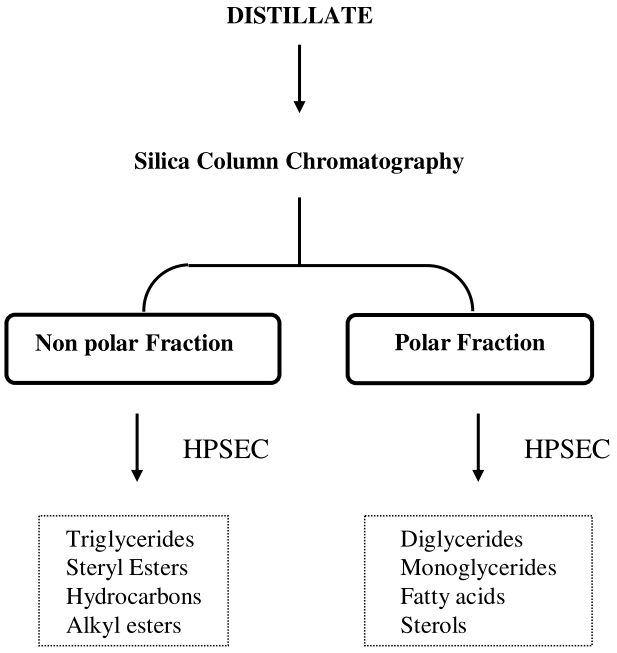

Fig. 1. Analytical scheme for the analysis of deodoriser distillates.

in molecular weight or size. In Fig. 2, the importance of the first separation is clarified. The figure shows HPSEC chromatograms for the total distillate from olive-pomace oil (A) and for the two fractions isolated by adsorption chromatography (B and C). As can be observed, in the total distillate sample, triglycerides (TG), with MW around 900, elute first, followed by a second peak, including compounds of MW around 600, namely, diglycerides (DG) and steryl esters (SE) when present in significant amounts. The third peak corresponds to monoglycerides (MG) and hydrocarbons (H), mainly squalene, and lastly, alkyl esters (AE), fatty acids (FFA) and sterols emerge as a single last peak. It is interesting to comment that the peak of TG does not overlap with any other group of compounds and, consequently, the direct analysis of the distillate by HPSEC could be highly valuable as a rapid approach to control mechanical entrainment of TG during the deodorisation step in less than 15 min.

HPSEC analysis of nonpolar and polar fractions allowed separation and quantification of groups of compounds, which co-eluted when analysed in the total sample by HPSEC. The possibilities of the analytical procedure proposed in this paper can be deduced from chromatograms B and C in Fig. 2 corresponding to those obtained after silica column separation. In the nonpolar fraction (B), triglycerides, steryl esters, hydrocarbons and alkyl esters – mainly methyl and ethyl esters – were obtained in well-defined peaks, while partial glycerides, fatty acids and sterols were eluted in the polar fraction (C). Only fatty acids and sterols co-eluted in the same peak, due to their similar MWs. Minor amounts of tocopherols can also be present in the peak corresponding to fatty acids and sterols.

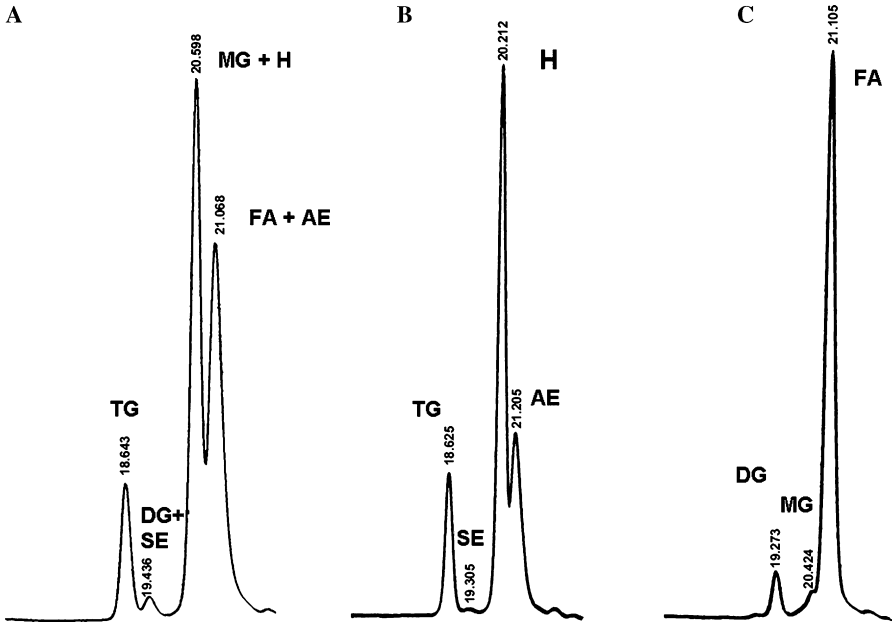

Fig. 2. HPSEC chromatograms corresponding to a total olive-pomace oil distillate (A) and to the nonpolar (B) and polar (C) fractions obtained by silica column. Abbreviations: TG, Triglycerides; DG, Diglycerides; SE, Steryl esters; H, Hydrocarbons; MG, Monoglycerides; FA, Fatty acids; AE, Alkyl esters.

However, they are more accurately quantified in the samples in less than 15 min by normal phase high-performance liquid chromatography, using fluorescence detection (IUPAC, 1992).

External calibration was applied, given the different response factors for the three main groups of compounds in the nonpolar fraction. A standard solution of triolein was used and the relative concentrations of hydrocarbons and alkyl esters in the samples were calculated from the areas obtained and from their relative response factors, given in Table 1.

The precision of the analytical procedure was studied in two samples of distillates representative of typical differences in composition. As is known, in chemical refining, fatty acids are eliminated by conventional caustic refining during the neutralisation step while, in physical refining, fatty acids are distilled in the deodorisation step. Consequently, higher temperatures are applied for physical refining and high amounts of free fatty acids are expected in the distillates obtained. Hence, the main difference in composition of distillates is established by the type of refining process applied, either chemical or physical. Table 2 shows the results obtained for two distillates from olive oil, physically refined, and from pomace olive oil, chemically refined, analysed in triplicate. As can be observed, substantial differences were found in the proportions of the two fractions separated by adsorption chromatography. The major compounds in the distillate from olive oil were the fatty acids isolated in the polar fraction. On the other hand, the major compounds in pomace olive oil distillate were the alkyl esters isolated in the nonpolar fraction. Concerning repeatability, RSD was below 4% for silica column separation while, as expected, the variation was higher for small peaks. Nevertheless, precision was excellent considering that RSD was always below 10% for groups of compounds in concentrations higher than 1% in the distillates.

Table 2
Precision of the combination of adsorption and size-exclusion chromatography for the analysis of deodoriser distillates

Sample		Nonpolar fraction (% on distillate)				Polar fraction (% on distillate)			
		Total	TG	H	AE	Total	DG	MG	FA
Olive oil (ph)[b]	Mean[a]	24.5	2.2	13.9	8.4	75.5	1.0	n.d.	74.4
	RSD (%)	1.9	7.9	4.9	6.8	0.7	14.8	–	1.3
Pomace olive oil	Mean	89.3	0.2	11.6	77.5	10.6	0.5	n.d.	10.1
	RSD (%)	0.6	21.2	4.7	1.2	3.9	10.8	–	5.0

Abbreviations: RDS (%), percentage of relative standard deviation; TG, triglycerides; H, hydrocarbons; AE, alkyl esters; DG, diglycerides; MG, monoglycerides; FA, fatty acids (the peak also includes sterols).

[a] $n = 3$.

[b] Distillate from physical refining.

Table 3
Quantitative evaluation of deodoriser distillates from different chemically refined oils by combination of adsorption and size-exclusion chromatography

Distillate	Nonpolar fraction (% on distillate)				Polar fraction (% on distillate)			
	Total	TG	H	AE	Total	DG	MG	FA
Soybean oil	53.5	31.9	5.8	15.8	45.8	9.5	3.4	32.9
Sunflower oil	44.3	27.2	4.6	10.5	55.2	6.3	n.d.	48.9
Olive oil	52.5	5.7	37.6	9.2	46.1	1.9	n.d.	43.2
Pomace olive oils								
1	89.3	0.2	11.6	77.5	10.6	0.5	n.d.	10.1
2	88.3	0.7	15.3	72.3	11.7	0.5	n.d.	11.2
3	69.6	2.3	8.3	59.0	30.3	2.2	1.2	26.9

For abbreviations see Table 2.

Table 3 shows the results obtained for distillates from different chemically refined oils and their differences in composition. As can be observed, two fractions of similar weight (total nonpolar and polar fractions) were obtained after silica column separation for soybean oil, sunflower oil and olive oil, although great differences in composition were found between them in the HPSEC analysis. In the nonpolar fraction, triglycerides arising from mechanical entrainment were the major compounds present in distillates from seed oils. However, in olive oil distillate, hydrocarbons were the major compounds, due to the high content of squalene in olive oils and its high volatility at the usual deodorisation temperatures. No significant amounts of steryl esters were found in the samples.

Pomace olive oil distillates represent a special case. The nonpolar fraction is considerably more abundant than the polar fraction, alkyl esters being the major constituents. The fermentation that occurs during olive-pomace storage in ponds results in esters of fatty acids with short chain linear alcohols (alkyl esters). They are completely lost during deodorisation, the only step in the refining process in which their elimination is foreseen (Ruiz-Méndez and Ramos-Hinojosa, 2003). In consequence, the amount of distillate obtained with this type of oil is much higher than that obtained with other chemically refined oils due to the quantitative importance of alkyl esters.

Finally, detailed quantification of the minor specific compounds of interest present in the fractions could be further achieved by gas chromatography after adding the adequate internal standards, namely, ethyl pentadecanoate, squalene, 5-α-cholestane-3β-ol and pentadecanoic acid, to quantify AE, hydrocarbons, sterols and FA, respectively. The quantification of the compounds present in deodoriser distillate by direct analysis is difficult when the constituents overlap or are present at low levels. The GC analysis of a distillate from pomace olive oil under the conditions described in the experimental part is shown in Fig. 3. As can be observed in the chromatogram corresponding to the total sample (Fig. 3a), the predominant peaks correspond to AE and squalene. Also, AE and FA overlap. After the separation of the major constituents in the nonpolar fraction (Fig. 3b), quantitation of fatty acids, sterols and alcohols in the polar fraction was very easy due to their significant concentration (Fig. 3c).

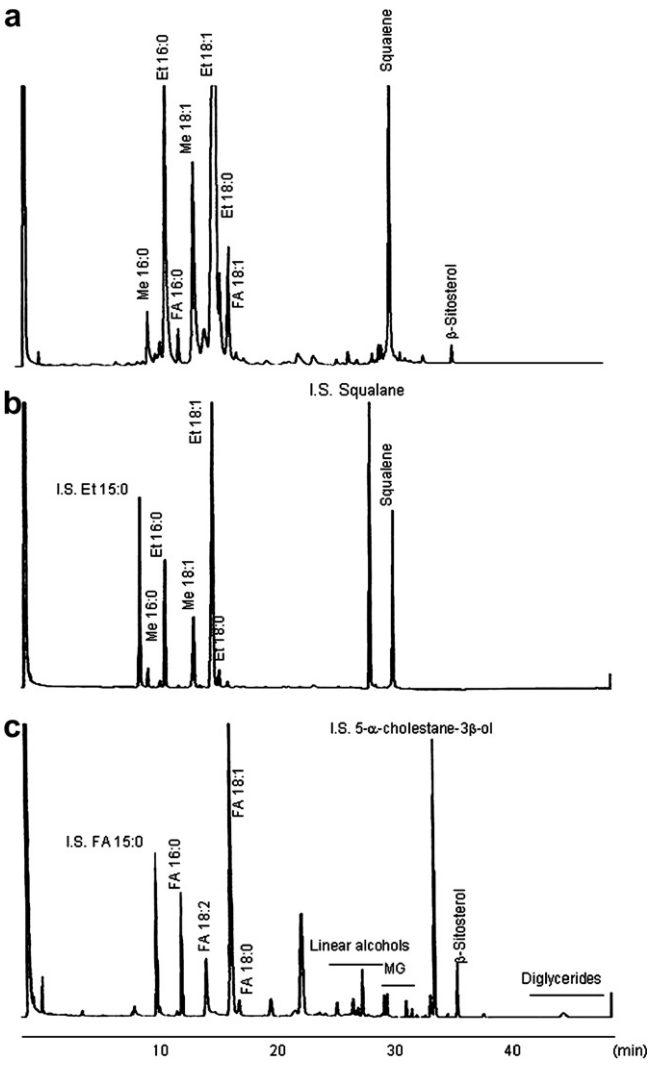

Fig. 3. Gas chromatograms corresponding to a total pomace olive oil distillate (a) and to the nonpolar (b) and polar (c) fractions obtained by silica column.

To sum up, the results obtained in this study indicate that a previous separation of deodoriser distillates into two fractions of differing polarity may be of great utility for the accurate quantitation of the groups of compounds of interest, whatever the type of refining process or the composition of the distillate.

Acknowledgements

This work was funded by Ministerio de Educación y Ciencia (Project AGL2000-0420-P4-02) and Junta de Andalucía.

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