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Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD

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Abstract

This paper describes an HPLC procedure for the determination of triacylglycerol (TAG) profile in vegetable oils. Sample preparation consisted in the dissolution of the oils in acetone and filtration. The chromatographic separation was achieved using a Kromasil 100 C_{18} column (at 25 °C) and gradient elution with acetone and acetonitrile. Elution was performed at a solvent flow rate of 1 mL/min. Detection was accomplished with an evaporative light scattering detector (ELSD), with the following settings: evaporator temperature 40 °C, air pressure 3.5 bars and photomultiplier sensitivity 6. TAG peaks were identified taking into account the logarithms of α in relation to homogeneous TAG (relative retention times to triolein) and their quantification was based on the internal normalization method.

The linearity, precision and relative response of the method were examined. A total of 15 peaks were separated, identified, and quantified based on the relative percentage peak area. After validation the methodology was applied to eight vegetable oils including olive oil in a total of 52 samples. The proposed method appears to be an adequate method for quality control and a useful tool for authenticity issues.

Keywords: HPLC/ELSD; Triacylglycerol profile; Vegetable oils; Olive oil

1. Introduction

Vegetable oils are used as salad oils, cooking oils, liquid and solid shortenings, spreads and ingredients in several foods, including bakery products and fried foods. Its prices may vary considerably from time to time, depending on production costs and availability. Occasionally can occur adulteration especially with oilseeds/raw materials more expensive. This is the case of olive oil, a product with high commercial value due to it nutritional value and association with beneficial health effects. Although some adulterations may not raise safety questions there are always, at least, related to unfair trading practices and mislabeling.

Due to the economic importance of oils and fats it is indispensable to develop methods to confirm authenticity, to detect adulteration and to define the composition of blends (Ashurt & Dennis, 1996; Carelli & Cert, 1993). Edible vegetable oils consist predominantly of triacylglycerols (TAG) that generally follow a unique and typical pattern in the glycerol molecule being characteristic in the different oilseeds. The advantage of using TAG profile comparing to fatty acid (FA) profiles is that the stereospecific distribution of FA on the glycerol molecule is genetically controlled and thus, the information of intact TAGs is usually higher (Aparicio & Aparicio-Ruíz, 2000).

Different analytical techniques have been used for studying the TAG profile of vegetable oils: thin-layer

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chromatography (TLC) (Christie, 1992), reserved-phase high-performance liquid chromatography (RP-HPLC) (Carelli & Cert, 1993; Parcerisa et al., 1995), RP-HPLC combined with silver chromatography (Ag-RP-HPLC) (Robison, Tsimidou, & Macrae, 1985) and high-temperature gas–liquid GLC (Aparicio & Aparicio-Ruíz, 2000; Carelli & Cert, 1993). RP-HPLC is the methodology most commonly employed for the separation of TAGs, because it operates on the principle of both chain length and degree of unsaturation of fatty acids, thus provided a better separation of individual TAG molecules.

The IUPAC standard method for TAG determination in vegetable oils involves an isocratic non-aqueous RP-HPLC with refractive index (RI) detection (Parcerisa et al., 1995). However, the RI is not compatible with gradient elution that is desirable for reducing retention times, for higher-molecular-mass saturated triacylglycerols and for improving chromatographic resolution (Pons, Bargalló, & Sabater, 1998). Other detector usually employed is the ultraviolet but this is incompatible with most suitable solvents (i.e. acetone) (Stolyhwo, Colin, Martin, & Guiochom, 1984). Therefore, several achievements have recently increased the interest in the evaporative light scattering detector (ELSD) (Carelli & Cert, 1993; Macher & Holmqtvist, 2001; Perona, Barrón, & Ruiz-Gutiérrez, 2001; Redden, Huang, Lin, & Horrobin, 1995; Semporé & Bézard, 1991). It seems to be an interesting alternative to the above-mentioned detectors (ultraviolet and refractive index), once no baseline drift occurs and there are no limitations on the use of mobile phase solvents. Nevertheless, quantification by ELSD may represent a problem, since the response factor is not linear at very low or high levels of TAGs (Christie, 1992).

The aim of this work was to optimize a HPLC/ELSD method for separation of TAGs in vegetable oils. Several chromatographic conditions were assayed in order to optimize the methodology: sample solubility, mobile phase, column temperature and mass detector oven temperature. The linearity, precision and relative response of the method were examined. A total of 15 peaks were separated and quantified based on the percentage peak area. After the methodology implementation and validation it was applied to the study of the TAG profiles of 8 vegetable oils (sunflower, corn, peanut, soybean, hazelnut, walnut, sesame and olive oil). The results obtained were analyzed by statistical multivariate analysis in order to achieve a better interpretation and understanding of the TAG profile of vegetable oils.

2. Experimental

2.1. Apparatus

The chromatographic analyses were performed with a Jasco (Japan) high-performance liquid chromatograph equipped with a PU-1580 quaternary pump and a 7125 Rheodyne injector (Rheodyne, Cotati, CA, USA) with a 10 μL loop. Detection was performed with an evaporative light scattering detector (ELSD) (model 75-Sedere, France). Data were analyzed using the Borwin-PDA Controller Software (JMBS, France).

2.2. Reagents and standards

Trilinolein (LLL), trimyristin (MMM), triolein (OOO), tripalmitin (PPP), tristearin (SSS), trilinolenin (LnLnLn), and tripalmitolein (PoPoPo) of purity greater than 98% were purchased from Sigma (St. Louis, MO, USA). The code letters used for the fatty acid are: Po, palmitoleic; L, linoleic; Ln, linolenic; M, myristic; O, oleic; P, palmitic; S, stearic; G, gondoic; A, arachidic. Acetonitrile and acetone were of HPLC grade and obtained from Merck, Darmstadt, Germany.

2.3. Oil samples

The samples (52) used in the work were seven vegetable oils of different origins (sunflower (10), corn (8), peanut (10), soybean (6), hazelnut (4), walnut (6) and sesame (4)), and 4 virgin olive oils. They were purchased on the local market and, until analyses, were maintained in the dark, at room temperature.

2.4. Sample preparation

Oil samples were dehydrated with anhydrous sodium sulphate and subsequently filtered through filter paper. A 0.2 g oil sample was dissolved in 4.0 mL of acetone and homogenized by stirring. The mixture was filtered through a 0.22 μm disposable LC filter disk and analyzed by HPLC under the following conditions.

All the standard solutions underwent the same treatment.

2.5. HPLC analysis

The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 μm; 250 × 4.6 mm) column from Teknokroma, (Spain) operating at ambient temperature.

The eluent used was a gradient of acetone (A) and acetonitrile (B). Elution was performed at a solvent flow rate of 1 mL/min with a linear gradient as follows: 0 min 30% B, 20 min 25% B, 35 min 20% B, keeping these conditions during 20 min and returning to the initial conditions within 3 min. The effluent was monitored with an ELSD detector, with the following settings: evaporator temperature 40 °C, air pressure 3.5 bar and photomultiplier sensitivity 6.

Taking into account the relative retention times to triolein the peaks were identified according to the

logarithms of α in relation to homogeneous TAGs (Hernández, Castellote, & Permonyer, 1991). Quantification was based on the internal normalization method, assuming that the detector response was the same for all compounds.

2.6. Statistical analysis

Statistical treatment of the data was performed using SPSS for windows version 12.0 (SPSS Inc, Chicago, IL). Descriptive analyses, one-way ANOVA, pair-wise comparison of mean values following Tukey's test and principal component analyses were used.

3. Results and discussion

3.1. Sample solubilization

Two experiments were carried out in order to optimize the sample solubilization for HPLC analysis. In the first experiment all samples (0.2 g) were eluted with a mixture of hexane and diethyl ether (87:13) through a silica gel column to remove oxidation products if present. After evaporation, the extract (ca. 0.5 mL) was dissolved in acetone (10 mL) before HPLC injection (IUPAC, 1987). In the second experiment 0.2 g of oil were dissolved in 2 mL of acetone: chloroform (1:1) and the solution was filtered by disposable filter (EC 2472/97). The second experiment was more economic, involved an easier sample preparation and revealed best results.

3.2. Optimisation of elution phase composition

The composition of the elution phase is the most important chromatographic factor affecting the separation of TAGs in RP-HPLC considering the low variability of stationary phases employed (Ruiz-Gutiérrez & Barron, 1995). The selected stationary phase require the use of an acetone–acetonitrile mixture for peak separation (Carelli & Cert, 1993; Héron & Tchapla, 1999; Hierro, Tomás, Fernández-Martín, & Santa-Maria, 1992; Pons et al., 1998; Semporé & Bézard, 1991). Different mixtures of these solvents (10:90; 20:80; 30:70; 40:60; 50:50; 60:40; 70:30; 80:20 90:10) were assayed at a flow rate of 1 mL/min. The peak separation decreased with the increased acetone content in mobile phase. The best separation was obtained with a linear gradient of acetone (A) – acetonitrile (B) as previously described (0 min 30% B, 20 min 25% B, 35 min 20% B during 20 min and returning to the initial conditions within 3 min). This gradient allowed the fall of retention times for highly saturated TAGs and, at the same time, achieved the best resolution.

3.3. Optimisation of column temperature

The ideal column temperature should ensure a good solubility of highly saturated TAGs and a good selectivity of critical pairs with the same partition number (Ruiz-Gutiérrez & Barron, 1995). In order to determine the effect of temperature, the sample and the TAG standard profiles were determined with the column at different temperatures (25, 30 and 40 °C). The best resolution for most of the TAGs was obtained at 25 °C. Higher column temperatures decreased retention times but simultaneously a decline of selectivity as described by several workers (Cárdenas, Gallego, & Valcárcel, 1999; Herslöf, Podlaha, & Toregard, 1979).

3.4. Detector temperature effects

The sensitivity of this method is affected by three instrumental parameters: detector temperature, nebulizing gas pressure and photomultiplier gain (Cárdenas et al., 1999). The effect of temperature was evaluated by injection of a known sample amount at different detector temperatures (30, 40, 50 and 60 °C). Fig. 1 presents the peak/area variation with detector temperature. The optimum temperature seems to be 40 °C. This temperature was enough to allow a complete solvent evaporation and a negligible baseline noise. The airflow rate (pressure) of the air compressor was set at 3.5 bar to reduce the baseline noise. A photomultiplier gain of 6 provided an average sensitivity for TAGs detection giving an adequate signal to noise ratio.

3.5. TAG identification

Identification of the peaks has been based in methods to predict the relative retention times (RRTs) of TAGs. They included partition number (PN) (Cárdenas et al.,

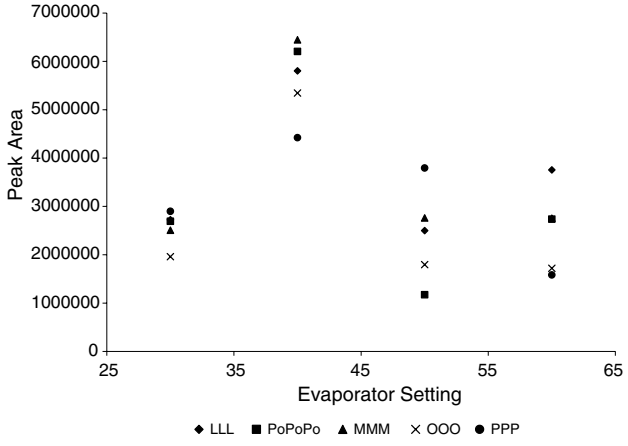

Fig. 1. Detector temperature effects in the analysis of a mixture of five TAGs: trilinolenin, (◆); tripalmitolein, (■); trimyristin, (▲); triolein, (×); tripalmitin, (●) following the experimental procedures described.

1999), equivalent carbon number (ECN) (Hierro et al., 1992), theoretical carbon number (TCN) (Héron & Tchapla, 1999) and matrix model (Herslöf et al., 1979). PN and ECN are generally used in RP-HPLC to characterize the TAGs. Their elution occur according to PN being PN = CN (carbon number)- 2ND (number of double bonds) (Stolyhwo et al., 1984). However, TAGs with the same PN can be differentiated by the ECN or by the log α. The ECN (ECN = CN + a' ND) is defined as the PN when $a' = 2$ (Hierro et al., 1992). In this study log α is calculated on the basis of the TAGs retention times relative to triolein.

In order to obtain reliable PNs for peak identification, homologous series of TAGs were injected and estimated the positional distribution for the different TAGs relative to OOO. The chromatographic profile of soybean oil is shown in Fig. 2.

3.6. TAG quantification

Several authors have established that the ELSD response is linear for a wide range of concentrations (Pons et al., 1998; Stolyhwo et al., 1984). However, some workers have demonstrated that ELSD provides a non-linear response, resulting from several light scattering mechanisms and particle size distribution (Hierro et al., 1992). The non-linear ELSD response is described by the widely used empirical model ($y = a/m^b$) where m represents the amount injected, a and b are numerical coefficients (Christie, 1992).

The linearity of the method was determined by the analysis of increasing amounts of aqueous standard solutions of six TAGs. Power relationships were detected in all instances with following coefficients of determination: 0.996 – LnLnLn, MMM and LLL; 0.994 – PoPoPo; 0.995 – PPP and 0.998 – OOO, in the range studied (3–200 μg).

Relative response factors (RRFs) calculated for pure homogeneous TAG standards in relation to triolein (OOO) are shown in Table 1. The response factor values close to unit allowed the quantification on the basis of percentage peak area.

Considering that in HPLC methods the limit of quantification typically requires peak heights, at least, 10 times higher than the baseline noise, the values obtained for homologous standard TAGs were: 0.15 μg/10 μL for OOO; 0.62 μg/10 μL for MMM, PoPoPo and PPP; and 1.2 μg/10μL for LLL.

Table 1
Relative response factors of standard triacylglycerols (TAGs)

TAGs	Response factor values[a]	Standard deviation	Coefficient of variation
LLL	1.086	0.76	0.49
PoPoPo	1.161	0.56	0.49
MMM	1.206	0.94	0.74
OOO	1.000	1.00	1.00
PPP	0.827	0.30	0.38

[a] Response factor values are given in relation to triolein (OOO).

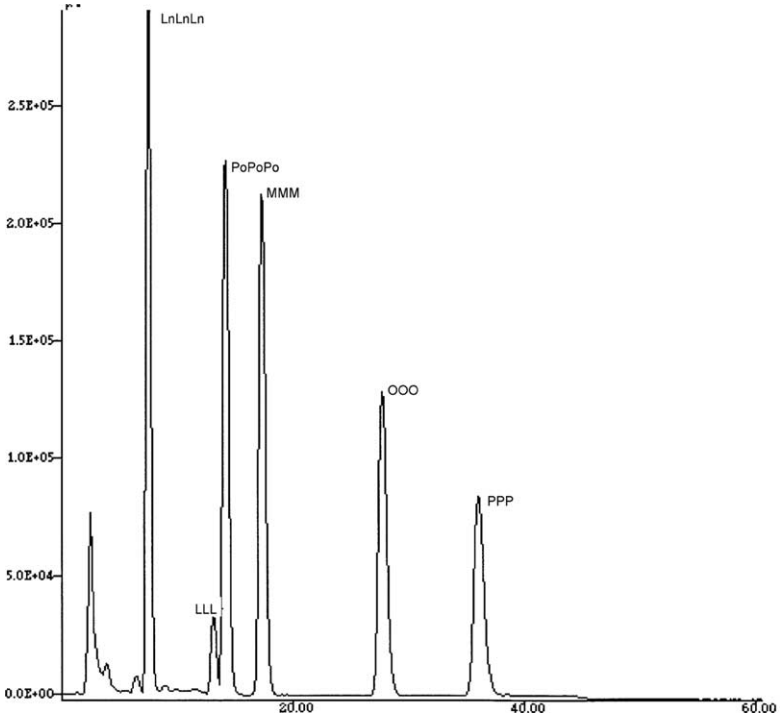

Fig. 2. TAG profile of a soybean oil sample.

The precision of the analytical method was evaluated by measuring the TAGs peak area of the same sample injected six times. In the walnut oil the standard deviation was 0.90% and 0.46%, and the coefficient of variation was 0.43% and 0.19% for trilinolein and triolein, respectively.

The mean values obtained for vegetable oils TAG profiles, together with ANOVA results are presented in Table 2. In general, the results were in good agreement with those described in literature. The trilinolein (LLL) content, in all vegetable oils, was higher than in the olive oil samples. The very low level ($\leqslant$0.5%) of LLL in olive oil is substantiated by analysis of authentic olive oils from the major Mediterranean countries. On other hand, the triolein (OOO) content in the olive oil was higher than in all vegetable oils.

The main TAGs in sunflower oil were OLL (27.77%) and LLL (19.72%). Similar contents have been reported by Carelli and Cert (1993), (28.57% to OLL and 20.06% to LLL).

TAGs profile data obtained for corn oil was very similar to reported by Ashurt and Dennis (1996).

OOO and OOL are the major TAGs in peanut oil representing 26.63% and 18.27% of the total content, respectively.

In what concerns to soybean oil, the high average content of OLL (19.51%) was also observed by Phillips, Erdahl, Schmitt, and Privett (1984).

The results for walnut TAGs qualitative profile are consistent with the ones reported by Héron and Tchapla (1999) and Ayorinde, Eribo, Balana, Johnson, and Wan (1999). Considering the quantified TAGs the major peaks contain, at least, one linoleic acid molecule. These data are therefore consistent with the described by Amaral, Casal, Pereira, Seabra, and Oliveira (2003) for fatty acid composition, in which linoleic acid represented 60% of the total fatty acid content.

The sesame oil shows higher OLL and OLnL contents.

The levels of OOO and LLO in hazelnut and olive oils are higher than those described by Parcerisa et al. (1995) with 35% (OOO) and 18% (LLO) to hazelnut oil and 32% (OOO) and 15% (LLO) to olive oil. This similarity justifies the consensual difficulty in the detection of adulterated olive oil with hazelnut oil.

A categorical principal component analysis (CATPCA) was performed to simplify data from TAG profile of vegetable oils. The results have been depicted on a two dimensional plot (Fig. 3) that explained 82.24% of the total of variance. Dimension 1 (k = 6.54) explained 50.33% of the variance, with a Cronbach's Alpha of 0.92; the negative is closely related to the contents of SPO, PLO and POO, whereas its positive counterpart is mainly related to LLL, PLL and OLLn. Dimension 2 (k = 4.15) explained 31.91% of the variance in data, with a Cronbach's Alpha of 0.82; this dimension is

Table 2
TAGs composition (%) in vegetable oils, with ANOVA results

TAGs	Sunflower (n = 10)	Corn (n = 8)	Peanut (n = 10)	Soybean (n = 6)	Hazelnut (n = 4)	Walnut (n = 6)	Sesame (n = 4)	Olive (n = 4)	F	p
LLnLn	$1.65^{b} \pm 0.35$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	0.00 ± 0.00	$0.00^{a} \pm 0.00$	$3.14^{e} \pm 2.02$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	18.21	10^{-4}
LLLn	$1.43^{a} \pm 0.56$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	1.09 ± 0.33	$0.00^{a} \pm 0.00$	$17.14^{b} \pm 4.17$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	104.30	10^{-4}
LLL	$19.72^{c} \pm 1.17$	$1.20^{a} \pm 0.51$	$1.76^{a} \pm 0.28$	$11.20^{b} \pm 0.70$	$1.27^{a} \pm 0.25$	$31.67^{d} \pm .68$	$7.90^{b} \pm 1.28$	$0.03^{a} \pm 0.00$	198.32	10^{-4}
OLnL	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$1.27^{b} \pm 0.33$	$0.00^{a} \pm 0.00$	$5.93^{c} \pm 0.74$	$14.29^{d} \pm 0.33$	$0.00^{a} \pm 0.00$	1473.80	10^{-4}
OLL	$27.77^{f} \pm 1.02$	$14.57^{d} \pm 0.73$	$10.57^{c} \pm 0.73$	$19.51^{e} \pm 0.61$	$5.76^{b} \pm 0.04$	$16.99^{e} \pm 1.24$	$9.61^{c} \pm 0.86$	$0.95^{a} \pm 0.42$	671.39	10^{-4}
PLL	$9.87^{d} \pm 1.64$	$0.46^{a,b} \pm 0.41$	$2.49^{b,c} \pm 0.34$	$2.87^{c} \pm 1.03$	$0.26^{a} \pm 0.01$	$11.67^{d} \pm 2.34$	$2.11^{a,b,c} \pm 0.18$	$0.23^{a} \pm 0.11$	101.09	10^{-4}
OOL	$18.09^{c,d} \pm 1.63$	$18.19^{c,d} \pm 0.32$	$18.27^{c,d} \pm 0.75$	$15.61^{b,c} \pm 1.42$	$19.76^{d} \pm 0.51$	$6.50^{a} \pm 4.67$	$17.43^{c,d} \pm 1.07$	$11.58^{b} \pm 4.14$	25.19	10^{-4}
PLO	$12.42^{b} \pm 0.79$	$13.36^{b} \pm 3.43$	$12.02^{b} \pm 0.51$	$14.40^{b} \pm 0.83$	$4.40^{a} \pm 0.31$	$4.09^{a} \pm 0.53$	$14.89^{b} \pm 1.40$	$2.28^{a} \pm 1.24$	56.79	10^{-4}
PPL	$0.27^{a} \pm 0.37$	$0.00^{a} \pm 0.00$	$0.30^{a} \pm 0.27$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.96^{b} \pm 0.20$	$0.00^{a} \pm 0.00$	11.29	10^{-4}
OOO	$4.21^{a} \pm 0.94$	$17.09^{c} \pm 0.69$	$26.63^{d} \pm 0.95$	$11.85^{b} \pm 1.24$	$46.68^{e} \pm 0.49$	$2.65^{a} \pm 0.47$	$14.22^{b} \pm 1.52$	$60.71^{f} \pm 4.10$	1076.43	10^{-4}
POO	$4.27^{b} \pm 0.33$	$18.71^{d,e} \pm 2.16$	$16.79^{d} \pm 0.43$	$16.87^{d} \pm 0.50$	$17.07^{d} \pm 0.17$	$0.00^{a} \pm 0.00$	$14.29^{e} \pm 2.42$	$19.88^{e} \pm 0.69$	286.11	10^{-4}
POP	$0.18^{a} \pm 0.16$	$1.32^{b,c} \pm 0.53$	$1.25^{b,c} \pm 0.30$	$1.46^{b,c} \pm 0.26$	$0.00^{a} \pm 0.00$	$0.07^{a} \pm 0.11$	$1.62^{c} \pm 1.37$	$0.66^{a,b} \pm 0.06$	13.16	10^{-4}
GOO	$0.00^{a} \pm 0.00$	$5.39^{b} \pm 1.16$	$0.79^{a} \pm 0.04$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	126.48	10^{-4}
SOO	$0.10^{a} \pm 0.13$	$8.12^{d} \pm 0.92$	$6.88^{d} \pm 0.50$	$1.69^{b} \pm 0.90$	$4.81^{d} \pm 0.12$	$0.00^{a} \pm 0.00$	$2.07^{b,c} \pm 0.33$	$3.28^{c} \pm 1.64$	153.45	10^{-4}
SPO	$0.00^{a} \pm 0.00$	$0.91^{c} \pm 0.58$	$0.54^{a,b,c} \pm 0.29$	$1.57^{d} \pm 0.37$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.61^{b,c} \pm 0.05$	$0.15^{a,b} \pm 0.07$	22.47	10^{-4}
SPP	$0.00^{a} \pm 0.00$	$0.09^{a} \pm 0.06$	$1.09^{c} \pm 0.21$	$0.66^{b} \pm 0.06$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	140.721	10^{-4}
AOO	$0.00^{a} \pm 0.00$	$0.48^{b} \pm 0.29$	$0.82^{c} \pm 0.22$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$	36.77	10^{-4}

a–f Means in lines without common letters are significantly different ($p < 0.05$).

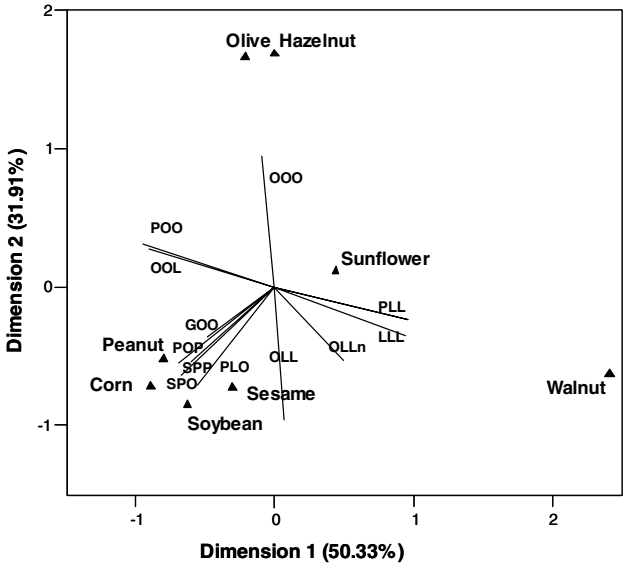

Fig. 3. Categorical principal component analysis biplot.

negatively related to OLL and positively related to OOO. As expected, vegetable oils can be easily distinguished, but olive and hazelnut oils cluster together. These oils can be differentiated from the others by its high levels of OOO. The similarity in TAG composition of olive and hazelnut oils explained the increased investigation in the field of detection of adulteration (Parcerisa et al., 1995; Christopoulou, Lazaraki, Komaitis, & Kaselimis, 2004).

Sunflower and walnut oils are discriminated from the others oils mainly to LLL and PLL contents, which also permit the differentiation between both (walnut oil presents the highest levels of these TAGs).

On the other hand, the other vegetable oils (peanut, corn, sesame and soybean) have different profiles mainly in what concerns the contents of PLO, SPO and POP.

HPLC using a solvent gradient and ELSD is an appropriate technique for the determination of TAG composition in oils. The method proposed is sensitive, rapid and precise and may be considered suitable for routine analyses.

It is also clear from these results that this parameter can be a useful tool in the identification and discrimination of vegetable oils. Furthermore, it may be an important parameter to detect the adulteration of such products during quality control.

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References

Amaral, J. S., Casal, S., Pereira, J. A., Seabra, R. M., & Oliveira, B. P. P. (2003). Determination of sterol and fatty acid compositions, oxidative stability and nutritional value of six walnut (*Juglans regia* L.) cultivars grown in Portugal. *Journal of Agriculture and Food Chemistry, 51*, 7698–7702.

Aparicio, R., & Aparicio-Ruíz, R. (2000). Authentication of vegetable oils by chromatographic techniques. *Journal of Chromatography, 881*, 93–104.

Ashurt, P. R., & Dennis, M. J. (1996). *Food authentication*. New York: Blackie academic & professional, Chap. 7 (pp. 198 258).

Ayorinde, F. O., Eribo, B. E., Balana, K. V., Johnson, J. H., & Wan, L. W. (1999). Determination of major triacylglycerol components of polyunsaturated specialty oils using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Communication and Mass Spectrometry, 13*, 937–942.

Cárdenas, S., Gallego, M., & Valcárcel, M. (1999). Evaporative light scattering detector: an new tool for screening purposes. *Analytical Chimica Acta, 402*, 1–5.

Carelli, A. A., & Cert, A. (1993). Comparative study of the determination of triacylglycerol in vegetable oils using chromatographic techniques. *Journal of Chromatography A, 630*, 213–222.

Christie, W. W. (Ed.). (1992). *In advances in lipid methodology* (pp. 239–271). Ayr, UK: The Oily Press.

Christopoulou, E., Lazaraki, M., Komaitis, M., & Kaselimis, K. (2004). Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oil with vegetable oils. *Food Chemistry, 84*, 463–474.

Hernández, B., Castellote, A. I., & Permonyer, J. J. (1991). Triglyceride analysis of cocoa beans from different geographic origins. *Journal of Food Chemistry, 41*, 269–276.

Héron, S., & Tchapla, A. (1999). Comparison of the responses of triacylglycerols with an evaporative visible light scattering detector used in conventional, micro and capillary liquid chromatography. *Journal of Chromatography A, 848*, 95–104.

Herslöf, B., Podlaha, O., & Toregard, B. (1979). HPLC of triglycerides with gradient elution and mass detection. *Journal of the American Oil Chemists Society, 56*, 864–876.

Hierro, M. T. G., Tomás, M. C., Fernández-Martín, F., & Santa-Maria, G. (1992). Determination of the triglyceride composition of avocado oil by high-performance liquid chromatography using a light-scattering detector. *Journal of Chromatography B, 607*, 329–338.

International Union of Pure and Applied Chemistry (IUPAC), in: C. Paquot, A. Hautfenne (Eds), Standard Methods for the Analysis of Oils and Derivatives, 7 th ed., Blackwell (1987) Method 2.323.Oxford.

Macher, M. B., & Holmqtvist, A. (2001). Triacylglycerol analysis of partially hydrogenated vegetable oils by silver ion HPLC. *Journal of Seperation Science, 24*, 179–185.

Parcerisa, J., Boatella, J., Codony, R., Rafecas, M., Castellote, A. I., Gracía, J., et al. (1995). Comparison of fatty acid and triacylglycerol composition of different Hazelnut varieties (*Corylus avellana* L.) cultivated in Catalonia (Spain). *Journal of Agriculture and Food Chemistry, 43*, 13–16.

Perona, J. S., Barrón, L. J. R., & Ruiz-Gutiérrez, V. (2001). Determination of rat liver triacylglycerides by gas-liquid chromatography and reversed-phase high-performance liquid chromatography. *Journal of Chromatography A, 706*, 173–180.

Phillips, F. C., Erdahl, W. L., Schmitt, J. A., & Privett, O. S. (1984). Quantitative analysis of triglyceride species of vegetable oils by high performance liquid chromatography via a flame ionization detector. *Lipids, 19*, 880–887.

Pons, S. M., Bargalló, A. I. C., & Sabater, M. C. L. (1998). Evaluation by high-performance liquid chromatography of the hydrolysis of

human milk triacylglycerides during storage at low temperatures. *Journal of Chromatography A, 823*, 475–482.

Redden, P. R., Huang, Y. S., Lin, X., & Horrobin, D. F. (1995). Separation and quantification of the triacylglycerols in evening primrose and borage oils by reversed-phase high-performance liquid chromatography. *Journal of Chromatography A, 694*, 381–389.

Robison, J. L., Tsimidou, M., & Macrae, R. (1985). Evaluation of the mass detector for quantitative detection of triglycerides and fatty acid methyl esters. *Journal of Chromatography A, 324*, 35–52.

Ruiz-Gutiérrez, V., & Barron, L. J. R. (1995). Methods for the analysis of triacylgylcerols. *Journal of Chromatography B, 671*, 133–168.

Semporé, G., & Bézard, J. (1991). Enantiomer separation by chiral-phase liquid chromatography of urethane derivatives of natural diacylglycerols previous fractionated by high-performance liquid chromatography. *Journal of Chromatography, 557*, 227–240.

Stolyhwo, A., Colin, H., Martin, M., & Guiochom, G. (1984). Study of the qualitative and quantitative properties of the light-scattering detector. *Journal of Chromatography A, 288*, 253–275.