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# Use of triacylglycerol profiles established by high performance liquid chromatography with ultraviolet–visible detection to predict the botanical origin of vegetable oils

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ABSTRACT

A method for the determination of triacylglycerols (TAGs) in vegetable oils from different botanical origins by HPLC with UV–vis detection has been developed. Using a core-shell particle packed column (C18, 2.6 μm), TAG separation was optimized in terms of mobile phase composition and column temperature. Using isocratic elution with acetonitrile/*n*-pentanol at 10 °C, excellent efficiency with good resolution between most of the TAG peak pairs, within a total analysis time of 15 min, was achieved. Using mass spectrometry detection, a total of 15 peaks, which were common to oils of six different botanical origins (corn, extra virgin olive, grapeseed, hazelnut, peanut and soybean) were identified. These peaks were used to construct linear discriminant analysis (LDA) models for botanical origin prediction. Ratios of the peak areas selected by pairs were used as predictors. All the oils were correctly classified with assignment probabilities higher than 95%.



## 1. Introduction

Vegetable oils are important commodities in the world trade and market, being widely used in several sectors of industry, including cosmetics and nutrition. Their production largely increased in the past decades, and it is still annually increasing with an estimated production of about 185 million tonnes on 2020 [1]. In particular, the production of crude vegetable oils has shown a net increase in the European Union along the last twenty years to reach about 13 million tonnes on 2008, without including olive oil, which accounted for about an additional 2 million tonnes in the 2008/2009 season [2,3].

Triacylglycerols (TAGs) are the main components of vegetable oils, as they are generally present in the 95–98% range of the whole oil composition. Their molecular structure, including the distribution of fatty acids between the different stereospecific positions of the glycerol skeleton, controls the functionality of oils and fats as food ingredients, influencing such physical properties as crystal structure and melting point. In addition, they have important physiological effects as components of the human diet, being an important source of essential fatty acids. Their imbalances can lead to several disorders such as coronary heart disease, obesity or dyslipidaemia. Thus, the development and improvement of analytical methods that enable the thorough identification of TAGs is very important to avoid adulteration of high-price quality vegetable oils with cheaper oils of lower quality and less beneficial nutritional effects.

Different chromatographic techniques are suitable for the analysis of TAG profiles in vegetable oils, but HPLC is the most employed [4]. Two HPLC techniques have been particularly used in the last years: $Ag^+$-HPLC in normal phase mode, based on TAG separation according to number and position of double bounds and *cis*/*trans* isomerism [5–7], and non-aqueous reversed-phase HPLC (NARP-HPLC) with mobile phase systems of low polarity, where TAG are separated according to the equivalent carbon number (ECN) [8–10]. Separation according to the different position of double bond(s) or within ECN groups is also possible [10–12]. In addition, comprehensive 2D HPLC, based on the combination of an anionic exchange column charged with silver ions followed by an RP-C18 silica monolithic column as second dimension has been recently applied for the characterization of TAGs in complex lipid matrices [13].

Several detectors are generally coupled with NARP-HPLC for TAG analysis. UV detection at low wavelengths (205 or 210 nm) provide a linear response; however, a low sensitivity for saturated TAG has been reported [10,14]. Evaporative light scattering

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is also employed, but it is not appropriate for quantitative analyses due to the non-linear response [10], while refractive index detection, although often used for routine quality control, cannot be applied with gradient elution [15]. Several applications reporting mass spectrometry (MS) detection, in combination with atmospheric pressure chemical ionization (APCI) as ionization source (which provides linear responses and excellent sensitivity with both saturated and unsaturated TAGs), have been recently published [12,16,17].

Finally, the application of multivariate statistical techniques to the TAG chromatographic data can lead to the discrimination of oils according to their botanical origin. Principal component analysis, linear discriminant analysis (LDA) and cluster hierarchical analysis have been all successfully applied for this purpose [8,17,18].

The aim of this work was the development of an analytical method based on the use of HPLC columns of the new core-shell particle class, for the prediction of the botanical origin of vegetable oils, based on the determination of the TAG profile. For this purpose, NARP-HPLC with UV–vis detection, with the aim of obtaining a good resolution within a short analysis time, was used. TAG profiles were used to construct LDA models addressed to the prediction of vegetable oil origins.

## 2. Experimental

### 2.1. Reagents and samples

The following analytical grade reagents were used: acetonitrile (ACN), 2-propanol, ethanol (EtOH), *n*-butanol, *n*-pentanol (Scharlau, Barcelona, Spain) and *n*-hexane (Riedel-de-Haën, Seelze, Germany); OOO (Sigma, Saint Louis, MO, USA) was used as standard. Deionized water (Barnstead deionizer, Sybron, Boston, MA, USA) was also used. A total of 36 vegetable oils were employed in this study, six for each botanical origin. These samples, which were either purchased in the local market or kindly donated by different manufacturers, were the following: corn oil from Guinama, Asua, Cristal, Gloria and Mazola; extra virgin olive oil (EVOO) from Borges, Carbonell, Coosur, Grupo Hojiblanca, Romanico and Torrereal; grapeseed oil from Coosur, Guinama and Paul Corcelet; hazelnut oil from Guinama and Percheron Freres; peanut oil from Coppini, Guinama and Maurel; and soybean oil from Coosur, Guinama, Biolasi and Coppini. In all cases, the botanical origin and quality grade of all the samples were guaranteed by the suppliers.

### 2.2. Instrumentation and working conditions

A 1100 series liquid chromatograph provided with a quaternary pump, a degasser, a thermostated column compartment, an automatic sampler and an UV–vis diode array detector (Agilent Technologies, Waldbronn, Germany) was used. Separation was carried out with a Kinetex™ C18 100A column (150 mm × 4.6 mm, 2.6 μm; Phenomenex, Torrance, CA, USA). The optimized separation conditions were: isocratic elution with a 70:30 ACN/*n*-pentanol mixture; UV–vis detection at 205 ± 10 nm (360 ± 60 nm as reference); column temperature, 10 °C; flow rate, 1.5 mL min$^{-1}$ and injection volume, 20 μL.

When required, the liquid chromatograph was also coupled (in series with the UV–vis detector) to the APCI source of an HP 1100 series quadrupole mass spectrometer (MS) (Agilent). The MS working conditions were as follows: nebulizer gas pressure, 35 psi; drying gas flow, 12 L min$^{-1}$ at 350 °C; vaporizer temperature, 350 °C; capillary voltage, 3 kV. Nitrogen was used as nebulizer and drying gas. The mass spectrometer was scanned within the *m*/*z* 300–1000 range in the positive-ion mode.

### 2.3. Sample preparation, data treatment and statistical analysis

Vegetable oil samples were chromatographed after a simple dilution to 3% in a 2:2:1 ACN/2-propanol/*n*-hexane (v/v/v) ternary mixture. All samples were injected three times. A data matrix was constructed measuring the APCI-MS peak areas of all peaks, and using these data as original variables. After normalization of the variables, statistical data treatment was performed using SPSS (v. 15.0, Statistical Package for the Social Sciences, Chicago, IL, USA). LDA, a supervised classificatory technique, is widely recognized as an excellent tool to obtain vectors showing the maximal resolution between a set of previously defined categories. In LDA, vectors minimizing the Wilks' lambda, $\lambda_w$, are obtained [19]. This parameter is calculated as the sum of squares of the distances between points belonging to the same category divided by the total sum of squares. Values of $\lambda_w$ approaching zero are obtained with well resolved categories, whereas overlapped categories made $\lambda_w$ to approach one. Up to $N-1$ discriminant vectors are constructed by LDA, being $N$ the lowest value for either the number of predictors or the number of categories. The selection of the predictors to be included in the LDA models was performed using the SPSS stepwise algorithm. According to this algorithm, a predictor is selected when the reduction of $\lambda_w$ produced after its inclusion in the model exceeds $F_{in}$, the entrance threshold of a test of comparison of variances or *F*-test. However, the entrance of a new predictor modifies the significance of those predictors which are already present in the model. For this reason, after the inclusion of a new predictor, a rejection threshold, $F_{out}$, is used to decide if one of the other predictors should be removed from the model. The process terminates when there are no predictors entering or being eliminated from the model. The probability values of $F_{in}$ and $F_{out}$, 0.01 and 0.10, respectively, were adopted.

## 3. Results and discussion

### 3.1. Optimization of the separation of TAGs

In order to obtain TAG separation with optimal resolution within a short analysis time, the use of a Kinetex™ core-shell particle column was tried. These columns are capable of maintaining high efficiencies at increasing flow rates with the subsequent reduction of analysis time. Also, these columns operate comfortably within the pressure limits of conventional LC instruments, rivalizing the performance obtained with sub-2 μm particle columns on UHPLC instruments.

Peanut oil was used to optimize TAG separation in terms of mobile phase composition and column temperature. A flow rate of 1.5 mL min$^{-1}$, which gave a moderate pressure, was selected. The influence of the length of the alkyl chain of different alcohols used to modify eluotropic strength was also investigated. For this purpose, a column temperature of 25 °C and binary mixtures of ACN with either EtOH, 2-propanol, *n*-butanol or *n*-pentanol, all at a 70:30 ratio, were tried. Using EtOH, poor resolution and long analysis times (*ca.* 90 min) were obtained (data not shown). Using 2-propanol (Fig. 1A), resolution largely improved, being analysis time shorter than with mobile phases containing EtOH. Analysis time further decreased using *n*-butanol and *n*-pentanol (Fig. 1B and C), being also resolution slightly improved. Pump pressures ranged from 16.7 to 20.0 MPa for mobile phases containing EtOH and *n*-pentanol, respectively. Thus, an ACN/*n*-pentanol mixture was selected for the following studies. Next, proportions of ACN/*n*-pentanol from 80:20 to 50:50 were tried in isocratic elution mode. With 50:50 ACN/*n*-pentanol, most peaks overlapped. Resolution slightly improved when ACN content was increased to 60%, and further increased with 70% ACN; however, resolution decreased