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Using the liquid-chromatographic-fingerprint of sterols fraction to discriminate virgin olive from other edible oils

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ABSTRACT

A method to discriminate virgin olive oil from other edible vegetable oils such as, sunflower, pomace olive, rapeseed, canola, corn and soybean, applying chemometric techniques to the liquid chromatographic representative fingerprint of sterols fraction, is proposed. After a pre-treatment of the LC chromatogram data – including baseline correction, smoothing signal and mean centering – different unsupervised and supervised pattern recognition procedures, such as principal component analysis (PCA), hierarchical cluster analysis (HCA), and partial least squares-discriminant analysis (PLSDA), have been applied. From the information obtained from PCA and HCA, two groups can be clearly distinguished (virgin olive and the rest of vegetable oils tested) which have been used to discriminate between two defined classes by means of a PLSDA model. Five latent variables (LVs) explained 76.88% of *X*-block variance and 95.47% of the defined classes block (γ-block) variance. A root mean square error for calibration and cross validation of 0.10 and 0.22 respectively, confirmed these results and a root mean square error for prediction of 0.15 evidences that the classification model proposed presents an adequate prediction capability. The contingency table also shows the good performance of the model, proving the capability of the LC-R-FpM, to discriminate virgin olive from other vegetable edible oils.

1. Introduction

According to several authors [1–3], a fingerprint can be defined as a characteristic profile reflecting the complex chemical composition of an analyzed sample and can be obtained by spectroscopic, chromatographic or electrophoretic techniques. The chromatographic methods are able to characterize the chemical composition of samples using the chromatographic signal as a fingerprint which describes a sample as unique (as a human fingerprint) [4]. In this sense, the comparison among chromatographic fingerprints (FINGERPRINTING) [5–7] can be used to uncover or explain the variability caused by differences in the chemical composition of samples, being useful for quality assessment and authentication of them [8–11].

Fingerprinting appears first in authentication of foods in the Project TRACE (FP6-2003-FOOD-2-A): Tracing Food Commodities in Europe [12], in which food analysis by fingerprinting techniques is described as part of Work Package 2 in analytical tools group. These techniques describe a variety of analytical techniques which can measure the composition of some foods in a non-selective way. The information obtained, defined as instrumental fingerprint (InF), is a signal (i.e. a spectrum or a chromatogram which are function of the chemical composition and have different specificity grade) provided and registered by an analytical instrument that requires a mathematical treatment, normally a chemometric approach including projection, clustering, modelling techniques, etc. [13–15] with the objective of characterizing the food.

In the last years the olive oil has won in popularity not only by its quality, but also for the potential benefits for health derived from its consumption. The interest in its chemical composition has increased due to different reasons among them, to assure its quality and origin, to guarantee the fulfilment of the current regulations and to detect possible adulterations or frauds. The olive oil can be defined as a matrix of great complexity and diversity and some of the methods that are in use for its analysis have been adopted and regulated by official institutions as the International Olive Oil Council (IOOC) or the Codex Alimentarius Commission [16,17].

The European Union (EU) [18,19] has published two regulations about the commercialization of olive oil and olive oil mixed with other vegetable edible oils. In the labelling of these products, the presence of olive oil must be indicated when its percentage is higher than 50%. Therefore, it would be interesting to have methods that

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allow establishing the presence of olive oil based on easily obtained instrumental fingerprints that, not being necessary to quantify, allow discriminating between this type of oil and other edible oils.

The oil fraction which permits to differentiate a type of oil from other is known as unsaponifiable fraction. In the olive oils it covers the 1–2% of its total content and it is rich in minor metabolites such as, triterpene dialcohols, phenolic compounds, tocopherols, hydrocarbons, pigments, terpenic acids, mono- and diacylglycerols, etc., being sterols the major proportion of this fraction and the most important olive oil minor compounds in authentication purposes [20].

In relation with these analytes, most of the chromatographic studies, as far as we concerned, have been made using the area/height of peaks (peak profiling) or concentration data (compositional profiling) rather than using raw data (InF). The most well-known chromatographic fingerprinting is focused on the utilization of well resolved InF [21–29].

In this paper, from poorly resolved chromatograms of unsaponifiable of different edible vegetable oils, the zone in which the sterols fraction appears, has been used as raw data to obtain the samples fingerprint matrix. A chemometric approach on the "representative fingerprint matrix" (R-FpM) obtained after a pretreatment of the chromatograms (base line correction, signal smoothing, and mean centering), using different unsupervised and supervised pattern recognition techniques, has been applied to discriminate virgin olive oil from the other oils studied.

2. Materials and methods

2.1. Chemicals and reagents

To do the saponification of the oils, a 2 M ethanolic solution of potassium hydroxide Panreac (Barcelona, Spain), was prepared by dissolving 130 g in 200 mL of distilled water and then, made up to 1 L with ethanol (96% (v/v), Panreac). This solution once prepared, must be kept in a well-stoppered dark glass bottle.

Other reagents, as diethyl ether (98% purity), anhydrous sodium sulphate (99.5% purity) and acetone (99.5% purity), were purchased from Panreac and all of them were of analytical grade. HPLC-grade solvents (hexane and tert-butylmethyl ether (TBME)) were from Merck (Darmstadt, Germany).

2.2. Samples

Fifty-one trademark edible vegetable oils, from different origins, i.e. Spain, Mexico, France and USA, were purchased in local markets or gourmet shops. The following oils (code, number of samples studied) were analyzed: Virgin olive (VOO, 25), pomace olive (POO, 4), sunflower (SFO, 11), rapeseed (RO, 3), canola (CanO, 2), soybean (SyO, 3) and corn (CoO, 3).

2.3. Instrumentation

The analysis was performed with a HPLC 1050 Series Chromatograph equipped with an UV–visible variable wavelength detector (VWD) Agilent Technologies (Palo Alto, CA, USA), and a Reodhyne (Reodhyne, Inc., Cotati, CA, USA) 7125 loop injector provided with a 20 μL sample loop. The main characteristics of the VWD used were: wavelength range, 190–600 nm; wavelength accuracy, ±2 nm; wavelength reproducibility, ±0.3 nm; band width, 6.5 nm; response time, 1 s.

The software used for acquisition and handling of the chromatographic data was an Agilent ChemStation (Rev. A.08.03).

2.4. Analytical procedures

2.4.1. Sample preparation

5 g of oil sample were saponified with 50 mL of a 2 M ethanolic potassium hydroxide solution by refluxing, at approximately, 80 °C, with constant shaking until the solution was clarified (ca. 1 h). The unsaponifiable fraction was extracted with ethyl ether, the extract dried with anhydrous sodium sulphate, and the solvent was evaporated to dryness in a rotary evaporator at 30 °C. The resulting residue was dissolved in 1 mL of mobile phase (80:20 v/v, n-hexane:TBME). Then, 20 μL of this solution were injected into the HPLC system.

2.4.2. HPLC-UV analysis

The chromatogram of the unsaponifiable fraction was obtained by HPLC [30], using a Lichrospher 100 CN column (250 mm × 4.0 mm i.d., 5 μm) with a Lichrospher guard column (10 mm × 4.0 mm i.d., 5 μm) from Merck, a mobile phase constituted by hexane:TBME, 80:20 (v/v), a flow rate of 0.8 mL min^{-1} and a detection wavelength of 208 nm [31,32].

The chromatograms were exported to csv format from ChemStation software and imported to MATLAB version 7.8.0347 R2009a (Mathworks Inc., Natick, MA, USA) to handle chromatographic data matrices.

2.5. Construction of the LC-fingerprints

In a previous work [30], it could be observed that the LC-chromatogram of the unsaponifiable fraction of an edible vegetable oil was a bad resolved chromatogram, morphologically dependent of the type of oil analyzed. The last registered peak, assigned to the sterols fraction (that comprised the zone of the chromatogram between retention times 7.5 and 9.5 min), could be considered as a specific fingerprint of the sterols fraction as well as the edible oil.

Fig. 1 shows the procedure used for the construction of the oil fingerprint based on its sterols fraction. It can be seen that for each chromatogram, a data matrix $IM_{"j"}$ (retention time × absorbance), which initially had a dimension of 6916 × 2 variables, was obtained.

Once the matrices were combined and transposed, all the TR rows were eliminated. For each sample, the variables related with the sterols fraction (2001), were selected, generating a fingerprint matrix (FpM) with a dimension of 51 × 2001.

2.6. Chemometric approach

The chemometric study was made using principal component analysis (PCA), as exploratory data analysis [13,14,26,33,34], combined with hierarchical cluster analysis (HCA) [35–37] to confirm the results obtained previously. Finally, a partial least squares-discriminant analysis (PLSDA) [38–42] was used to discriminate virgin olive oil from other edible oils.

All chemometric treatment was performed using PLS_Toolbox Version 7.0.3. (Eigenvector Research, Inc., West Eaglerock Drive, Wenatchee, WA).

3. Results and discussion

3.1. Obtaining the representative fingerprint matrix (R-FpM)

To obtain the representative fingerprint matrix (R-FpM), a tree steps pre-processing of the FpM (51 × 2001) was carried out (see Fig. 1) using a baseline correction, a signal smoothing and finally a mean centering.

(i) In order to separate the analytical signal of interest from the signals due to other factors, a baseline correction was made.