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Chemometric classification and quantification of olive oil in blends with any edible vegetable oils using FTIR-ATR and Raman spectroscopy

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ABSTRACT

Samples of olive oils (n = 67) from different qualities and samples of other vegetable edible oils (including soybean, sunflower, rapeseed, corn oil etc; n = 79) were used in this study as pure oils. Previous to spectroscopy analysis, a transesterification step was applied to the pure vegetable oil samples and all the different oil blends were then prepared to create in-house blended samples. Spectral acquisition was performed with typical parameters to collect the FTIR and Raman fingerprints. For the olive/non-olive classification model, three classification strategies have been applied: (i) one input-class (1iC) classification; (ii) two input-class (2iC) classification; and (iii) one input-class plus one 'dummy' class classification (or *pseudo* two input-class (*p*2iC) classification). The multivariate classification methods used were k-nearest neighbours (kNN), partial least squared-discriminant analysis (PLS-DA), one-class partial least squares (OCPLS), support vector machine classification (SVM-C), and soft independent modelling of class analogies (SIMCA). The multivariate quantification method used was partial least square-regression (PLS-R). FTIR fingerprints showed excellent classification ability to distinguish pure olive from non-olive oil. When PLS-DA or SVM-C techniques are applied, 100% of olive oil samples and 92% of other vegetable edible oils are correctly classified. In general FTIR fingerprints were more discriminative than Raman's in both classification and regression scenarios.

1. Introduction

As a natural product that is produced using 'only mechanical means' from olive drupes, olive oil is protected by various regulations and institutions such as the EU Regulations (Commission Regulation EEC, 2016; Regulation UE, 2011; Regulation UE, 2016) and Codex Alimentarius (Codex Stan, 2015). Due to its increasing popularity, it has always been the target for fraudulent practises such as substitution fraud with cheaper oils (blends). To prevent that, authenticity of olive oil is described adequately in the legislation. The top two qualities of olive oil that exist are the extra-virgin and the virgin olive oil and both of them must comply to certain well defined physical, chemical and sensorial parameters. There are several standard methods that are used to determine these parameters. For example, with the use of chromatographic techniques detection of several major and minor constituents of olive oil (fatty acids, tocopherols, carotenoids etc.) is achieved. Nowadays rapid and novel methods are continuously developed (such as those based on spectroscopy), as alternatives to the standard methods offering speed, efficiency (less resources required) and accuracy in authenticity testing.

Actually, studies about authentication of olive oil using spectroscopic techniques are based on the application of chemometric tools to develop multivariate models that are able to differentiate pure olive oils from adulterated olive oil with other vegetable edible oil. Then, the proportion of olive oil in these blends is quantified; therefore, although blends of olive oil with other vegetable oils are allowed by the legislation, there is a restriction of labelling them as "olive oils" if the olive oil in the blend does not exceed 50% (Regulation UE, 2016). Consequently, a proper method of control must be established. Sun, Lin, Li, Shen, and Luo (2015) reported: (i) a principal component analysis (PCA) model to

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discriminate extra virgin olive oil from binary blends of olive oil with camellia oil, soybean oil, sunflower oil and corn oil; and (ii) a quantification model using partial least squares (PLS) to quantify the olive oil in binary blends. López-Diez, Bianchi & Goodacre, 2003 described a PCA model to differentiate pure extra virgin olive oil from adulterated olive oil with hazelnut oil, and a PLS model to quantify the amount of olive oil in the mixtures. Similar studies to the above mentioned ones are shown in Table 1. This table shows five papers using FTIR to detect adulteration of olive oil with other vegetable oil in blends binary, only Gurdeniz and Ozen (2009) develop a model to quantify olive oil in ternary blends. For Raman spectroscopy five works are reported, as in FTIR all the authors detect and quantify olive oil in blends binary, except Rohman and Che Man (2012) which quantifies olive oil in quaternary blends.

The main disadvantage of the reported models to authenticate olive oil using spectroscopic techniques, such as FTIR and Raman, is the low number of different botanical species used to build the blends of olive oil with other edible vegetable oils. Most authors employ a small set of oils to elaborate the blends, and sometimes using a single olive oil or a limited number of vegetable edible oil (non-olive oil) in the different mixtures prepared. For example, Tay, Singh, Krishnan, and Gore (2002) reported a method to authenticate olive oil using only thirty two olive oil and seven vegetable edible oils (non-olive oil) to build the different blends (Tay et al., 2002). Thus, the resulting models cannot be considered as global methods to detect adulteration of olive oil (independently of the cultivars) with any edible vegetable oil. Moreover, some authors erroneously apply PCA as discriminant analysis technique to develop and validate classification models of olive oil (Sun et al., 2015). PCA is an unsupervised data analysis technique used to explore the variability in the dataset and to evaluate if there are different groups of samples when the dimensionality of the data decreases. This exercise should not be used for classification purposes. In the literature there is only one published study where it is developed a classification model to distinguish pure olive oil from other pure vegetable oil using FTIR or Raman spectroscopy. De la Mata et al. (2012) reported a partial least squares discriminant analysis (PLS-DA) aiming to distinguishing between olive oil and binary mixture of non-olive samples applying ATR-FTIR.

The aims of this study are: (i) discrimination of pure olive oil/non-pure olive oil, (ii) detection of adulterated olive oil and (iii) quantification of olive oil in blends (from binary to heptenary mixtures) with other vegetable edible oils using a number of chemometric techniques. For this purpose, we have developed a global and comprehensive analytical method to differentiate, detect and quantify olive oil in blends with any edible oils. The number of oils used in this work is wide, and spread worldwide. Although, in the "real world" the usual blends of olive oil with other seed oil are binary, a quality control laboratory does not know which was and/or how many were the seed oils used in adulteration, if any. For this reason, the proposed method aims at covering binary and higher-order blends which could be found.

2. Materials and methods

2.1. Chemicals

Isopropanol, *n*-hexane, methanol and *tert*-butyl methyl ether (TBME) were purchased from VWR International Eurolab, S.L. (Barcelona, Spain) and all of them were of HPLC grade. Other reagents, such as sodium methoxide, citric acid monohydrate, and anhydrous sodium sulphate were purchased from Merck (Darmstadt, Germany). The nitrogen (99.9999%) used was provided by Air Liquid (Madrid, Spain).

2.2. Instrumentation

FT-IR spectra were obtained on a NICOLET iS5 spectrometer (Thermo Scientific, Waltham, Massachusetts, USA) equipped with a DTGS detector and KBr beam splitter. Spectra were obtained in the range of 4000 cm^{-1} to 550 cm^{-1} with a resolution of 2 cm^{-1} using a monolithic diamond attenuated total reflectance (ATR iD7) accessory. All the spectra were recorded at room temperature with 32 scans.

Raman measurements were carried out using IDRAMAN Reader (Ocean Optics, Oxford, UK) with 785 nm emission of a laser (23.4 mW at sample) for excitation. The laser was focused on the sample contained in 2 mL vial. For signal detection, a 2048-element NIR-enhanced CCD array with thermoelectric cooling to 10 °C was employed. An averaged spectrum for each sample was recorded in the range of 200–3200 cm^{-1}, using an integration time of 10 s each 3 scans.

NIR spectra were obtained using Antaris II (Thermo Electron Corporation, Waltham, Massachusetts, USA) FT-NIR analyzer, equipped with a diffuse reflection fibre optic and InGaAs detector. All the spectra, in the range of 4000–10000 cm^{-1}, were recorded at room temperature with 32 scans.

In all cases, each sample was analysed in triplicate.

2.3. Samples

2.3.1. Pure vegetable edible oils used to the classification models

67 samples of olive oils and 79 samples of other vegetable edible oils were used in this study. The samples of olive oils were constituted by 52 extra virgin olive oils (EVOO) samples, including 41 samples from 10 different monovarietals ("Arbequina", "Hojiblanca", "Picual", "Royal", "Manzanilla", "Cornicabra", "Empeltre", "Frantoio", "Verdial" and "Blanqueta") and 26 samples of varietal mixtures, 4 virgin olive oil samples (VOO), 5 olive oils, blend of virgin and refined (OO) and 6 pomace olive oil samples (POO). Vegetable edible oil samples (non-olive oils) consisted of 8 hazelnut oils, 5 peanut oils, 10 canola oils, 2 safflower oils, 12 sunflower oils, 2 flax oils, 5 corn oils, 9 palm oils, 8 seeds oils (marketing mixture of unidentified seeds), 4 sesame oils, 8 soybean oils, 1 wheat oil and 4 grapeseed oils. In addition, a speciality olive oil extracted from previously dehydrated olive fruits was also added in this group. All samples were collected from marketed edible oils, purchased in food stores and sourced from respective partners from multiple geographical locations.

2.3.2. Blends of olive oil with other vegetable edible oils

To build the blends were used 27 olive oil samples, of which 22 EVOO (including 16 monovarietal oils), 3 VOO and 2 OO. In addition, 52 edible oils samples of 8 botanical origins, obtained each one from different suppliers, were used: 8 soybean oils, 11 sunflower oils, 10 rapeseed (canola) oils, 5 corn oils, 5 seeds oils (commercial blends of unknown seed oils), 5 peanut oils, 4 sesame oils and 4 grapeseed oils. Table 2 shows details on the composition of the different blends.

All the oil samples were stored at 4 °C until the sample preparation in order to provide realistic testing conditions.

2.4. Sample preparation

Previous to the spectrometric analysis, a transesterification reaction was applied to the pure vegetable oil samples and all the different oil blends prepared. This reaction was carried out using 0.1 g/mL sodium methoxide in a methanol/TBME mixture, 4:6 (mL:mL), and then the extraction was performed with *n*-hexane. In this alkaline medium, the free fatty acids presents in the oil are not