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Identification of Adulteration of Olive Oil with Other Edible Oils by LED-induced Fluorescence and Multivariate Calibration

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Abstract

The most common adulterants found in extra-virgin olive oil are refined olive oil and other vegetable oils, such as sunflower, soybean, corn, and canola. In addition to constituting economic fraud, adulteration can cause serious damage to the health of the consumer. This study focuses on the detection and quantification of the adulteration of extra-virgin olive oil with edible oils, using spectrofluorimetry and chemometrics. The data were analyzed by Principal Components Analysis (PCA) and Partial Least Squares (PLS) analysis. Through PCA, it was possible to separate the samples into two distinct areas, olive oil and other edible oils, based on their chemical composition. The PLS model, built with the spectra of mixtures of soybean oil in extra-virgin olive oil, exhibited an R^2 of 0.99412 and low RMSEP (Root Mean Square Error of Prediction) (3.59), RMSEC (Root Mean Square Error of Calibration) (2.32) and bias (4.77. 10^{-7}) values. Thus, the PLS model was considered exact for calibration and prediction.

Keywords: virgin and extra-virgin olive oil, adulterations, fluorescence, PCA, PLS.

1. Introduction

Olive oil is a type of oil produced from the fruit of the olive tree. The name “olive oil” can only be applied to pure oil obtained from olives, not to mixtures composed of this oil and other oils such as soybean or corn, which are generically known as oil compounds. The use of olive oil dates back millennia, but its exact origin is unknown. Olive cultivation has occurred for more than 5000 years. The Phoenicians, Syrians, and Armenians were the first people to consume olive oil, and it was introduced to Europe and the West by the Greeks and Romans. For a long time, the consumption of olive oil was restricted to the Mediterranean, but in the sixteenth century, the Spaniards reported its use in the regions of South America, Central America, and the United States. There are records detailing the use of olive oil 5000 years ago by Mesopotamian peoples, who anointed their bodies with oil to protect themselves from extreme cold. In the Roman Empire, olive oil was used to soften skin and dry hair. In Greece, athletes used the oil to improve their performance. In the sixteenth century, olive oil was the basis for many drugs (Oliva, 2011).

The importance of olive oil has grown over the years due to its multiple uses in food, medicine, hygiene, and cosmetics. Many of the benefits of olive oil vaunted by popular wisdom have been proven by several scientific studies. The major differentiation of olive oil from other edible oils is associated with the higher content of monounsaturated fatty acids such as oleic acid, and reduced content of saturated fatty acids, which assist in the control of cholesterol in the blood, helping to reduce “bad” cholesterol (LDL) while maintaining an appropriate level of “good” cholesterol (HDL) (Huang & Sumpio, 2008; Van Tol *et al*., 1999). Olive oil also distinguishes

itself by its high level of the triterpene squalene, which promotes the excretion of toxins and has anticarcinogenic effects. The presence of steroids such as ß-sitosterol helps to lower cholesterol and to aid in the prevention and treatment of prostate cancer, colon cancer and breast cancer (Menendez *et al*., 2006). Olive oil also has phenolic compounds, vitamin E and β-carotene, which are powerful antioxidants that react with free radicals, thus inhibiting platelet aggregation and preventing LDL oxidation (Aguilera *et al*., 2004). Due to its composition, olive oil provides a major contribution to the prevention and treatment of many diseases such as atherosclerosis (Acń *et al*., 2005), thrombosis (De La Cruz *et al*., 2000), diabetes mellitus (De La Cruz *et al*., 2010), biliary disease, cataracts and eye diseases (Aparicio-Ruiz, Mínguez-Mosquera & Gandul-Rojas, 2011), depression (Logan, 2005), bone mineralization (Coxam, Puel & Davicco, 2010), hypertension (Perona *et al*., 2004), and cancer (breast, prostate, digestive tract) (Fabiani & Morozzi, 2010; Fernández-Arroyo *et al*., 2012; Flynn & Mega, 2010; Menendez *et al*., 2006). In addition, regular consumption of olive oil has a protective effect against free radicals in the skin, and increases life expectancy because it strengthens the immune system and protects against memory loss due to age (Viola & Viola, 2009; Baccouri *et al*., 2008).

According to the Brazilian Association of Producers, Importers and Traders of Olive Oil (Oliva, 2011), olive oil is graded based on organoleptic characteristics (taste and aroma), chemistry (acidity and other chemical data) and the following three types of extraction:

- Extra-virgin olive oil is produced by a mechanical pressing extraction process. It has flawless flavor and taste and less than 1% acidity.
- Olive oil is also produced by a mechanical pressing extraction process. It has outstanding flavor and aroma with acidity below 2%.
- Pure olive oil is formed by blending refined olive oil and virgin olive oil and has less than 1.5% acidity.

Olive pomace is one of the main sub-products of the processing of olives after pressing to extract the oil. The olive residue has a low oil content and low oxidative stability due to a moisture content that accelerates the hydrolysis of triacylglycerol. Generally, olive oil from the pomace is extracted with solvent and subjected to the refining process, including neutralization, bleaching and deodorizing.

Due to its low price, sometimes refined oil is used to adulterate olive oil of better quality, such as pure, virgin and extra-virgin olive oil. Similarly, due to lower market prices, edible oils such as soybean, corn, canola, cotton, sunflower, peanut and almond are likely to be used as illicit adulterants of olive oil. Therefore, a rapid method to detect adulteration is important for purposes of quality control and labeling olive oils of better quality (Guimet, Ferré & Boqué, 2005).

Several analytical methodologies have been developed in recent years to ensure the authenticity of olive oil. These methods include chromatographic techniques (Bosque-Sendra *et al*., 2012; Baccouri *et al*., 2008) and spectroscopic techniques, such as mass spectrometry (Calvano *et al*., 2012), Nuclear Magnetic Resonance (NMR) (Fragaki *et al*., 2005), near-infrared spectroscopy (Mignani *et al*., 2011), Raman spectroscopy (Dong *et al*., 2012), chemiluminescence (Papadopoulos *et al*., 2002), fluorescence spectroscopy (Sikorska, Khmelinskii & Sikorski, 2012), and synchronous fluorescence (Poulli, Mousdis & Georgiou, 2007).

This study focuses on the detection and quantification of adulteration of extra-virgin olive oil with edible oils, using a combination of LED-induced spectrofluorimetry and chemometrics.

2. Materials and Methods

2.1 Samples

Oils were purchased from a supplier located in Salvador in Bahia, Brazil.We used 56 samples of oils, 10 being of extra-virgin olive oil, 10 of soybean, 13 of corn, 10 of canola and 13 of sunflower.

Mixtures of extra-virgin olive oil with adulterants

Mixtures of extra-virgin olive oil with soybean oil as an adulterant were analyzed at 0%, 5%, 10%, 15%, 30%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90% and 100%.

2.2 Principal Component Analysis

A Perkin Elmer-LS55 spectrofluorometer (U.S.A.) and 1 cm quartz cuvettes were used. The fluorescent emission spectra of the samples were obtained at 230-800 nm in intervals of 0.5 nm, while the sample was excited at wavelengths from 200-775 nm with increments of 25 nm. A total of 24 excitation wavelengths and 1142 emission wavelengths were obtained for each sample. The measurements were made with a slit of 2.5 nm and a scan speed of 1200 nm/min. The spectrofluorimetric maps were generated with Origin8.0®.

For PCA of pure samples, the spectra were initially organized in a cube with the following dimensions: 5 samples x 1142 emission wavelengths x 24 excitation wavelengths. The cube was transformed by the command unfoldm in MatLab6.1® to generate a general matrix with dimensions of 5 x 27408. This matrix was mean centered and then subjected to multivariate analysis with MatLab6.1®.

2.3 PLS Analysis

For the PLS analysis, mixtures of extra-virgin olive oil with soybean oil were assessed using a Quimis LED spectrofluorometer model Q-798FIL (Brazil) equipped with a violet LED centered at 400 nm, and the emission was captured in the range of 400-1018 nm. The amount of soybean oil added to the extra-virgin olive oil is described in Table 1.

Table 1. Mixtures of extra-virgin olive oil and soybean oil in the range of 0-100%.

Sample number	% Soybean oil	% Extra-virgin olive oil
1	0	100
2	5	95
3	10	90
4	15	85
5	30	70
6	35	65
7	45	55
8	50	50
9	55	45
10	60	40
11	65	35
12	70	30
13	75	25
14	85	15
15	90	10
16	100	0

3. Results and Discussion

3.1 Fluorescence Maps

Comparing the fluorescence maps of extra-virgin olive oil (Figure 1) with other oils (Figure 2) shows that the extra-virgin olive oil has an emission-excitation band concentrated in three distinct regions, with peaks at 375 nm excitation and 520 nm emission, 325 nm excitation and 400 nm emission, and 375 nm excitation and 430 nm emission. The other oils have concentrated bands at approximately 350 nm excitation and 450 nm emission.

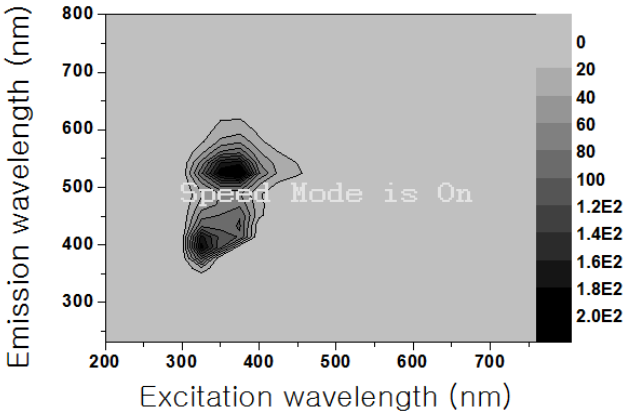

Figure 1. Contour map of the fluorescence spectra of extra-virgin olive oil.

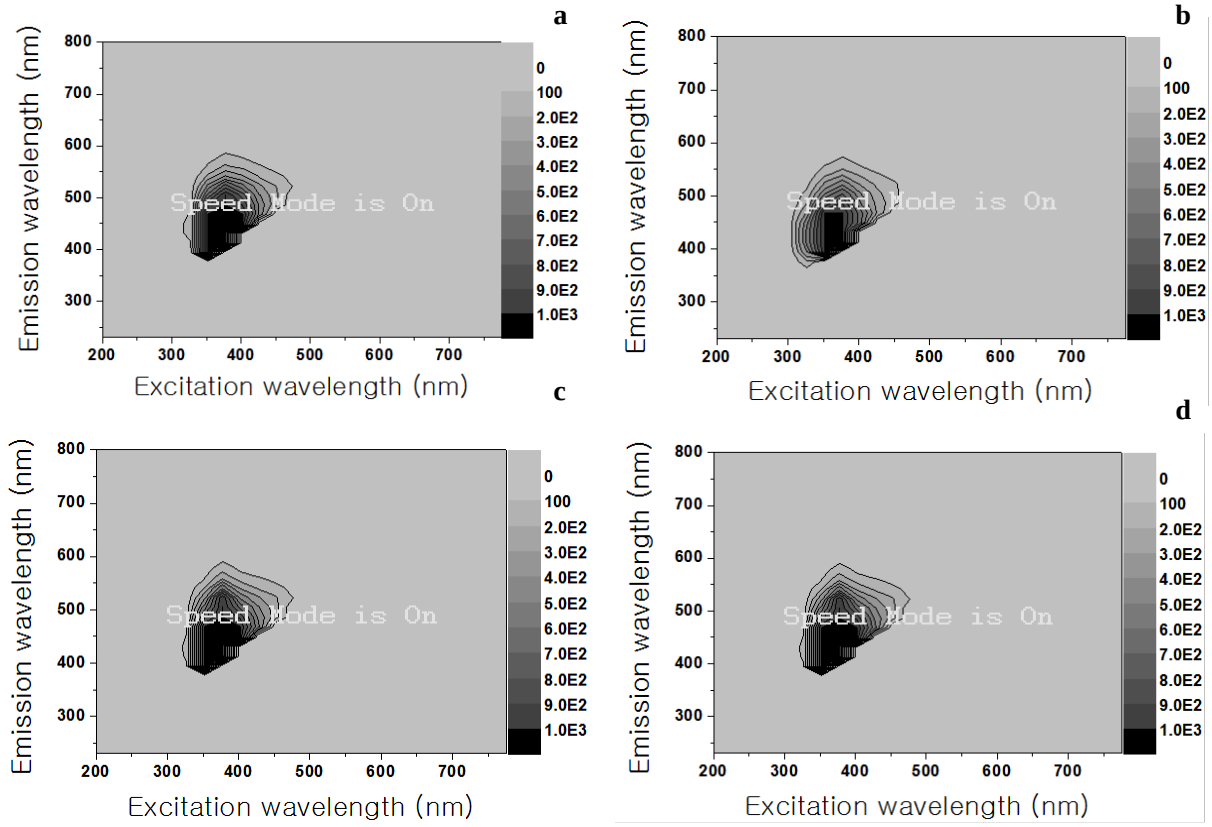

Figure 2. Contour maps of the fluorescence spectra of canola (a), sunflower (b), corn (c) and soybean (d).

3.2 PCA of Pure Samples

Through PCA, we found that only two principal components (PC) explained 89.87 % of the variance of the data, 79.33 % by PC1 and 10.54% by PC2. PC1 separated the samples by their chemical composition into two distinct areas, olive oil and other edible oils (Figure 3). The sample of extra-virgin olive oil has negative scores, and the samples of vegetable oils have positive scores.

The different fatty acid compositions of olive oil and other oils (ANVISA, 1999; Lee *et al*., 1998; Kim *et al*., 2010) may explain the differences found among the excitation-emission matrices shown by PCA. Extra-virgin olive oil is rich in oleic acid (55-83%), which is monounsaturated, while corn, soybean and sunflower oils predominantly contain polyunsaturated fatty acids. Sunflower oil has the highest content of linoleic acid (55-75%), which has two double bonds. Canola oil has an oleic acid composition similar to that of olive oil. However, canola oil has 5-13% linolenic acid (three double bonds), while olive oil has less than 0.9%. In addition, because extra-virgin olive oil is pressed, it retains higher levels of other fluorophores such as tocopherols, β-carotene and phenolic compounds that are refined out of other oils.

The spectra that most influenced the separation of extra-virgin olive oil (PC1 negative) from other vegetable oils (PC1 positive) were the peaks at 325 nm excitation and 382, 523 nm emission on the negative axis, and at 350, 375, 400 nm excitation and 440, 452, 459 nm emission, respectively, on the positive axis.

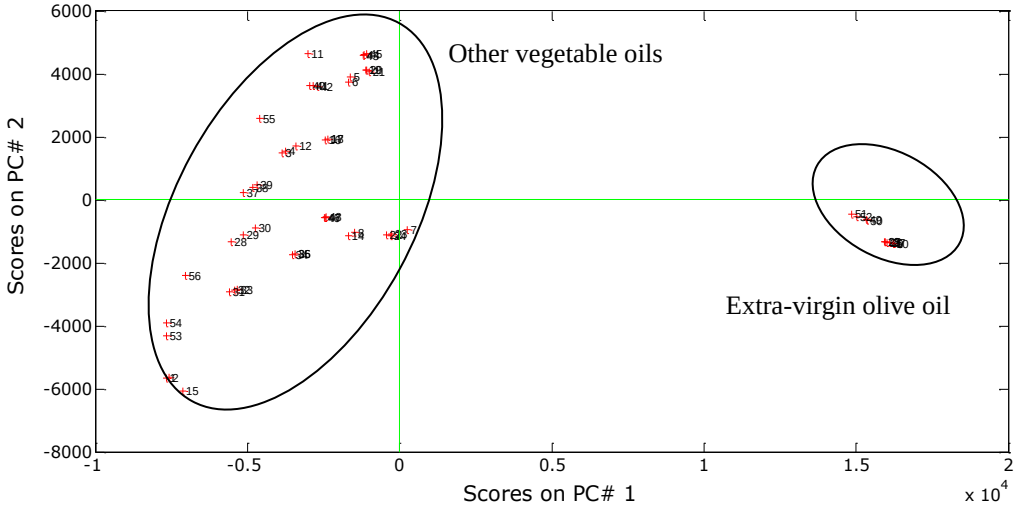

Figure 3. PC1 x PC2 scores for samples of extra virgin olive oil and other vegetable oils (corn, soybean, canola and sunflower)

3.3 PLS of spectra of mixtures of soybean oil in extra-virgin olive oil

Through the Partial Least Squares (PLS) multivariate calibration technique, it was possible to build a model correlating the concentration of extra-virgin olive oil with the fluorescence spectra. The PLS model had an R^2 of 0.99412 and low RMSEP (3.59), RMSEC (2.32) and bias (4.77. 10-7) values. Thus, one can consider the model exact for both calibration and prediction (Figure 4).

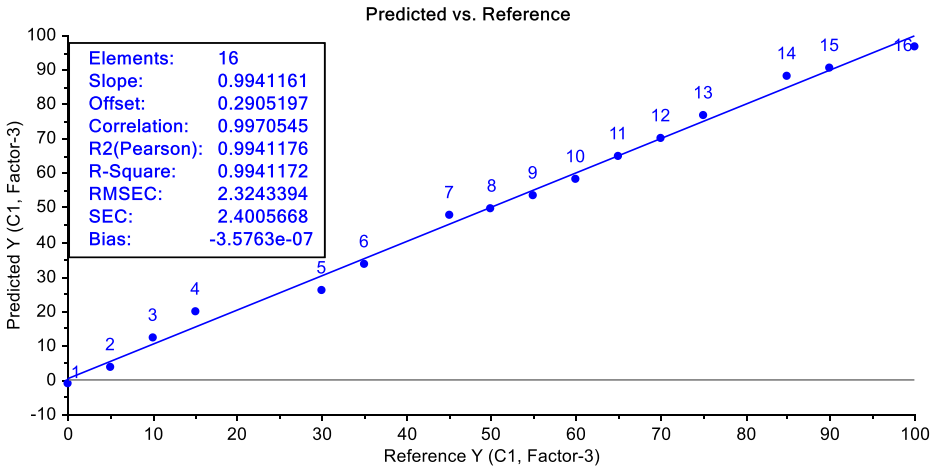

Figure 4. PLS mixtures of olive oil, extra-virgin olive oil and soybean oil in the range of 0-100%

4. Conclusions

Due to lower market prices, edible oils such as soybean, corn, canola, and sunflower are likely to be used as adulterants of extra-virgin olive oil for illicit enrichment. The method proposed in this paper, which combined spectrofluorimetry with PCA and PLS, was characterized as fast and accurate in detecting tampering and has the potential to be used for quality control and labeling of the best-quality olive oils.

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