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FINGERPRINTING OF OLIVE OIL FROM SPECTRAL DATA

Najratun Nayem Pinky [a,b], Krikor B. Ozanyan [b]
a. University of Calgary, Canada; b. University of Manchester, UK

ABSTRACT

Olive oil is an extensively used product and extra virgin olive oil is much costlier than other edible oils. Hence, purity of olive oil is a very significant issue. Fluorescence spectroscopy is a largely acceptable, simple, reliable and quick technique for adulteration detection and fingerprinting of olive oil. In this project, principal component analysis has been performed on fluorescence spectral data of 100 samples including pure extra virgin olive oil and adulterated ones with sunflower oil. The analysis has been able to successfully map the samples in a clear pattern for adulteration detection. The maximum tolerance limit for detection of adulteration is ±4.71% for the range of 0%-80% adulterated samples and ±5.67% for the range of 80%-100% adulterated samples. Also, by using two third of the samples as training set, this system can detect the rest one third samples (test set) quite accurately with an average tolerance of only ±3.42%. It has also been found that, short time exposure to laser, as a crude indication of possible long time exposure to sunlight, can definitely affect the fluorescence emission spectra. The two most significant wavelengths have been found (using variability) and validated (by principal component loading), that can replace the use of spectrometer with two color fiber optic probe. In this way, the computational complexity can be reduced to a great extent to make the adulteration detection system more affordable at retailer level.

INTRODUCTION

Olive oil is a monounsaturated fat obtained from the fruit of olive trees (*Olea europaea*). The organoleptic properties of this oil depend on the quality of the fruits and extraction procedure. Among the several types, extra virgin olive oil (EVOO) is considered as the best, which is obtained only by mechanical or physical methods, without involving any thermal alteration or treatment and contain definite organoleptic score. The worldwide production of olive oil in 2015/16 is expected to be 2,988,000 tons, where the average price of EVOO produced in Italy is 2903 Euros per ton. Therefore, it can easily be imaginable what a huge market this olive oil has itself [1]. Due to the high price of EVOO, it is often adulterated with different kinds of cheaper oil.

FINGERPRINTNG METHODS OF OLIVE OIL

The history of adulteration detection of EVOO is quite long. The Initial tests were mainly based on values of iodine, saponification, density, viscosity, ultraviolet absorbance, fluorescence, refractive index etc. Later, fractionization of components by liquid and gas chromatographic methods and a following quantitative determination of fatty acid, triacylglycerol, sterol or tocol compositions resulted in more definite information [2]. However, in spite of the high resolution and reliability, these methods were practically unsuitable for widespread use due to various characteristics such as time required, cost, need for skilled operation, generation of hazardous chemical waste [3], sample preparation, preprocessing etc [2]. Other analytical methods such as stable carbon isotope analysis, elemental analysis-isotope ratio mass spectrometry, gas chromatography-isotope ratio mass spectrometry [4], MOS electronic nose with SMPE mass spectrometry etc. also require time consuming sample preparation. However, spectroscopic methods such as UV spectrophotometry, pyrolysis mass spectrometry, vibrational spectroscopy etc. solved this problem; as these do not require demanding sample preparation or

preprocessing [2]. In addition, to deal with the large number of variables and data, different mathematical and statistical approaches as well as computer aided techniques such as artificial neural networks, multiple linear regression model, multivariate principal component analysis etc. became more popular in spectroscopic methods Luminescent methods are also being widely used for fingerprinting, oxidation detection and adulteration detection as they are simple in nature, sensitive for low limits of detection, non destructive and relatively low cost for instrumentation. These methods are based on the excitation of molecules either by absorption of light, such as photo luminescence and fluorescence, or by a chemical reaction such as chemiluminescence. Relevant works use fluorescence of chlorophylls, tocopherols and oxidation products to discriminate edible oils [5], adulteration detection of virgin olive oil with crude or refined hazelnut oil [6], combined analysis of the emission spectra and excitation spectra with the use of principal component analysis and artificial neural networks to detect adulteration of refined olive oils with refined hazelnut oils [7], synchronous excitation-emission fluorescence spectroscopy for origin determination of virgin olive oil [8], EEM spectra to detect soybean, rapeseed, corn, sunflower, linseed, and olive oil samples [9] etc. Fluorescence is also being used for detection of other properties of edible oils such as phenolics compound in olive oil, for non-invasive oxygen determination to evaluate the shelf-life etc. Thus, fluorescent spectra seem to be a very reliable method for fingerprinting of olive oil and the combination of principal component analysis with it can provide reliable information in this regard.

EXPERIMENTAL SETUP

The experimental set-up of this study has mainly two parts: the portable sensor developed in the Sensing, Imaging and Signal Processing group (SISP) of the School of EEE, University of Manchester and an interfaced computer system installed with the spectrometer software. The former requires a 12V DC power supply, measures the spectral data and sends the data to a PC or PLC through a USB port. The excitation source is a semiconductor LASER diode based on GaN. It has temperature and current control system from Power Technology Inc. (PPM LD1380-T). It emits at 404 nm and is regulated to deliver less than 5mW CW power. The built-in thermo electric cooler keeps the laser operating temperature at 20°C. A 30 dB Faraday opto-isolator is used to ensure proper protection of the laser from back reflection to prevent instability. The laser radiation is coupled into an optical fiber with a lens of 16mm focal length. A reflectance bifurcated fiber probe is used (Ocean Optics R400-7 UV/VIS), which is specially designed for measuring fluorescence in liquids. A diffraction grating monochromator provides spectral decomposition into its component wavelengths which is then analysed on a detector array. The small Ocean Optic USB 2000 portable monochromator is used in this setup as it provides sufficient resolution and needs a little power which it can draw through the USB port, also used for data transfer. It has detection range of 200-1100 nm. This system has a sensitivity of 86 photons/ count, 16 bit A/D resolution and a signal to noise ratio of 250:1. Its dimension is 89.1 mm x 63.3 mm x 34.4 mm and weight is 190 g. Hence, this spectrometer satisfies all the concerns of size, performance and power requirements for a portable sensor. The cuvettes used here are made of disposable plastic which allows the wavelength range of 230nm-900nm with a light path of 1cm. It has two plain walls for passing the light and two grated walls to prevent scattering. Each cuvette is capable of holding up to 4.5ml oil. 100 olive oil samples were carefully prepared for different adulterations (from pure olive oil, to pure sunflower oil in steps of 1% volume fraction) in these cuvettes and they were organized in a cuvette holder with proper labeling. Each sample is given a case number similar to its adulteration percentage, e.g. 04 denotes 4% adulteration and 90 means 90% adulteration. Pure olive oil is named as OO and pure sunflower oil is named as SO.

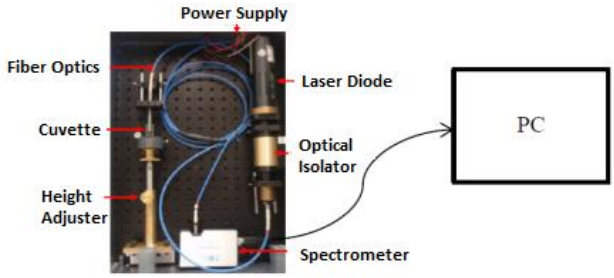

Figure 1: Components of the sensor system

RESULTS AND ANALYSIS

The fluorescence emission spectra of these samples for a range of 450 to 705 nm wavelength are plotted in figure 2. Here, the emission spectra of pure EVOO samples exhibit a clear distinguishable fluorescence peak in the range of 650-700 nm (maximum at 674.1 which can be assigned to chlorophyll groups. It should be mentioned that, olive oils shows another peak in the range of 275-297 nm wavelengths due to the presence of tocopherols. However, this is not visible here, as the excitation wavelength used in this experiment is 408 nm. On the other hand, pure sunflower oil shows fluorescence peak in the range of 450 to 550 nm (maximum at 492.7 nm) which is interpreted as higher content of linoleic acid [3].

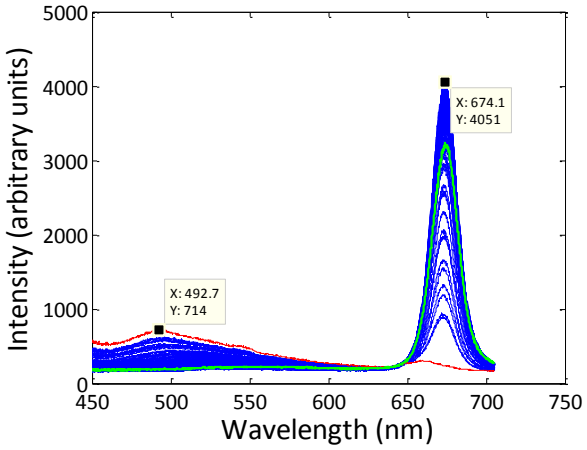

Figure 2: Fluorescence spectra of 100 samples: green for EVOO, blue for adulterated oil, red for sunflower oil

Based on the fluorescence spectra, principal component analysis is performed, on the samples in figure 3, by using orthogonal transformation to convert the spectral data of the samples into principal components, a set of values of linearly uncorrelated variables. Emission wavelengths in the range from 450.15 nm to 705.5 nm with an interval of 0.17nm have been considered; hence the total number of variables for wavelength is 1731. The number of principal components can be equal or less than the number of variables. Among the large number of principal components, we use the first two which exhibit the highest variability. In figure 3, 66.6% of the samples are training set (black) and 33.3% of the samples are used as a test set (red). The position of the training samples shows a visible pattern according to their adulteration percentage.

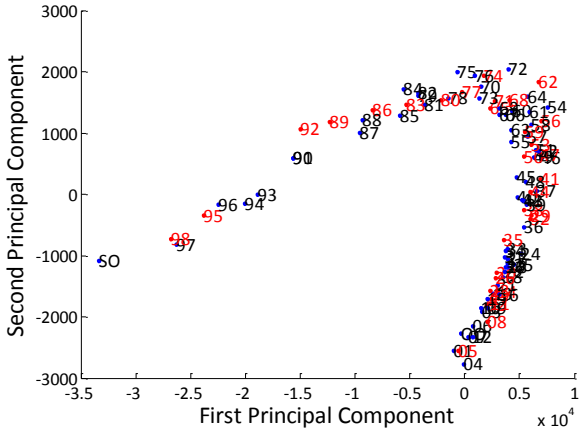

Figure 3: Principal Component Scatter Plot: numbers on plot correspond to case numbers; Black for training set, red for test set

The performance on the test set to determine the samples' adulteration, based on their nearest training sample's position on the map, is shown in figure 4. It shows that the adulteration percentage is determined with nominal average error (±3.42%) across the whole adulteration range. However, the error being different at the two extremes, the analysis will also have different sensitivity.

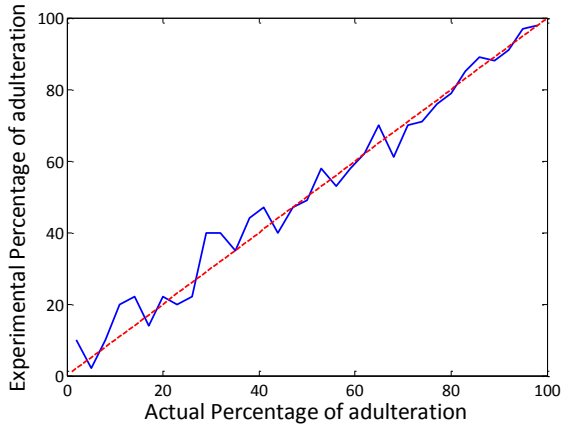

Figure 4: detected result vs. expected result for test set

Figure 5 plots the variances of the 100 samples vs. wavelength to determine the most important wavelengths for fingerprinting. Here, the maximum variance is at 674.9 nm and second highest variance is at 496.1 nm.

These wavelengths can also be validated by plotting the loading spectra for the first two principal components. Using only these two wavelengths, the test samples' adulteration detection average error becomes ±4.29%, which is slightly higher than that of the PCA analysis (3.42%). However, for a robust, low cost and accessible adulteration detection system, this approach can be more preferable.

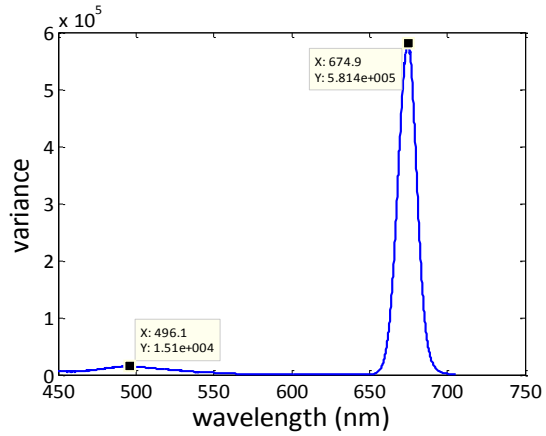

Figure 5: variance at different wavelengths

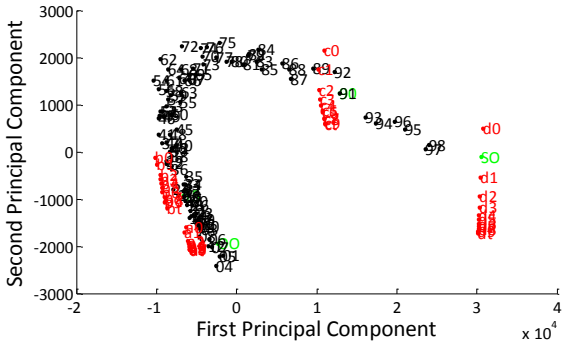

Figure 6:Principal Component Scatter Plot: Effect of laser

Figure 6 shows the effect of exposure to the UV laser radiation on pure EVOO, 15% adulterated EVOO, 90% adulterated EVOO and sunflower oil. This is a crude indication of the reliability of UV-induced fluorescence fingerprinting and the possible long time exposure to sunlight. The green numbers are for original non-exposed samples and the red points symbolize the effect for exposure to laser for durations of up to 10 minutes, respectively. It shows that exposure to laser can definitely affect the fluorescence emission spectra and more work is needed to validate the approach, possibly establishing the minimum UV irradiation intensity. The flipping of the display on figure 6 is equivalent to changing the sign of the first principle component.

CONCLUSION

This work clearly demonstrates the applicability of UV-induced fluorescence fingerprinting for adulterated olive oil. Normalizing the distances between neighboring samples, it has also been found that the error in adulteration detection can be ± 4.71% for 0% to 80% adulterated samples and ± 5.67% for 80% to 100% adulterated samples. Compared to the other available detection methods for adulteration of EVOO by sunflower oil, the fluorescence emission spectral method is faster and performs better than the high gradient diffusion NMR spectroscopy (10% sensitivity for sunflower oil) [10] and is in the same range with MOS electronic nose with SMPE mass spectrometry (sensitivity down to 5%) [11] and Rapid synchronous fluorescence with Partial least-squares regression model (4.3% sensitivity for sunflower oil) [12]. Therefore, considering a range of characteristics of interest such sensitivity (error), speed of obtaining a result, portability, as well as the potential for reasonably low cost and low service systems using only two wavelengths, the fluorescence emission method appears to be very promising for research and development of dedicated instruments.

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