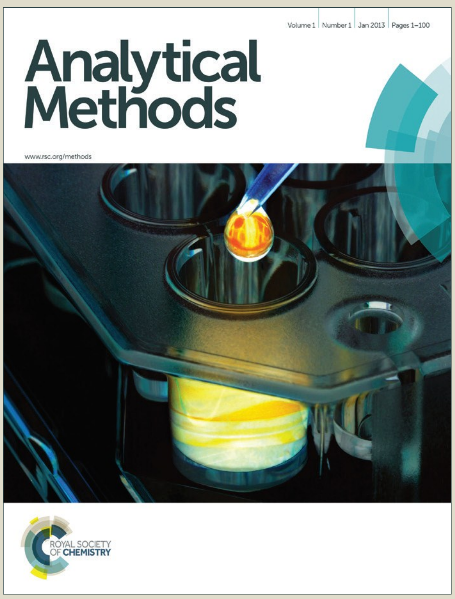
Analytical Methods

Accepted Manuscript



Fluorescence Polarization Technique: A New Method for Vegetable Oils Classification

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Concern about classification of different edible oils has roused recently in food safety. Developing an effective oil classification method is essential for public health. In this paper, a novel fluorescence polarization technique is developed to classify various types of oils. The degree of polarization(DOP) spectrums of seven vegetable oils are collected and analyzed. Classification of olive oil, walnut oil, and the other types of oils is successfully achieved with DOP spectrums under 532nm laser excitation. A classification accuracy of 100% is obtained for the investigated samples. The method is non-destructive and requires no sample preparation, which provides insight to develop a novel potable monitor system concerning food safety.

1 Introduction

Edible oils are necessary for daily life and provide foremost nutrients components including vitamins, essential fatty acids, tocopherols, and phytosterols to human beings.[1–3] However, Frauds of edible oils occur frequently, including replaced relative high-quality edible oil with low quality products, causing potential hazardous to public health and a series society issue. It is well known that the classification of edible oils is the basis of authentication identification. Thus, concern about fast classification of edible oils has roused recently.[4] Therefore, more investigation need to carry out to classify the various types of oils.[4]

Fatty acids have been frequently used as model components for classifying various kinds of oils in this field. Several analysis methods have been used to achieve this target.[3,5] In recent years, electronic nose has been developed to classify fresh edible oils combined with pattern recognition.The classifications accuracy is above 99% for the vegetable oil test samples.[6,7] H-1NMR and GC/MS have also been illustrated as powerful tool in edible oils analysis.[4,8,9]

Spectroscopic techniques have also been widely applied in many field.[10] It is demonstrated that mid-infrared spectra combined with principal component analysis(PCA) has been successfully used to classify fatty acids composition of olive oil samples.[11] In addition, The Fourier transform infrared spectra (FTIR) has been employed in oils classification[12] In conjunction with the 2D-PCA, the olive oil samples can be distinguished as distinct from other vegetable oils based on their FTIR spectra.[13]

However, these methods are either requiring expensive instrumentation or time consuming, which inhibit their large scale industry application. Fluorescence based technology has been used in various fields.[14–16] Specially, such methods are non-destructive, requiring no sample pre-treatment. They are becoming significant analytical methods for quality detection for oil products but not limit to oils.[17–19]

Synchronous scanning fluorescence spectroscopy has been shown to give good results for classification of different oils.[20,21] Together with PCA, cluster analysis, artificial neural networks(ANN), or partial least-squares, Raman spectroscopy has been achieved in the classification of vegetable oils.[22–26] However,Synchronous scanning fluorescence spectroscopy is time consuming and Raman need sample preparation, which limit the fast and potable detection.

Laser induced fluorescence(LIF) is fast and potable. But the classification result is unsatisfactory. In our present work, LIF polarization technology has been proposed to distinguish and classify various vegetable oils(including olive oil, walnut oil, peanut oil, corn oil, rapeseed oil, sunflower oil, and linseed oil) based on LIF. When polarized light is applied to a group of randomly oriented fluorophores, most of the excited molecules will be those oriented within a particular range of angles to the applied polarization. The emitted light will also be polarized within a particular range angles to the excited light. Fluorescence polarization (anisotropy) is usually applied to microscopy to study the local viscosity of the cytosol or membranes.

It has been found that this polarization information produce a larger variance among different edible oils. Also it can detect the trace of chlorophyll b. The fluorescence polarization characteristic of olive oil is presented and expounded in detail in our previous work.[27] With the degress of polarization(DOP) spectra detection system, the DOP spectrums of various vegetable oils have been acquired. The seven oils can be easily distinguished by the distinct DOP spectra with the help of PCA, ANN, and support vetor machine(SVM). The DOP spectroscopy technique are more fast and potable than other methods in classification of edible oils. Also the DOP spectra

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of samples are distinct from each other. Our method provide opportunity to develop a potable fluorescence based food analysis technology for food industry application.

2 Material and methods

2.1 Materials

In this work, seven types of vegetable oils have been measured by the fluorescence polarization detection system. Different brand samples, walnut(RONGS), Peanut(LuHua, FuLinMen), Sunflower(Arawana), Rapeseed(LuHua, XianCan),Linseed(XingHaiHu), Corn(Arawana , GoldEmbryo), Olive(AGRIC , BELLINA , GAFO), are purchased from a local supermarket and stored in the room temperature until use. The storage and preservation conditions are the same for all the samples to avoid interferences.

2.2 Detection system

532nm laser is used as excitation light source in the system for measurement.(Figure 1). The direction of polarization of laser light should be parallel to Z axis as it travels through the polarizer. Then the samples in the10×10mm fused-quartz cuvette can be excited by the vertically polarized light. The emission fluorescence is collected by a spectrograph (Avantes). A 532nm long-pass edge filter is placed before the spectrograph to avoid the strong elastic scattering light. A polarizer with a large aperture 25 mm, which is rotated in measurement process, is inserted between the cuvette and filter to control the polarization direction of emission fluorescence. The spectrum spanning wavelengths is from 540nm to 730 nm and the spectral resolution is 1.25nm .

2.3 Degree of polarization spectrums

DOP is determined using following equation[27,30,31]

$$DOP = \frac{\sqrt{S_1^2+S_2^2}}{S_0} \quad (1)$$

In Eq. (1). S_0, S_1, S_2 are defined as

$$S_0 = I(0^\circ)+I(90^\circ) \quad (2)$$
$$S_1 = I(0^\circ)-I(90^\circ) \quad (3)$$
$$S_2 = I(45^\circ)-I(135^\circ) \quad (4)$$

Where $I(0)$,$I(45)$,$I(90)$,$I(135)$ are fluorescence spectra respectively measured after rotating the rotatable polarized by $0^\circ, 45^\circ, 90^\circ$, and 135° from the initial direction in a clockwise direction. The initial direction could be arbitrary. But in this

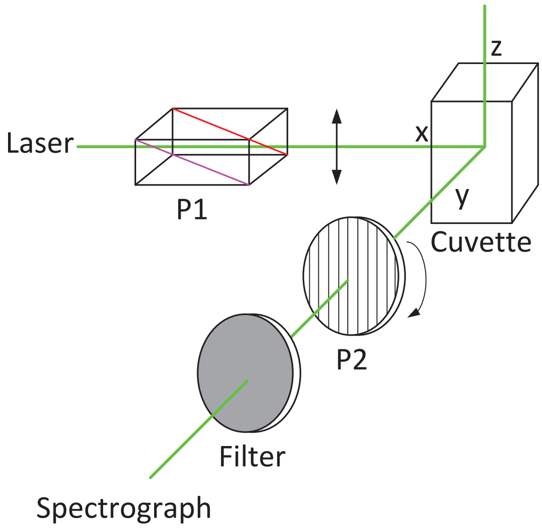

Fig. 1 The Schematic for measurement DOP spectrums. P1: Polarizer oriented parallel to z-axis; P2: polarizer with a large aperture 25 mm; Filter: 532 nm long pass edge filter.

paper z-axis is selected as the initial direction for convenience.

2.4 Multivariate Analysis

Total 280 sets of fluorescence data (40 sets for each type of oil) are collected. Data analysis is performed by classical multivariate procedures including PCA, ANN, and SVM with Matlab2010.All the spectra are normalized in pre-processing step. ANN and SVM are then applied for the classification of the samples by the extracted feature vectors.

2D-PCA plot is used in this study.[4] In addition, cluster analysis is employed as a classification technique to group a set of similar objects (which are more similar to each other than to those in other groups) into the same group. It is a technique for statistical data analysis, including data mining, bioinformatics, machine learning, image analysis, information retrieval and pattern recognition.[28]

ANN are a family of statistical learning algorithms inspired by biological neural networks. the greatest advantage of ANNs is their ability to be used as an arbitrary function approximation mechanism that 'learns' from observed data. With the correct implementation, ANNs can be used naturally in online recognition and large data set applications. Support vector machine (SVM), a supervised learning model, is another analysis method used in this study. Through the kernel function, the nonlinear data can be divided into different groups.[29] Here, the C-SVC model and RBF Kernel are used for the training of SVM. Then training patterns and kernel determine the decision function of SVM with following equation:

$$Plabel = sgn(\sum_{i=1}^{n} w_i exp(-\gamma \|x_i - x\|^2) + b) \quad (5)$$

Where, w_i is the corresponding weights; x_i is the training inputs; x is the test data; Here, $\gamma = 1/2\sigma^2$(σ is the variance); b is the intercept.

3 Results and discussion

3.1 DOP spectrums of seven vegetable oils

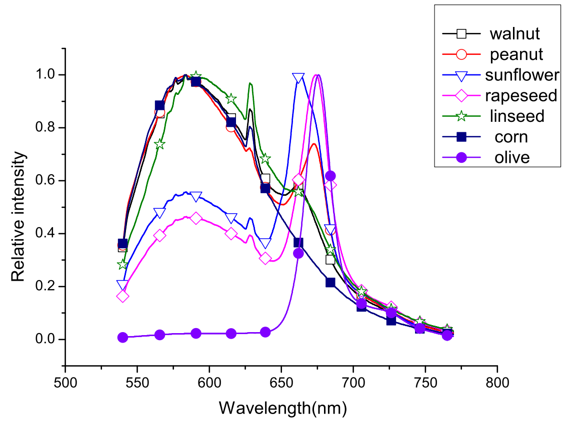

Fig. 2 The vegetable oils emission spectrums under 532 nm excitation laser.

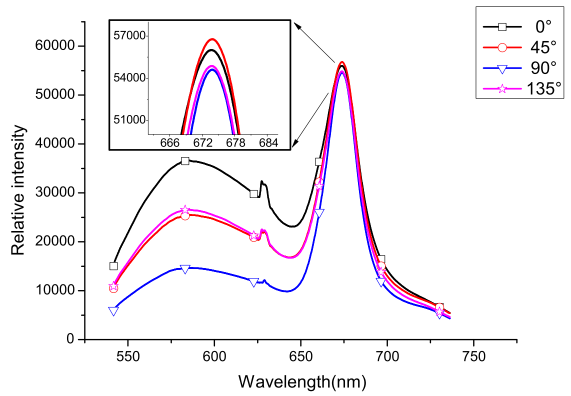

Fig. 3 The rapeseed oil emission spectrums of 0°, 45°, 90°, and 135° under 532 nm excitation laser.

The LIF of vegetable oils when excited by 532nm laser is shown in Figure 2. It can be found that the emission spectra of some edible oils is too similar to classify, especially for walnut and linseed oils. This has only become more evident as we add the numbers of samples. Fortunately,by adding two polarizers to the LIF system, the polarization fluorescence spectra of edible oils can be acquired by rotating the polarizer from 0°to 135° at an increment of 45° Figure 3. Vegetable oils fluorescence spectrums have two main bands centered at 580nm and 675 nm when the samples are irradiated under the 532nm wavelength laser.

As concluded by E. Sikorska, the peak at ∼ 675 nm and ∼725 is due to chlorophyll (a) , and chlorophyll (b), respectively. The peak round at 580nm is caused by oxidation products.[32,33]These oxidation products are converted from the unsaturated fatty acids. Unsaturated fatty acids of vegetable oils can be oxidized when they are exposed to high temperature or light. But, Obviously, the two spikes at 627nm and 630nm are Raman peaks, which correspond to be 2937 cm-1 (C-H stretching (asymmetry)),2856 cm-1 (C-H stretching (symmetry)).[34] The fluorescence spectra of different intensity (Fig. 3) indicate that the DOP vary with wavelength (Fig. 3).

The difference between DOP of different brand oils are enough for classification(Fig. 4). The varying curves of DOP acquired in this paper are due to more than one type of fluorophore (chlorophyll (a) , chlorophyll (b) and oxidation production) contained in the sample.

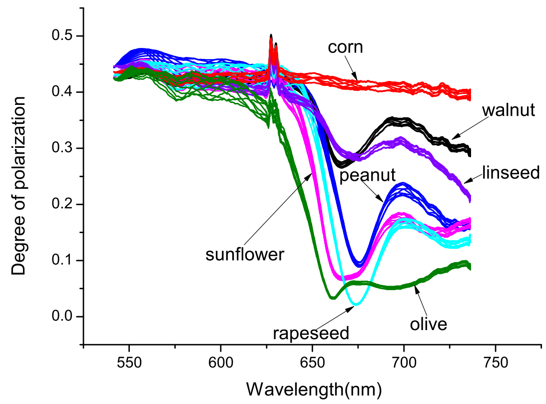

Fig. 4 The DOP spectrums of different vegetable oils, including walnut, peanut, sunflower, rapeseed, linseed, corn, olive under 532nm laser.

Due to the different content of various types of fluorophore, the DOP curves are different from each other. Then fluorescence polarization technique is suitable for studying edible oils. Compared to other vegetable oils, corn oils contain little chlorophyll, which produce the obviously different DOP spectrum in Fig. 4.[32]

3.2 Classification vegetable oils

PCA is initially performed to reduce the dimensionality of the data and examine the intrinsic variation in the data sets by finding key attributes (Figure 5). PCA models built with DOP spectrums data have been used to classify various types of oil samples. It is shown that the first two factors of Z-scores explained 76.02% of the total variance (67.27% and 8.75%). It is clearly shown that seven types of edible and oils are sufficiently separated, without any classification error. Moreover, it is shown that edible oils contain more chlorophyll have smaller scores in the first principal component (Factor1).

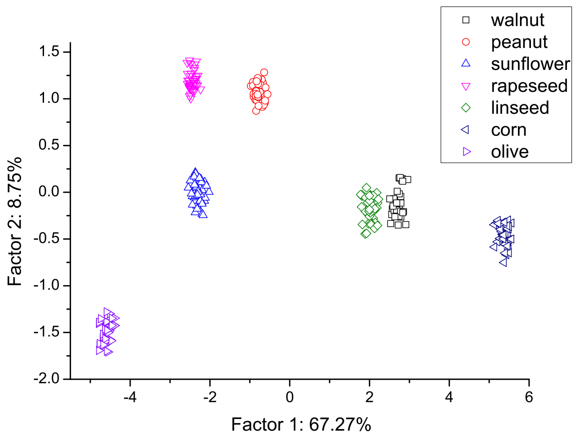

Fig. 5 The PCA scores plot of seven vegetable oils, including walnut, peanut, sunflower, rapeseed, linseed, corn, olive oils

The loadings plot of the first two principle components are shown in Fig. 6. It can be seen that there is a strong correlation between the first principle component and chlorophyll group (chlorophyll a (650-700nm), chlorophyll b (700-730nm)). However, there is no correlation between the first principle component and oxidation products (540-620nm). Unlike the first principle component, the second principle is mainly negative correlative with chlorophyll group.

Then ANN and SVM are employed to recognize seven types of samples. Training set consisted of 210 set of data (30 sets for each type of oil) and test set consisted of 70 sets of data (10 sets for each type of oil). A classification accuracy of 100% are obtained by not only ANN but also SVM for the investigated samples (Table 1). Experimental results indicate that DOP spectrum is effective in classifying vegetable oils together with multivariate statistical methods.

Table 1 The recognition rates of vegetable oils

Samples	Number for train	Number for test	Recognition number	Recognition rates
Wannut	30	10	10	100%
Peanut	30	10	10	100%
Sunflower	30	10	10	100%
Rapeseed	30	10	10	100%
Linseed	30	10	10	100%
Corn	30	10	10	100%
Olive	30	10	10	100%
Total	210	70	70	100%

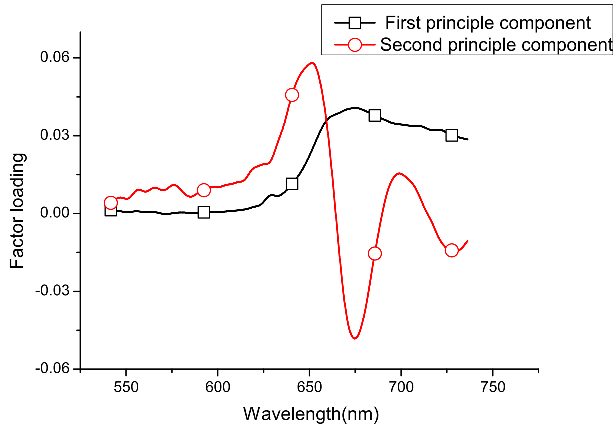

Fig. 6 The loadings plot of the first two principle components of seven vegetable oils, including walnut, peanut, sunflower, rapeseed, linseed, corn, olive oils

4 Conclusions

In conclusion, The classification of edible oils is the basis of authentication identification. Therefore, a new method, DOP spectroscopy,has been proposed to classify the various types of oils. It is demonstrate that DOP is better than LIF, but still have the benefit of LIF(fast and portable).In this paper, a DOP spectrums detection system is created and various types of edible oils are classified. The results indicated that DOP spectrums of different oils are distinct from each other. We can completely classify the seven kinds of edible oils into seven groups with the help of PCA, ANN, and SVM. Due to the fast, portable, and non-destructive characteric, this method can be effectively employed for food analysis.

Acknowledgements

The authors thanks Lin Chih-Jen for version one of LIBSVM. Also, the authors thanks the National Natural Science Foundation of China, No.61178072.

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Graphical Abstract

Polarization of fluorescence is firstly employed in edible oils analysis. It is found that the degrees of polarization of edible oils are distinct from each other which provide favorable conditions for edible oils classification.