

Classification of Edible Oils and Uncovering Adulteration of Virgin Olive Oil Using FTIR with the Aid of Chemometrics

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Abstract: olive oil adulteration with less expensive edible oils is a major issue for the olive oil industry. In this study we have used fourier transform infrared (FTIR) spectroscopy to classify different edible oils including the virgin olive oil. We also have identified adulteration of virgin olive oil with different volume ratios (25, 50 and 75 %) of both corn and sunflower oils quantitatively. The total spectral data in region of (4000 -400) cm^{-1} for all oil samples was recorded and then analyzed using different chemometric tools such as principal component analysis (PCA), clustering and partial least square discriminant analysis (PLSDA).

Key words: FTIR, edible oils, adulteration, PCA, clustering, PLSDA.

INTRODUCTION

Olive oil is an important product in the Mediterranean countries. It is well known for its health benefits. For example, it prevents several cancer types and reduces the risk factors for coronary heart disease (Stark, A.H., Z. Madar, 2002). Virgin olive oil is obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to any chemical change (Haward, J., R. Aparicio, 2000). Acidity is the most important quality parameter for virgin olive oil that gives the oil its organoleptic characteristics. Therefore virgin olive oil might be the most expensive edible oil (Allam M.A. and S.F. Hamed, 2007). This makes unscrupulous sellers adulterate it with less expensive edible oils, mainly sunflower and corn oils due to their close resemblance to virgin olive oil composition (Poulli, K.I., G.A. Mousdis, 2006). Other oils may also be used for adulteration (cottonseed, rapeseed, soy, hazelnut oils and others). However, the relative high prices of these oils besides their low availability in the market compared to the sunflower and corn oils make this type of adulteration less common. Adulteration of olive oil with other edible oils may cause serious health problems (Guimet, F., J. Ferré, 2005). Many analytical techniques can be used to discriminate among different oils as well as to detect adulteration. Most of these techniques rely upon chromatographic methods such as gas and liquid chromatography (GC and HPLC). However, these methods are time consuming, expensive, require skilled operator and produce hazardous chemical waste. In addition, these techniques rely on determination of fatty acids. This renders detection of olive oil purity quite complicated due to the overlapping of fatty acid compositions from other oils especially in case of adulterating with corn and sunflower oils (Aparicio, R., R. Aparicio-RuËe, 2000; Tsimidou, M., R. Macrae, 1987).

Spectroscopic techniques require no or limited sample preparation. Also they provide rapid and noninvasive analytical methods for several products. Nuclear magnetic resonance (NMR) (Mavromoustakos, T., M. Zervou, 1997), spectrophotometry (Kapoulas, V.M., N.K. Andrikopoulos, 1987) and Raman scattering (Guimet, F., J. Ferré, 2005) were used for discriminating among some edible oils and detecting possible adulteration. Synchronous fluorescence combined with chemometrics to improve the sensitivity was also used for detecting virgin olive oil adulteration with sunflower oil (Poulli, K.I., G.A. Mousdis, 2006). Fourier transform infrared (FTIR) was also used for detecting olive oil adulteration (Allam M.A. and S.F. Hamed, 2007), but the reported method of FTIR is subjective due to the poor data analysis method used. It is well known that vibrational spectroscopic techniques such as FTIR lack sensitivity (Skoulika, S.G., C.A. Georgiou, 2003; Ozgul-Yucel, S., A. Proctor, 2004). In this work FTIR combined with chemometrics is used to discriminate among virgin olive oil and other edible oils (sunflower, corn and cottonseed) and determination of virgin oil adulteration with sunflower and corn oils quantitatively.

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To overcome the low sensitivity of the FTIR, a wide scanning range (4000-400) cm^{-1} and chemometric methods (PCA, PLSDA and classification) for data analysis were involved.

MATERIALS AND METHODS

Materials:

Virgin olive, sunflower, corn and cottonseed oils samples used in this study were purchased from local markets. All oils were additives-free based on their labels. For the purpose of differentiation of edible oils, ten samples of each of the above oils were studied. For the quantitative detection of the virgin olive oil adulteration with sunflower and corn oils, a total of 60 samples were prepared and studied. These samples were prepared by mixing the virgin olive oil with different volume ratios (25, 50, and 75 %) of each of sunflower and corn oils. The composition details of the mixed samples are presented in Table 1. The absorbance for all samples including the pure and the mixture oils was measured in triplicates.

Table 1: Six groups of volume ratio combinations of virgin olive oil with sunflower and corn oils.

Oil Type	Group Number					
	G 1	G 2	G 3	G 4	G 5	G 6
Virgin olive	75 %	50 %	25 %	75 %	50 %	25 %
Sun flower	25 %	50 %	75 %	-	-	-
Corn	-	-	-	25 %	50 %	75 %

Spectra Acquisition:

Measurements were carried out by the fully computer-controlled Nicolet iS10 FT-IR spectrometer (Thermo Scientific, MA, USA) with tungsten/halogen source and equipped with fast recovery deuterated triglycine sulfate (DTGS) detector and KBr/Ge mid infrared optimized beam splitter. The FTIR spectral data for all samples (300 replicates) were recorded in the region between (4000-400) cm^{-1} .

Data Analysis:

MatLab 7.0.4 (MathWorks, MA, USA) and PLS_Toolbox 4.0 (Eigenvector Research, Inc, WA, USA) were used for data processing and analysis. Chemometrics involve mathematical, statistical and other methods of formal logic to extract maximum information from chemical data (Lavine, B.K., 2000). Chemometrics consists of several tools for data analysis. Pattern recognition and classification tools may be the most common chemometric tools (Wise, B.M., N.B. Gallagher, 2007). Principal component analysis (PCA) is a pattern recognition tool commonly used for finding differences and similarities in the data. PCA is often used to reduce multidimensional data sets to lower dimensions (PCs) with minimum information loss. Mathematically, PCA represents the eigenvectors for the covariance or correlation matrix of the original data matrix containing the measured variables. Number of eigenvectors is related to variable numbers. The eigenvector associated with the largest eigenvalue has the same direction as the first principal component. The eigenvector associated with the second largest eigenvalue determines the direction of the second principal component. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible (Poulli, K.I., G.A. Mousdis, 2005; Obeidat, S.M., T. Glasser, 2007; Wise, B.M., N.B. Gallagher, 1999). Partial least square discriminant analysis (PLSDA) is a classification method which identifies the differences between two or more classes. It is often used for classifying similar samples (spectroscopically) together in a separate cluster away from other classes through finding the fundamental relations between the data sets (Poulli, K.I., G.A. Mousdis, 2005; Trygg, J., S. Wold, 2002). In cluster analysis, each sample is initially assumed to be a lone cluster. All the distances between samples are then calculated (*e.g.* via Mahalanobis distance), and the samples with the smallest distance are identified. These samples are then joined together. The procedure is repeated and the samples with the next closest distance are found and linked. The results are often displayed as a connection dendrogram (Wise, B.M., N.B. Gallagher, 2007; Jolliffe, T., O.B. Allen, 1989).

RESULTS AND DISCUSSION

Oil Identification:

The first task of this work was to discriminate among four edible oils. For this purpose, 10 samples of each of (olive, corn, sunflower and cottonseed) oils were investigated. Triplicate trials of each sample were created and randomized prior to FTIR scan. The FTIR spectra in the range of (4000 - 400) cm^{-1} of all samples (a total of 120 samples) were recorded. The spectral data of each sample was saved in one excel file. All

spectra were background corrected. The three replicates of each sample were averaged to produce 40 excel files (10 files for each oil type). Then all the 40 files were concatenated in a single two dimensional data matrix using MatLab 7.0.4. The resulted data consisted of 40 columns and 1860 rows. Each column contains the spectral data (wavenumber) of one averaged sample and the rows represent the absorption intensities. MatLab codes for data processing were written in our laboratories. PCA, PLSDA and clustering tests were applied to the resulted matrix.

PCA:

Prior to PCA application the data were normalized and mean centered. The first three PCs were found to carry the maximum variation among the data matrix. In order to show the differences among the edible oils spectra, the score plot using the first and the third principal components was used (Fig. 1). Four clusters can be clearly identified. Each cluster represents one type of oil. The farther the distance between the clusters the more different the spectra are. This result clearly shows the ability of this technique to discriminate among the four edible oils used in this study. This model (Fig. 1) can be then used as a calibration model for identifying test oils by applying the model on other spectral data which belong to test samples (Obeidat, S., B. Bai, 2008). Also it can be used to detect adulterated virgin olive oil qualitatively.

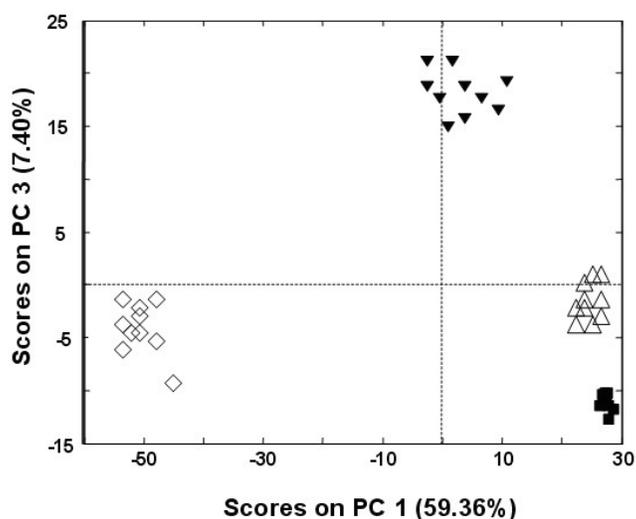


Fig. 1: Score plot of PCA model, Corn oil (▼), Sunflower (□), Cottonseed oil (Δ) and Olive oil (◊).

Hierarchical Cluster Analysis (HCA):

HCA is an unsupervised technique that uses the information obtained from measured variables to reveal the natural clusters existing between the studied samples (Peterson, L.E., 2002; Skrobot, V.L. E.V.R. Castro, 2005). Figure 2 shows the resulted dendrogram obtained from HCA, where four clusters can be identified. Cluster A contains 10 corn oil samples, cluster B contains 10 sunflower samples, cluster C contains 10 cottonseeds oil samples and cluster D contains 10 virgin olive oil samples. The dendrogram shows that the four types of oil including the virgin olive oil are well separated with no overlapping between any two types of oil. This indicates 100% correct classification.

PLSDA:

The pure oil samples were investigated by another chemometric tool, PLSDA. Four latent variables (LVs) were found to carry the maximum variation among the dataset. Like PCA, in PLSDA model four separate clusters (each cluster represents one oil type) were identified clearly using the first and the fourth LVs (Figure not shown). For further inspection the prediction of each oil samples was explored within the four LVs PLSDA model. The samples of each oil type clustered independently of other oil samples within the estimated threshold calculated from Bayes Theorem. The results in this part of study came out to be consistent with the previous results.

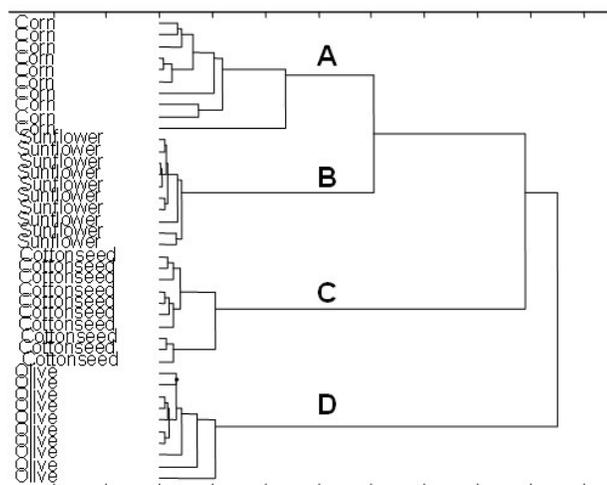


Fig. 2: HCA dendrogram of corn oil (class A), Sunflower oil (class B), Cottonseed oil (class C) and virgin olive oil (class D) using Mahalanobis distances.

Quantitative Detection of Virgin Olive Oil Adulteration:

The most common type of virgin olive oil adulteration is via mixing it with different quantities of either corn or sunflower oils (Allam M.A. and S.F. Hamed, 2007). In this study virgin olive oil was mixed with different volume ratios (25, 50 and 75) % of corn oil (groups 1, 2 and 3) and sunflower oil (groups 4, 5 and 6). The exact mixture combinations are presented in Table 1. Ten samples of each mixture combination in Table 1 were prepared. These mixtures represent adulterated virgin olive oil samples. FTIR spectrum for each of the 10 samples of all combinations in Table 1 was run in three replicates. The replicates were averaged after subtracting the background from each replicate spectrum. The spectral data of the mixture samples (groups 1 to 6) were concatenated in a single data matrix with dimensions of (60 x 1860). The resulted data matrix is used for testing the ability of chemometric tools to detect such mixtures. A new PLSDA model was created using only the pure samples of virgin olive, corn and sunflower oils. Again, a two dimensional PLSDA model was created using the first and the fourth LVs. Each of the pure oils clustered clearly in the model. The resulted model was used as a calibration model for testing the mixtures in groups 1 to 6. The PLSDA model was applied to data matrix containing the mixture samples (groups 1-6). The result of this application is shown in Figure 3. It can be seen that each group of the mixture samples clustered by itself away from the pure oil clusters and other mixtures. This indicates the ability of such model to distinguish among pure oil types including the virgin olive oil and the adulterated olive oil samples (group 1 to 6) clearly within a 95% confidence limit.

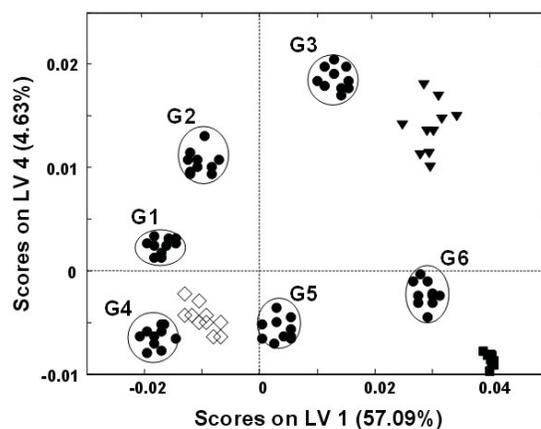


Fig. 3: Application of Score plot of PLSDA calibration model using Corn oil (▼), Sunflower (□) and olive oil (◇) on the mixture samples (G1-G6) (see Table 1).

From the separated clusters of the adulterated samples (groups 1 to 6), it is obvious that the present technique is able to make a qualitative detection of adulterated olive oil with corn and sun flower oils. The PLSDA model including the six adulterated samples (Fig.3) can be used as a calibration model for testing suspected adulterated virgin olive oil samples with corn or sunflower oils. For further investigation, the mixtures can be tested in the former PCA model to give comparable results to these obtained using the PLSDA model.

Conclusion:

The current study shows that the presented technique (FTIR-Chemometrics) is powerful in discriminating among edible oils (Corn, sunflower, cottonseed and olive) through comparing the spectral data of these oils in the range of (4000 – 400) cm^{-1} , without the need of the direct method of studying the FTIR spectra for individual oils which could be time consuming and highly subjective. Also the present study suggests a reliable quantitatively method for detecting adulteration of the virgin olive oil with corn or sunflower oils.

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