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Detection of adulteration of coconut oil using Fourier transform infrared spectroscopy and chemometrics

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Abstract
Virgin coconut oil may be adulterated with cheaper oils, such as palm oil and mustard oil. The detection and quantification of virgin coconut oil adulteration with palm oil and mustard oil were monitored using Fourier transform infrared spectroscopy, combined with chemometrics of Principle component analysis, Hierarchical cluster analysis and Discriminant analysis of frequency regions of 4000-400cm^{-1}. The principal component analysis, hierarchical cluster analysis and discriminant analysis using two principal components were able to classify virgin coconut oil and the same adulterated with palm oil and mustard oil.

Keywords: FTIR spectroscopy, adulterants, coconut oil, mustard oil, chemometrics, principle component analysis

Introduction

The adulteration is sometimes acted deliberately and sometimes accidentally (Shukla *et al.,* 2005) [10] which involves the replacement of high price oils with cheaper ones. The adulteration of fats and oils is not easy to detect when the oil adulterant has a composition approaching to that of the original oil (Rossell *et al.*, 1983) [9]. Most initial researches conducted to analyze the oil adulterant, involved time consuming and laborious processes such as wet chemical methods and chromatography techniques. Therefore, there is a demand to develop rapid and sensitive method for detecting and quantifying the adulterants.

Fourier transform infrared (FTIR) spectroscopy combined with chemometric tools has received a great attention to be used in the quantitative analysis and to analyze virgin oils in the mixture with other edible oils (Che Man and Mirghani, 2001). In the case of studies of authentication, FTIR combined with chemometrics has been explored to detect adulterants such as lard in cod-liver oil (Rohman and Che Man, 2009a) [7], palm oil (Rohman and Che Man, 2009b) [8], and palm kernel oil (Manaf *et al.,* 2007) [6] in virgin coconut oil, sunflower, corn, soybean, and hazelnut oils (Lerma *et al.*, 2010) [5], corn oil (Gurdeniz and Ozen, 2009) [3], and sunflower oil (Downey *et al.*, 2002) [2] in extra virgin olive oil (EVOO), soybean oil in camellia oil (Wang *et al.*, 2006) [12], and some vegetable oils in olive oil (Tay *et al*., 2002) [11]. Therefore, this study is directed to analyze the deduction of the adulteration of coconut oil with palm oil and mustard oil using chemometrics of principal component analysis, hierarchical cluster analysis and discriminant analysis for quantification and classification.

Materials and Methods
Sample Preparation

Vegetable oils, such as virgin coconut oil, palm oil and mustard oil were purchased from the local market. The virgin coconut oil was mixed with palm oil and mustard oil in different proportions i.e., 5, 10 and 15% v/v in the neat forms. To complete the homogenization, these mixtures were shaken vigorously.

All samples were analyzed using an FTIR spectrometer. The spectral regions where the variations were observed between the virgin coconut oil and the same adulterated with palm oil and mustard oil were chosen for developing the chemometric tools.

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2.2 Statistical analysis

For each adulterant a different set of samples containing three concentration levels of adulterant, were used for calibration; the corresponding admixtures were used for validation. Principal Component Analysis, Hierarchical Cluster Analysis and Discriminant Analysis were used for each blend with the respective training set samples by using the SPSS version 17.0.

3.1 Principal Component Analysis

Before proceeding with the factor analysis of the present data, the adequacy test was applied. The rule of thumb, index, provided by the Kaiser measure of sampling adequacy was applied to acquire a rough idea of whether the data are adequate for the technique or not.

The following table.1 provides the KMO index for the virgin coconut oil with adulterants of palm oil and mustard oil. The KMO measure indicates the marvelous level of coconut oil adulterant (0.805) and Barlett's value of 2066 with a significance of 0.000. Hence, the factor analysis was carried out further to differentiate against adulterants.

Table 1: Kaiser-Meyer-Olkin Measure of Sampling Adequacy

Kaiser-Meyer-Olkin Measure of Sampling Adequacy	0.805
Bartlett's Test of Sphericity Approx. Chi-Square	2066.00
df	21
Sig.	0.000

As a first step, the correlation matrix for the 7 different adulterant groups on edible oils was examined. The results clearly indicate that all the values are positive and much variation was also noticed.

In the factor extraction phase, the numbers of common factors exhibited to adequately describe the data are determined. The decision is based on the eigenvalues and percentage of total variance accounted for by different number of factors.

Several methods have been proposed for determining the number of factors to be used in the given model. One criterion suggests that the only factors that account for eigenvalue greater than 1.0 should be included in the analysis. The cumulative percentage of variance and percentage of variance accounted for each factor with eigenvalue are summarized in the following table.2. From the above table.2, it is evident that the only two factor component was exhibited with eigenvalues of 6.269 and 1.713.

Table 2: Factor extraction and its relative variance

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.269	89.553	89.553	6.269	89.553	89.553	3.791	54.157	54.157
2	1.713	10.183	99.736	1.713	10.183	99.736	3.191	45.579	99.736
3	.017	.238	99.974						
4	.001	.019	99.993						
5	.000	.007	100.000						
6	0.0000006346	0.000009066	100.000						
7	0.0000003183	0.000004547	100.000						

The first factor (component) accounts for 89.55% of the total variance and the second factor accounts for 10.18% of the variance. Hence, the total variance explained by these two factors accounts for 99.73%. So only a meager 0.264% of variance was not explained with the remaining components. In order to confirm the initial factors extracted in the principal component analysis, the rotation was carried out using VARIMAX rotation. After the initial factor extraction, the varimax rotation was carried out and the results show that, even in this face, only two component models were exhibited with an eigenvalue of 3.791 and 3.191 with the percentage of variance of 54.15% for the first component and 45.57% for the second component (Table.2). Hence, the total variance explained by these two component accounts for 99.73%.

Therefore, it can be stated that using principal component analysis, the variables of spectral data can be described by the principal components because the first two principal components can describe more than 90% of the variation. The scree plot (Fig.1) indicates clear identification of only two components. It can be concluded that FTIR spectral data in combination with chemometrics of principal component analysis can be exploited for differentiation and classification of virgin coconut oil from their adulterants. From the scatter points, the samples could be classified into two groups, indicating a clear differentiation between adulterations blended with virgin coconut oil at different proportions.
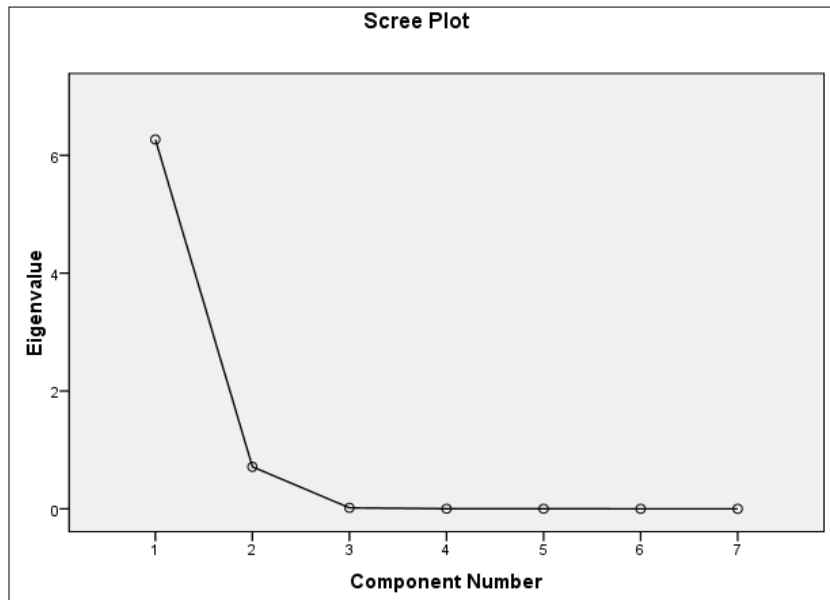
Fig 1: Scree plot for adulterated oils

Factor analysis is used to find out different groups of attributes which are important in quantifying the adulteration of oils. The principal component analysis finds a linear combination of attributes explaining the variability, contributed by individual variables.

After identifying the number of factors, the variables included in each factor are distinguished. The factor loadings are considered as the best measure to include variables into factors. The factor loading is considered to be the best method associated with a specific factor and a specific state and it is simply the correlation between the factor and the statement's standardized score. The table shows the factor loading on the 7 variables (Table. 3).

The factor - matrix pattern reflects a reasonably clear loading structure. Reference groups can be very potent and influential.

Table 3: Factor loading structure of virgin coconut oil and adulterants

	Component	
	1	2
Palm oil 5%	0.963	-0.267
Palm oil 10%	0.963	-0.267
Palm oil 15%	0.963	-0.268
Virgin Coconut oil%	0.953	-0.281
Mustard oil 5%	0.928	0.372
Mustard oil 15%	0.927	0.375
Mustard oil 10%	0.927	0.375
Total	3.791	3.191
% of variance	54.157	45.579
Cumulative %	54.157	99.736

In the present study, the samples of virgin coconut oil adulterated with palm oil 15% (with a loading value 0.963), palm oil 10% (with a loading value 0.963) and palm oil 5% (with a loading value 0.963) and the virgin coconut oil are associated and form into factor 1. In the second component, adulterants of virgin coconut oil with mustard oil 15% (with a loading value 0.375), mustard oil 10% (with a loading value 0.375 and mustard oil 5% (with a loading value 0.372) are grouped into factor 2 (Table.3). This clearly indicates that in the case of coconut oil, the FTIR spectral data of the palm oil adulterants did not show any significant difference, whereas, the mustard oil adulterants of 5, 10 and 15% with the coconut oil will not come closer to the earlier factor.

The factor matrix pattern also reflects the reasonable clear loading structure which is given in Fig.2. Two factor exhibits combining virgin coconut oil and palm oil of different percentage as an isolated factor.
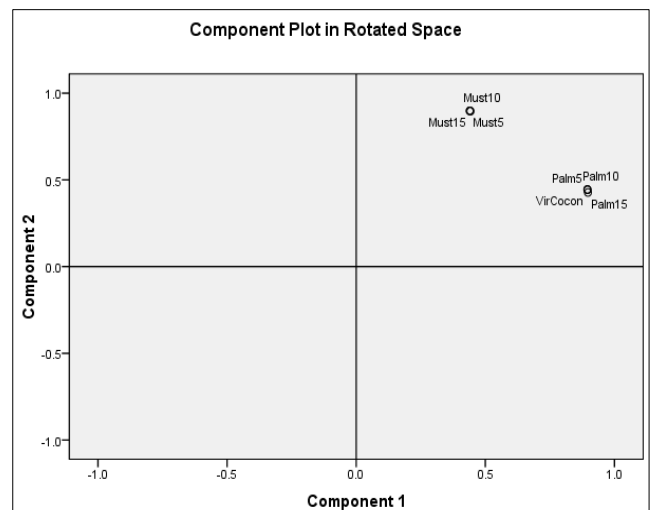

Fig 2: Principal Component analysis (PCA) under varimax rotation for the virgin coconut oil adulterants

Hierarchical Cluster Analysis

The agglomeration schedule table shows the change in the coefficient which deals the cases which are closer and forming into different clusters. Table.4 comprises six clusters. The first stage of agglomeration schedule shows that the oil samples 6 and 7 are combined to form the first cluster with a close relationship of a minimum coefficient value of30.820. At stage 2, a new cluster (second cluster) is formed with the oil sample numbers 1 and 2 with a value of 2947.706. At stage 3, the oil samples 1 and 3 are formed into a third cluster with a coefficient value of 21577.584. At stage, 4 the oil sample 5 and 6 are formed in the fourth cluster with a coefficient value of 89948.563. At stage 5, the oil samples 1 and 4 are formed in a fifth cluster with a coefficient value of 999395.736. In the last stage, the oils 1 and 5 are joined to form the sixth cluster with a coefficient value of 180200000.00. The coefficient columns give the mean distances for combining the clusters.

Table 4: Agglomeration Schedule of virgin coconut oil adulterated with palm oil and mustard oil

Stage	Cluster Combined		Coefficients	Stage Cluster First Appears		Next Stage
	Cluster 1	Cluster 2		Cluster 1	Cluster 2	
1	6	7	30.820	0	0	4
2	1	2	2947.706	0	0	3
3	1	3	21577.584	2	0	5
4	5	6	89948.563	0	1	6
5	1	4	999395.736	3	0	6
6	1	5	180200000.00	5	4	0

These coefficients depend on the method which is chosen for forming the cluster. The graphical presentation of the hierarchical clustering results (Fig.3) can be represented in the form of a dendrogram, where two clusters can be identified. Since the distance is very close, only two major visible clusters are formed which were termed as cluster A and cluster B.

The hierarchical cluster analysis, at the distance of oils can be grouped as follows:
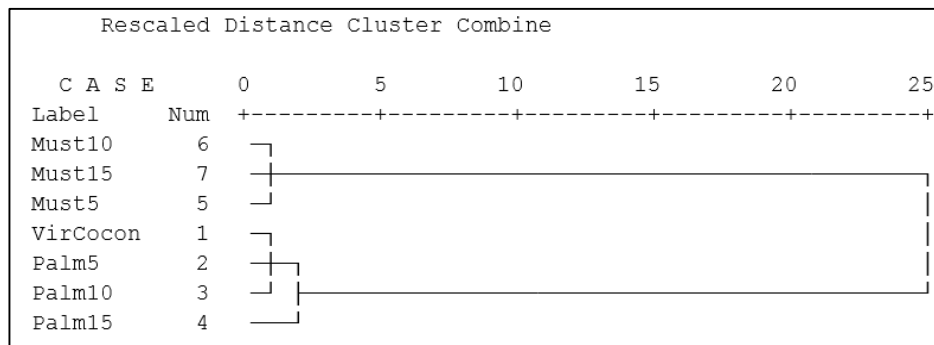
Cluster A comprises of mustard oil 10%, mustard oil 15% and mustard oil 5%

Cluster B comprises of virgin coconut oil, palm oil 5%, palm oil 10% and palm oil 15%.

The dendrogram shows that the samples of palm oil 15% clustered with palm oil 10% forms into a homogenous cluster.

At the second cluster level, the virgin coconut oil is clustered with palm oil 5% level and the other clusters are with the long distance (Fig.3). The dendrogram shows that the virgin coconut oil is quite homogeneous. From the above results it is clear that mustard oil used as an adulterant with the coconut oil can easily be identified, whereas palm oil by 10% adulterated with the coconut oil is very difficult to identify. Detection of such natural groupings suggests that discrimination between at least some of them may be possible. Hence, blending of palm oil with coconut oil as an adulterant is hard to find with the help of the spectral data.

Dendrogram using Single Linkage

```
        Rescaled Distance Cluster Combine

   C A S E       0         5        10        15        20        25
  Label    Num   +---------+---------+---------+---------+---------+
  Must10     6     ─┐
  Must15     7     ─┤─────────────────────────────────────────────┐
  Must5      5     ─┘                                              │
  VirCocon   1     ─┐                                              │
  Palm5      2     ─┤─┐                                            │
  Palm10     3     ─┘ ├──────────────────────────────────────────┘
  Palm15     4     ───┘
```

Fig 3: Dendrogram of edible oil data set showing two natural groupings using the single linkage method.

Discriminant analysis

The edible oil variable is independent of the study that comprises of two factors, namely virgin coconut oil and adulterants namely palm oil and mustard oil. From this output; we can see that virgin coconut oil, palm oil and mustard oil differ noticeably from group to group. One attribute of edible oil construct representing the virgin coconut oil. Meanwhile the adulterants contain three attributes as follows; palm oil consists of three attributes, namely palm oil 5%, palm oil 10% and palm oil 15% in proportion and the

mustard oil consist of three attributes, namely mustard oil 5%, mustard oil 10% and mustard oil 15% in different proportion. The dependent variable for the present study is the adulterant which consists of three categories 5%, 10% and 15% in different proportions.

In order to determine whether the edible oils are capable for subsequent analysis, the results of the present study based on Multiple Discriminant analysis on edible oil are discussed below.

Table 5: Mean value of spectral wave number of virgin coconut oil, palm oil and mustard oil adulterants

Group		Mean	Std. Deviation	Valid N (list wise)	
				Unweighted	Weighted
Coconut	Percent5	2582.57	997.104	52	52.000
	Percent10	2582.57	997.104	52	52.000
	Percent15	2582.57	997.104	52	52.000
Palm	Percent5	2517.91	1052.561	52	52.000
	Percent10	2580.15	996.445	52	52.000
	Percent15	2563.79	989.259	52	52.000
Mustard	Percent5	2596.32	1014.750	52	52.000
	Percent10	2590.51	1011.476	52	52.000
	Percent15	2590.66	1011.647	52	52.000
Total	Percent5	2565.60	1015.700	156	156.000
	Percent10	2584.41	995.225	156	156.000
	Percent15	2579.01	992.975	156	156.000

Table.5 displays the means for three different categories of edible oil with regard to three attributes. An examination of Table.6 shows the existence of several significant mean differences across the oil adulterants. Multiple discriminant analysis was used to explore the degree to which FTIR spectra of oils can match with the functional groups. Only the first discriminant function (Wilk's $\lambda=0.978$ $x^2=3.373$; DF=6,

p=0.761) is significant and explains 99.8% variance of adulterant oil (Table.6). The result of the second function was Wilk's $\lambda=1.000$; $x^2=0.005$; DF=2, p=0.997. Since the second function is not significant, its associated statistics will not be used in the interpretation of the ability of the seven constructs to discriminate among three categories of edible oils.

Table 6: Eigen values and Wilks' Lambda

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation	Wilks' Lambda	Chi-square	df	Sig.
1	.022[a]	99.8	99.8	.148	.978	3.373	6	.761
2	.000[a]	.2	100.0	.006	1.000	.005	2	.997

The standardized coefficients and discriminant loadings for each attribute of edible oils are provided in Table.7. The standardized coefficients denote the partial contribution of each of the attributes of subjective norm construct to the discriminant function. The largest subjective norm constructs to the discrimination between categories of the edible oils.

Table 7: Standardized Canonical Discriminant function Coefficients

	Function	
	1	2
Percent5	2.826	2.005
Percent10	-10.767	6.582
Percent15	8.093	-8.011

Table 8: Structure Matrix

	Function	
	1	2
Percent5	.224	.656*
Percent10	.021	.528*
Percent15	.074	.473*

As it can be seen from Table.8, palm oil was the main contributing attribute of blending with coconut oil (discriminate loading=0.224) in discriminating between all categories of edible oil. Substantive loadings (>±., 30, Hair *et al*., 1998) were also obtained from palm oil adulterant at 5% v/v proportion (0.224). *F*-tests of the equality of group mean, illustrated in Table.9, supported these results. All *p*-values >0.001 indicate that, for each of the other three attributes of the edible oil construct, means are equal across the two adopter categories of adulterants (Table.9).

Table 9: Tests of Equality of Group Means

	Wilks' Lambda	F	Sig.
Percent5	.999	.087	.916
Percent10	1.000	.002	.998
Percent15	1.000	.010	.990

Table 10: Functions at Group centroids

Group	Function	
	1	2
Coconut oil	.096	-.007
Palm oil	-.209	.000
Mustard oil	.114	.007

An examination of group centroids clearly suggests that the function 1 discriminates between palm oil and coconut oil. When compared to coconut oil, mustard oil blend offer higher influence for mixing(Table.10).There is not much influence of palm oil blend with coconut oil. The mustard oil blend with groundnut oil influences them for positive response to be used as adulterants.

Further, discrimination was successful in classifying 36.5% of original grouped cases correctly classified and 7.7% cross-validated grouped cases correctly classified (Tables.11& 12).

Table 11: Classification function coefficients

	Group		
	Coconut oil	Palmoil	Mustard oil
Percent5	.001	.000	.001
Percent10	.000	.003	.000
Percent15	.002	.000	.002
(Constant)	-4.452	-4.418	-4.477

Table 12: Classification Results (a)

Group	Predicted Group Membership			Total
	Coconut oil	Palm oil	Mustard oil	
Original Count				
Coconut oil	17	4	31	52
Palm oil	16	7	29	52
Mustard oil	15	4	33	52
%				
Coconut oil	32.7	7.7	69.5	100.0
Palm oil	30.8	13.5	55.8	100.0
Mustard oil	28.8	7.7	63.5	100.0
Cross-validated[a] Count	3	11	38	52
Coconut oil Palm oil	23	0	29	52
Mustard oil	38	5	9	52
Coconut oil %	5.8	21.2	73.1	100.0
Palm oil	44.2	0	55.8	100.0
Mustard oil	73.1	9.6	17.3	100.0

4. Conclusion

The present study reveals that the FTIR spectroscopy, in combination with the multivariate chemometric methods, represents an effective strategy for fast and reliable identification and detecting adulteration of virgin coconut oil with palm oil and mustard oil as a potential adulterant. Chemometrics combined with FT-IR spectroscopy proved to be an excellent method for quality control of coconut oil and identifying substances by providing unique fingerprint spectra in classifying oils and detecting adulteration of palm oil and mustard oil. The results of the present study, which concluded with the detection of oils using FTIR spectral data and chemometric analysis indicates a necessity for food related organizations to develop and utilize more reliable methods to detect such adulterations, which can make consumers and markets more certain on the authenticity and purity of edible oils.

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