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Application of Multivariate Analysis for Detection of Crude Palm Oil Adulteration through Fatty Acid Composition and Triacylglycerol Profile

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ABSTRACT

This study focused on developing a reliable procedure for the identification of the adulteration of crude palm oil (CPO) by blending sludge oils (SO) and used vegetable oils (UVO) ranging from 1 to 20% (v/v). Fatty acids methyl esters (FAME) and Triacylglycerol composition consisting of all single and blended CPO were analysed using a gas chromatography (GC)-flame ionisation detector (GC-FID) and high performance liquid chromatography evaporative light scattering detector (HPLC-ELSD), respectively. The results were processed using the multivariate analysis i.e. principal component analysis (PCA) and cluster observation (CO) to discriminate the most applicable factors useful for detecting this adulteration. The results revealed that the combination of chemical properties and multivariate analysis resulted in a strong differentiation between the blends according to the amount of adulterant in the CPO. PCA and CO provided good results, allowing detection of the adulteration of the CPO with the SO and UVO as low as 5% and 2% respectively for each multivariate analysis.

Keywords: Adulteration, crude palm oil, fatty acid methyl esters, GC-FID, HPLC-ELSD, principal component analysis, triacylglycerol

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INTRODUCTION

The oil palm is believed to be originated from Africa, but at present, production is mainly based in the tropical areas of

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America, Africa and Asia, with Malaysia and Indonesia being the most productive countries (Corley, 2003; Sundram, 2005). Oil palm has the highest yield per hectare compared to any other oil crops. In the year 2012, Malaysia accounted for 39% of world oil palm production and 44% of world exports. Generally, oil palm produces two types of oil from its fruit: mesocarp oil or crude palm oil (CPO) and palm kernel oil (PKO). CPO consists of 50% saturated and 50% unsaturated fatty acids. Its major triglycerides are tri-saturated (10.2%), di-saturated (48%), mono-saturated (34.6%), and triunsaturated (6.8%) fatty acids (Corley, 2003; Sundram, 2005; Soh, 2008).

Palm oil is one of the most commonly used vegetable oils in the world today, which accounts for 33% of all oils consumed globally (Kumar, 2013). This situation demands for a high quality of palm oil to be produced for domestic and industrial applications. However, the quality of palm oil is affected by various factors from harvesting, handling, processing fresh fruit bunches and the storing methods of crude palm oil. Recently, there has been speculation that crude palm oil is being adulterated in order to increase profit margin. Countries like Nigeria and India have been found guilty with adulterated cases that have an impact on consumer's health (Soh, 2008; Okonkwa, 2012). Since there is variability within the fatty acid and triacylglycerol composition of crude palm oil, mixing crude palm oil with other oils with similar composition while examining the oil on the physical, chemical and nutritional properties is an analytical challenge.

Principal component analysis (PCA) is widely used for the evaluation of olive oil and virgin olive oil (Pizarro, 2011; Rohman, 2012; Salces, 2010). The use of PCA treated data enables the detection of adulterants in olive oil and virgin olive at different levels. On the other hand, through cluster analysis, samples are clustered together based on the similarity of their chemical and physical properties and the results are displayed as a connection dendrogram. Previous researchers (Fragaki, 2005; Obeidat, 2009) reported that a clear and independent cluster was obtained for further prediction of the adulterant in the virgin olive oil blends.

Therefore, the aim of this study was to verify the effectiveness of fatty acid composition determined by GC-FID and triglyceride composition identified by HPLC-ELSD. This is followed by chemometric tools, principal component analysis and cluster observation to detect the adulteration of crude palm oil with sludge oil and recycled oil in the level of 1 to 20% (v/v). To our best knowledge, there is no published report investigating the adulteration of crude palm oil using multivariate analysis. Most of the studies on the authentication and quality of oil that were profoundly investigated and thereafter published focused on olive oil or virgin olive oil. (Christopoulou, 2002; Gamazo-Vazquez, 2003; Diza, 2005; Jafari, 2009; Bucci, 2002; Capote, 2007; Zabaras, 2004; Gurdeniz, 2009).

MATERIAL AND METHODS

Materials

All reagents used were analytical grade. The analysed samples were crude palm oil (CPO), sludge oil (SO) and used vegetable oil (UVO) and admixtures of CPO. CPO samples were obtained from Genting Ayeh Item Oil Mill (GAIOM, Ayeh Hitam, Johor, Malaysia) and used vegetable oil was obtained from oils used in the cooking of french fries and chicken nuggets in the laboratory. To avoid oxidation and chemical composition changes, all samples were kept at -80^{o}C in the freezer immediately after arrival in the laboratory. Mixtures of the CPO sample with each one of the adulterant oils were prepared. For each adulterant oil, five mixtures with double replicate (Treatment=7, replicate=2) were prepared with percentages 1, 2, 5, 10 and 20% of the respective oil in the genuine CPO. Altogether, n=28 samples of genuine oil and admixtures were prepared. These admixtures together with the genuine oil were analysed after the preparation.

Analysis of Fatty Acid Composition (FAC)

Fatty acid composition of oil sample was determined by taking 1g oil sample and refluxing with 40mL 0.01 M of sodium methoxide solution and 1M methanolic hydrochloric acid solution for 30 minutes (Ainie *et al.,* 2005). Hexane was added in the solution and shaken vigorously. The upper layer of the solution was then analysed using a gas chromatograph (Model: 2014, Shimadzu, Fisher Scientific, Kyoto, Japan). The analysis was performed on a 30mx0.2 capillary column using a gas chromatograph (GC) connected to a flame ionisation detector (FID). The GC conditions used were as follows: injection volume 1 uL, split injection 50:1 at 240^{o}C; oven temperature set at 90^{o}C, then ramped to 165^{o}C at 5^{o}C min^{-1}, then ramped again to 205^{o}C at 2^{o}C min^{-1} and final ramping to 220^{o}C at 15^{o}C min^{-1}(hold 4min). The total run time was approximately 40 minutes. The helium gas carrier was held at a constant flow rate of 1mL min^{-1}, whilst the detector was set at a temperature of 300^{o}C. Individual peaks of fatty acid methyl esters were determined using Supelco F.A.M.E. Mix, C8 –C24 fatty acids standards.

Analysis of Triacylglycerol (TAG) Profile

Triacylglycerol composition was determined by a high performance liquid chromatograph-evaporative light scattering detector (HPLC-ELSD, Model 2695, Waters, Massachusetts, USA). The HPLC system used was equipped with an autoinjector and *evaporative light scattering* detector. The mobile phase was a mixture of acetone/acetonitrile (63.5:36.5 v/v) and the flow rate was 1mL/min. The injection volume was 10µL of 5% (w/v) oil in acetone. Triglyceride peaks were identified based on the retention time of supelco lipid standards of triglyceride mixtures.

Experimental Design and Multivariate Data Analysis

Fatty acid and triglycerides compositions were the main components applied in

this experiment to detect the adulteration of the CPO with the SO and UVO. Five treatments with double replicate were assigned based on the applied combination levels. The experimental design, data analysis, optimisation procedure and method validation were performed using the Minitab v. 14.0 statistical package (Minitab Inc., State College, PA, USA).

Principal component analysis (PCA) and cluster observation (CO) were applied to obtain an overview of correlation between the samples. The correlation matrix was applied with multivariate analysis using Minitab 14.0. Multivariate analysis for processing chromatographic data is an efficient tool for classification and searches for similarities of oil samples, and this provides good quality control.

RESULTS AND DISCUSSION

Detection of Adulterants in Crude Palm Oil Based on FAC and TAG

Table 1 and 2 present the results of the analysis of the crude palm oil (CPO), genuine adulterant oil (Sludge Oil (SO) and used vegetable oil (UVO)) and their admixtures with CPO. The values of fatty acid and triglycerides in the CPO were similar as that reported by Sundram, 2005. However, there were no official values of fatty acid and triglycerides for SO and UVO that have been reported. Comparison between the CPO with SO and UVO showed that there were similarities with regards to triglycerides with ECN of 46, 48 and 50 and fatty acid compositions (C12-C120). Sludge oil (SO) or palm oil mill effluent is the voluminous liquid waste that comes from the sterilisation and clarification sections of the oil palm milling process whereas UVO is produced from used cooking oil derived from refined CPO. Since both SO and UVO are derivatives from the CPO milling process and refined, their triglycerides and fatty acid compositions are similar to that of the native CPO.

In the present study, detection of adulteration of CPO up to the concentration of 20% was investigated. The results for the fatty acid and triglyceride composition of admixtures of CPO with SO and UVO are presented in Table 1 and 2. Based on the results, parameters for detecting adulteration were examined. From the results presented in Table 1 and 2, it could be concluded that the analysis of fatty acid and triglyceride composition does not provide satisfactory results and could not be used as a basis for detecting the adulteration. As the level of adulteration increased gradually from 1 to 20 % there were no major changes in the compositions observed. Changes were very minor in the admixtures of CPO. This is mainly due to the dominant effect of the CPO, which suppressed the adulterants' chemical profiles even at 20% concentration level.

Additionally, a one-way ANOVA was performed on the whole data set in Tables 1 and 2 in order to compare for each variable and the variance within any category. The results showed that there were no differences among the treatments. Hence, a one-sample T-test (df=6 and CI=90%) was applied. The results from the T-test revealed that

TABLE 1
Fatty Acid Composition (%) and Triacylglycerol ECN (%) of Crude Palm Oil (CPO) and Admixtures with Sludge Oil (SO)

Sample	Fatty Acid Composition					Triglycerides (ECN)		
	Carbon 12*	Carbon 14	Carbon 16	Carbon 18*	Carbon 20	46	48*	50*
Genuine Crude Palm Oil (CPO)	0.42	1.09	44.47	53.67	0.35	17.28	69.70	4.00
99 CPO : 1 SO	0.42	1.09	44.44	53.70	0.35	17.28	69.69	3.99
98 CPO : 2 SO	0.43	1.09	44.41	53.72	0.35	17.28	69.68	3.99
95 CPO : 5 SO	0.44	1.09	44.31	53.80	0.35	17.29	69.66	3.97
90 CPO : 10 SO	0.47	1.10	44.15	53.93	0.35	17.30	69.62	3.95
80 CPO : 20 SO	0.53	1.11	43.82	54.19	0.35	17.32	69.54	3.89
Genuine Sludge Oil (SO)	0.96	1.21	41.19	56.29	0.35	17.48	68.92	3.46

Each value in the table represents the mean of fatty acids (n=2)
Carbon 12: Methly Laurate; Carbon 14:Methyl Myristate; Carbon 16:Combination of Methyl Palmitate, Palmitoleic Acid Methyl Ester; Carbon 18:combination of Cis-9-oleic methyl acid, Methyl Linoleate, Methyl Linolenate; Carbon 20:Methyl Eicosenoate. ECN Equivalent Carbon Number; ECN 46:Combination of MPL , PLO, PPL; ECN 48:Combination of MMP, OOO, OOP, PPO,PPP; ECN 50:Combination of OOS, POS (P:Palmitic, M:Myristic, O:Oleic, L:Linoleic)

TABLE 2
Fatty Acid Composition (%) and Triacylglycerol ECN (%) of Crude Palm Oil (CPO) and Admixtures with Used Vegetable Oil (UVO)

Sample	Fatty Acid Composition					Triglycerides (ECN)		
	Carbon 12*	Carbon 14	Carbon 16	Carbon 18*	Carbon 20	46	48*	50*
Genuine Crude Palm Oil (CPO)	0.42	1.09	44.47	53.67	0.35	17.28	69.70	4 .00
99 CPO : 1 UVO	0.41	1.09	44.43	53.72	0.35	17.27	69.72	4.00
98 CPO : 2 UVO	0.41	1.09	44.39	53.76	0.35	17.25	69.73	3.99
95 CPO : 5 UVO	0.41	1.09	44.26	53.90	0.35	17.21	69.78	3.99
90 CPO : 10 UVO	0.40	1.09	44.04	54.12	0.36	17.15	69.85	3.97
80 CPO : 20 UVO	0.39	1.08	43.61	54.57	0.37	17.01	69.99	3.94
Used Vegetable Oil (UVO)	0.30	1.03	40.14	58.14	0.40	15.92	71.16	3.68

Each value in the table represents the mean of fatty acids (n=2)
Carbon 12: Methly Laurate; Carbon 14:Methyl Myristate; Carbon 16:Combination of Methyl Palmitate, Palmitoleic Acid Methyl Ester; Carbon 18:combination of Cis-9-oleic methyl acid, Methyl Linoleate, Methyl Linolenate; Carbon 20:Methyl Eicosenoate. ECN Equivalent Carbon Number; ECN 46: Combination of MPL , PLO, PPL; ECN 48: Combination of MMP, OOO, OOP, PPO,PPP; ECN 50:Combination of OOS, POS (P:Palmitic , M:Myristic, O:Oleic, L:Linoleic)

there were significant differences between CPO and admixtures with adulterant on fatty acids and triacylglycerols at different concentrations of adulterant. The parameters that were identified as discriminatory markers based on the T-test for CPO adulteration are Carbon 12, 18 and ECN 48 and 50 for fatty acid and triacylglycerols composition, respectively.

Detection of Adulterants in Crude Palm Oil by Principal Component Analysis (PCA)

Based on the shortlisted chemical properties of CPO and admixtures with SO and UVO, PCA modelling was carried out and the calculation was tabulated. The PCA score plots were used to determine segregation between CPO, SO and UVO (Fig.1). The results indicated that the first two principal components explained 55.4% and 44.6% of the total variability, respectively. Despite the first two principal components that showed 100% total variation, the remaining principal components did not account for any variability and was not important.

Variables with positive loading on PC1 (Fig.2) were Carbon 12 and 18 whereas variables with negative loadings towards PC1 were ECN 48 and 50. Such distribution was strongly related towards the fatty acid and triacylglycerol (TAG) profiles of each oil. A combination of chemical profile markers and PCA was able to segregate CPO and admixtures (Fig.1) into three main clusters.

Each point across the cluster represents the concentration of adulterant in the crude palm oil ranging from 1 to 20% (v/v). As the concentration of adulterant was reduced to 1% (v/v), clusters were formed closer to the crude palm oil. This explains that those blends containing a lower concentration of adulterant exhibited similar profiles with genuine CPO. A similar observation was reported by Monfreda *et al*. (2012) in vegetable oils and by Kim *et al*. (2014) for discriminating cheeses. Blends with 5% adulterant concentration and higher was able to be identified and were distinguishable. With this proposed method it is possible to distinguish pure oils from mixtures. It also should be possible to predict the type and the percentage of an oil used to adulterate pure CPO. This has been demonstrated by Monfreda *et al*. (2014), whose results were obtained from PCA on adulteration of olive olive with seed oils (peanut, corn, rice and grape seeds) dividing based on type of seed oil and percentage of adulterant.

Detection of Adulterants in Crude Palm Oil by Dendrogram

Dendrograms were constructed using the single linkage of the individual adulterant (SO and UVO) with CPO (Fig.2). The dendrograms were fragmented into five main clusters; cluster one comprised genuine CPO and admixtures of adulterant less than 2% (v/v). Clusters 2, 3 and 4 comprised CPO adulterated with 5, 10 and 20% adulterant concentration, respectively and the final cluster was the genuine adulterant (Kostadinović, 2010; Mirhosseini, 2010).

The segregation level was able to be observed according to the concentration of

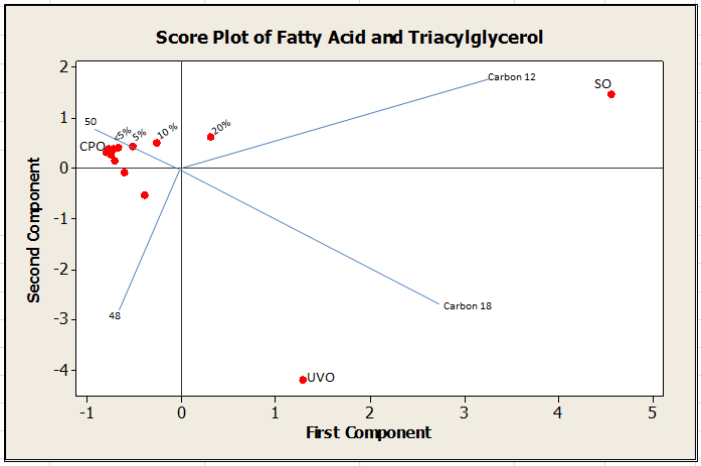

Fig.1: Principal Component Analysis (PCA) scores of crude palm oil (CPO) and blends; PCA of CPO, sludge oil (SO), used vegetable oil (UVO) and PCA loading distribution of fatty acid composition and triacylglycerol

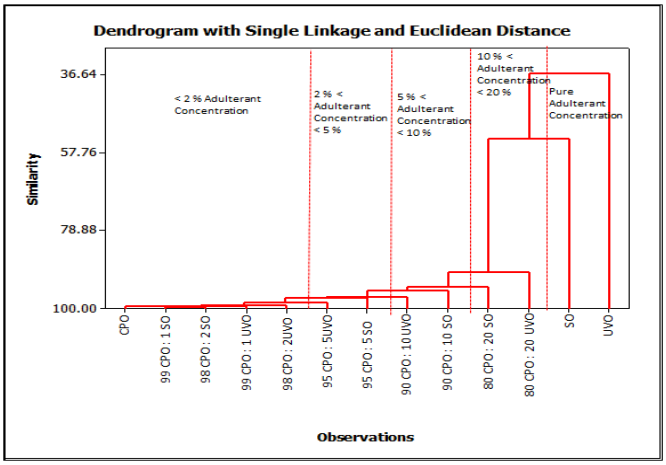

Fig.2: Cluster dendrogram of crude palm oil and adulterant admixtures.

adulterant added to the CPO. Lower level (<2% v/v) adulteration exhibited a similar chemical profile with the genuine crude palm oil that resulted in a single cluster due to the dominant effect of crude palm oil. Such groups are, in fact, well separated despite the existence of adulterant in CPO with less than 2% that was not able to be separated. Thus, detection of adulterant lower than 2% was difficult to be identified.

CONCLUSION

Gas chromatography (GC) equipped with a flame ionisation detector (FID) and high performance liquid chromatograph (HPLC) was employed to analyse the

chemical properties of adulterated crude palm oil (CPO) with sludge oil (SO) and used vegetable oil (UVO). PCA allowed the segregation between CPO and adulterant (SO and UVO) and gave variable loadings for each separated group. Apart from that, the dendrogram cluster allowed the construction of a linkage between crude palm oil and the adulterant that was possible to be used for the identification of blends. Overall, the results of both analyses showed a good indication. The adulterant level as low as 5% and 2% (v/v) was able to be detected by PCA and dendrogram correspondingly. Although this method showed a great potential of CPO quality assessment, the level of detection is still unfavourable. This was due to the dominant effect and similarity of the chemical properties of the adulterant with CPO at the lower level of the adulterant content. The findings of this study can be made more accurate if precision of detection is improved by increasing the number of replications and treatments used as well as by using alternative analytical instruments such as thermal and infrared equipment.

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