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THE DETECTION OF ADULTERATED COCONUT OIL BY USING DIFFERENT ANALYTICAL APPROACHES

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APPROVAL

I hereby declare that this thesis entitled "THE DETECTION OF ADULTERATED COCONUT OIL BY USING DIFFERENT ANALYTICAL APPROACHES" has been prepared and sent to the Department of Biochemistry by NORKHAIRUNISA BTE MOHD KAMIL as a requirement for BCH Project 4999 course of the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia.

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ABSTRACT

Adulteration is a fraudulent practice with the intention to cut costs and increase profits. Oils and fats frequently become subjects for food frauds since it does not require much work; the perpetrators just need to replace expensive and high quality oils with cheaper and low quality oils. Since the demand for Virgin Coconut Oils (VCO) in oils and fats market has increased, they are vulnerable to adulteration with other oils and fats. Therefore, it is essential to establish an appropriate method in detecting adulteration in VCO. The attempt to discover the most effective, simple, straight forward and most importantly chemical free method without affecting the sensitivity and specificity is needed. Thus, the objective of this research is to apply four instruments namely Gas Chromatography-Flame Ionization Detector (GC-FID), High Performance Liquid Chromatography (HPLC), Fourier Transform Infrared (FTIR) spectroscopy and Differential Scanning Calorimetry (DSC) to detect palm olein (PO) adulteration in VCO. VCO that has been used in this research was produced by MARDI while PO used was of Buruh brand obtained from local market. In the first phase, different mixture samples (w/w %) containing 5%, 10%, 15%, 20%, 25% and 30% of PO with VCO were prepared. A set of sample containing 100% of VCO was prepared as a positive control and another set containing 100% of PO as negative control. Each sample was subjected to analysis using GC-FID, HPLC, FTIR and DSC. The analysis of fatty acid (FA) and triacylglycerol (TAG) using GC-FID and HPLC were conducted as a quantitative analysis while spectroscopic and thermal analysis by FTIR and DSC as a qualitative analysis. Based on the study, the linear regression of GC-FID demonstrated $Y = -2.02E\text{-}03C_{14:0} + 0.19$ as the best prediction model for FA. For TAG analysis, POO was assigned as a good predictive model in the prediction of PO in VCO with the equation of $Y = 0.00261POO + 0.00486$ with R^2 value 0.857. Meanwhile, for DSC, the peak temperature was found to have good prediction ability for determination of PO % adulteration in VCO for both curves. For heating curve, the best regression model for peak 1 was temperature parameter with equation of $Y = -0.161Temperature + 14.0$ and R^2 value of 0.993 while for peak 2 onset parameter was the best model with equation of $Y = - 0.0847Onset + 17.0$ and R^2 value of 0.957. As for cooling curve, onset and temperature parameter were again becoming the best prediction model in which $Y = -0.102Onset + 5.52$ ($R^2 = 0.953$) for peak 1 while $Y = -0.253Temperature - 0.771$ ($R^2 = 0.997$) for peak 2. Lastly, FTIR spectrum can be used as a potential tool in determination of adulterant in pure oils and fats. It allows one to make a first differentiation among oils and fats because of its capability as fingerprint technique hence making the analysis process much easier. To conclude, the overall analysis showed that all four techniques could be applied in the detection of changes in the compositions and other characteristics of VCO. The findings indicate the potential use of GC-FID, HPLC, FTIR and DSC as reliable tests for PO detection.

ABSTRAK

Pencemaran dalam makanan adalah amalan penipuan dengan tujuan untuk mengurangkan kos dan meningkatkan keuntungan. Minyak dan lemak sering menjadi subjek untuk penipuan makanan kerana ia tidak memerlukan banyak kerja dimana pelaku hanya perlu menggantikan minyak yang mahal dan berkualiti tinggi dengan minyak yang murah dan rendah kualitinya. Oleh kerana permintaan untuk minyak kelapa dara (MKD) dalam pasaran minyak dan lemak bertambah maka MKD lebih terdedah kepada pencemaran dengan minyak dan lemak lain. Oleh itu, adalah penting untuk mewujudkan kaedah yang sesuai dalam mengesan penemaran dalam MKD. Usaha untuk mencari kaedah yang paling berkesan, mudah, tidak memerlukan banyak kerja dan yang paling penting bebas daripada bahan kimia tanpa menjejaskan sensitiviti dan spesifisiti adalah amat diperlukan. Oleh itu, objektif kajian ini adalah untuk menggunakan empat instrumen iaitu Kromatografi Gas Pengesan Pengionan Nyala (KG-PPN), Kromatografi Cecair Berprestasi Tinggi (KCBT), Pengubah Fourier Inframerah (PFIM) spektroskopi dan Kalorimeter Pengimbasan Perbezaan (KPP) bagi mengesan pencemaran olein sawit (OS) dalam MKD. MKD yang telah digunakan dalam kajian ini dihasilkan oleh MARDI manakala OS dari jenama Buruh dan diperolehi daripada pasaran tempatan. Dalam fasa pertama, campuran sampel yang mengandungi 5%, 10%, 15%, 20%, 25 % dan 30% PO dengan VCO telah disediakan. Satu set sampel yang mengandungi 100% VCO telah disediakan sebagai kawalan positif manakala 100% PO kawalan negatif. Setiap sampel tertakluk kepada analisis menggunakan KG-PPN, KCBT, PFIM speltroskopi dan KPP. Analisis asid lemak (AL) dan triasigliserol (TAG) menggunakan KG-PPN dan KCBT dilakukan bertujuan untuk analisis kuantitatif manakala spektroskopi dan analisis terma oleh PFIM spektroskopi dan KPP untuk analisis kualitatif. Berdasarkan kajian itu, regresi linear KG-PPN menunjukkan bahawa, $Y = -2.02E\text{-}03C_{14:0} + 0.19$ sebagai model ramalan terbaik AL. Untuk analisis TAG, POO dipilih sebagai model ramalan yang baik dalam meramal pencampuran OS dalam MKD dengan persamaan Y = 0.00261POO + 0.00486 dengan nilai R^2 ialah 0.857. Sementara itu, bagi KPP, suhu puncak didapati mempunyai keupayaan ramalan yang baik untuk mengenalpasti peratus pencampuran OS dalam MKD untuk kedua-dua lengkung. Untuk lengkung pemanasan, model regresi yang terbaik untuk puncak 1 adalah parameter suhu dengan persamaan Y = -0.161suhu + 14.0 dan R^2 nilai 0.993 manakala bagi puncak 2 permulaan parameter adalah model yang terbaik dengan persamaan Y = -0.0847permulaan + 17.0 dan R^2 nilai daripada 0.957. Bagi lengkung penyejukan, permulaan dan parameter suhu sekali lagi menjadi model ramalan terbaik di mana Y = -0.102permulaan + 5.52 ($R^2 = 0.953$) bagi puncak 1 manakala Y = -0.253suhu - 0.771 ($R^2 = 0.997$) bagi puncak 2. Akhir sekali, Spektrum PFIM boleh digunakan sebagai alat yang berpotensi dalam penentuan bahan asing dalam minyak dan lemak tulen. Ia membolehkan seseorang untuk membuat pembezaan pertama di antara minyak dan lemak kerana keupayaannya sebagai teknik cap jari dan membuatkan proses analisis lebih mudah dan senang. Secara kesimpulannya, analisis keseluruhan menunjukkan bahawa keempat-empat teknik boleh digunakan dalam mengesan perubahan dalam komposisi dan ciri-ciri lain MKD. Hasil kajian menunjukkan potensi penggunaan KG-PPN, KCBT, PFIM spektroskopi dan KPP sebagai ujian yang boleh dipercayai untuk mengesan kehadiran OS.

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VCO	Virgin coconut oil
PO	Palm olein
FA	Fatty acid
TAG	Triacylglycerol
%	Percent
w/w	By Weight
G	Gram
µl	Microliter
Psi	Pounds Per Square Inch
Mm	Millimeter
µm	Micrometer
mL/ml	Milliliter
Min	Minute
^{o}C	Degree Celcius
Mg	Milligram
S	Seconds
Cm	Centimeter
<	Less than

CHAPTER 1

INTRODUCTION

Virgin coconut oil (VCO) is defined as oil obtained from the fresh endosperm of mature coconut (*Cocos nucifera* L) by mechanical or natural means, either with the application of heat or without, and in which the process does not alter or change the nature of the oil (APCC, 2003). Unlike commercial coconut oils, the extraction process of VCO occurs via wet milling process directly from coconut milk, which does not have to go through any deodorizing or bleaching process and can be consumed without any refining, thus retaining its natural volatile and chemical components as well as their functional components such as polyphenols, vitamin E and pro-vitamin A (Xu *et al*., 2015). The price of VCO in market is higher compared to other vegetable oils because of the difficulty in their production. Recently, the demand for VCO became higher due to its great flavor as well as their potential health benefits. Although they are a newcomer in the oil markets, VCO has already earned their place. The efficacy of VCO has been known since the days of our ancestors, but it has been produced on a small scale only. Along with advances in biotechnology, VCO has been commercialized worldwide and is beginning to receive attention and is treated as valuable as olive oil. There are many research articles showing the benefits of VCO to human health. Fatty acids in VCO are distinct from animal fats due to the presence of high levels of lauric acid, which is medium chain fatty acid (MCFA). MCFA content in VCO is good for digestibility (Marina *et al*., 2009[b]). VCO showed potential as an anti-obesity treatment and according to (St-Onge and Jones, 2002), MCFA increases energy expenditure. It is directly absorbed and burnt as energy in the liver, thus resulting in early satiety. Consumption of VCO in the long term will eventually lead to weight loss. In addition, it can also reduce total cholesterols, triglycerides, phospholipids, low density lipoprotein (LDL) cholesterols, and very low density lipoprotein (VLDL) cholesterols in the serum and tissues with a corresponding increase in high density lipoprotein (HDL) (Nevin and Rajamohan, 2004). Apart from that, VCO has been applied widely in cosmetics manufacturing and medical fields due to its high content of MCFA.

Owing to its therapeutic value, it is important to know the exact composition of pure VCO so that it becomes a marker to identify their purity. Because of their high market demand, VCO is vulnerable to adulterations with less expensive oil. This will change the chemical composition and their therapeutic properties. Food adulteration is not only an economic fraud, but it also might cause severe health implications such as Spanish toxic oil syndrome (TOS) to consumers because of the substitution with cheap ingredients (Lee *et al*., 1998). Thus, the analysis of adulteration in VCO is vital not only for labelling purposes, but also to ensure the quality and prevent negative implications on the health of the consumers.

The characterization of VCO to detect adulteration can be done using two different analytical approaches. The first is by identification of the major constituents of VCO and their properties that exist at certain levels while the second approach is by identification of the constituents and properties of adulterants and their presence in VCO. Recently, there are multiple instrument approaches, such as differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), gas chromatography (GC) and high performance liquid chromatography (HPLC) which have been employed to encounter fraudulent practices (Marikkar and Rana, 2014; Manaf *et al*., 2007).

Thermal analysis has been applied for the authentication of fats and oils, and DSC is known as the most widely used thermo analytic technique (Marina *et al*., 2009[c]). DSC has been explored in the detection of adulteration in vegetable oils, such as the detection of hazelnut oil in extra virgin olive oil and adulteration of VCO by palm kernel oil (PKO) and soybean oil (SBO) (Marina *et al*., 2009[a]). In the food industry, FTIR spectroscopy is well known to differentiate and quantify fats and oils. It provides qualitative information regarding the functional groups present in the food sample. FTIR spectroscopy has been used in food authentication purposes, such as in the study of VCO adulterate with palm kernel olein (PKO) (Manaf *et al*., 2007) where the distinguishing is based on their infra-red spectra. GC analysis was first reported by James and Martin (Lee *et al*., 1998) and has been used extensively in fatty acid (FA) and triacylglycerol (TAG) compositional determination.

GC analysis is a good technique in detecting adulteration by comparing the peak and the peak height of chromatogram. The analysis of FA and TAG by GC is usually coupled with a flame ionisation detector (FID), a detector with high sensitivity and robustness. Next, HPLC is the most popular liquid chromatography that has been used for the analysis of a wide range of food compounds. It is known as a straightforward, robust and reproducible technique in food authentication. HPLC has also been used as a tool to check the presence of adulterant in food, such as in olive oil.

Since VCO is the miracle oil for health and has multifunction uses in human life; thus it is important to ensure the legitimacy of these virgin oils. There are not many research have done to detect the presence of adulterant especially palm olein (PO) in VCO at present.

Therefore, the objective of this study is to detect the presence of PO in VCO by investigating the changes in the composition and other characteristics of VCO in relation to adulterants using different analytical approaches:

i. Gas Chromatography-Flame Ionization Detector (GC-FID)
ii. High Performance Liquid Chromatography (HPLC)
iii. Fourier Transform Infrared (FTIR) spectroscopy
iv. Differential Scanning Calorimetry (DSC)

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