Perah Oil: A Potential Substitute for Omega-3 Oils and its Chemical Properties

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Abstract: The perah seed oil is a potential source for high unsaturated and omega 3 oil. It can be an alternative choice for regular vegetable oil. The chemical and physical properties need to be investigated and evaluated for it to be used feasibly in the food industries and health care products. Modern day's faster and sophisticated instruments and techniques are used to evaluate the physicochemical properties of edible or vegetable oils. This study includes FTIR spectroscopy GC-MS and GC to evaluate the chemical properties of it while colour was used to study the physical property. The peaks by FTIR were found at 3300-3000 cm^{-1}, 2960-2850 cm^{-1}, 1760-1670 cm^{-1}, 1650-1580 cm^{-1}, and 1340-1020 cm^{-1}. The GC-MS discover the potential beneficial compounds that available in extracted perah oil which are squalene, Vitamin E,and L.beta.-sitosterol. The GC data demonstrate about the free fatty acid profile which showed the high unsaturation in all oil samples extracted by different solvents. However, the colour measure for the visual suitable acceptability use for food or cosmetic application.

Keywords: Perah seed oil, Chemical properties, Physical properties, Omega 3, Skin care.

1. INTRODUCTION

In modern days the increasing demands of good quality edible oil has led the researchers to find out new sources. Especially the high unsaturated and ω-3/6 containing vegetable oils are always on top of choice. Past 20 years researches showed that consumption of vegetable oil rich in ω-3 and 6 increases the resistance for cardiac problem and minimize the risk [1-3]. The essential oils, on the other hand. Besides, cheap and renewable sources for the same have been explored and researches are still on going for this thrust. Past few years, researches brought a variety of seed oil to light in terms of food and health care products. Perah (*Elasteiospermum Tapos)* seed is one of those few oil seeds that have potentiality to emerge as a major source of vegetable oil. Although there are very few literatures available on this seed oil and about its applications, it has been known as useful and abundant. The much information found on it, shows the high content of linoleneic acid (18%) along with oleic acid (32.5%) and linoleic acid (31.8%) makes this seed a good contender for being used as a counterpart of the available oil grains [4]. The high content of unsaturated fatty acids in perah seed oil and relatively higher in alpha linoleneic acid (ALA) it is good oil that reduces bad cholesterol (LDL-Low density lipoprotein) and increase HDL (good cholesterol) [5]. Producing functional food is another emerging sector in last decade for a healthy diet, where additional health value in food becomes an interest for the industries [6,7]. The ingredients along with its fatty acids and TGA (triglycerides) Perah seed oil might have positive health effect and well-being beyond the provision of basic nutritional requirements. It has a promising future to be used as functional food. Due to its high unsaturation it has a low melting temperature which is desired for salad dressing, baking cakes and cooking [8]. Moreover, the unsaturated fatty acids in oil protect the skin from drying. The water permeability of oils is related to the degree of unsaturated fatty acids; this makes the high unsaturated fatty acid content in oil effective in preventing dry skin, by forming a thin film on the skin [9]. To this point, in addition, the availability and abundance of this oil will leave the industries an option to formulate better and cheaper skin care and health care products. However, this oil has a drawback which has been reported elsewhere by the researchers that this seed contains a little amount of hydrocyanic acid and cyanogen glycosides. The amount of these chemical constituents have not been reported nor exactly explained whether the seed flour or oil contains

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it. In this study these chemical constituents along with chemical properties was investigated on perah oil extracted by different solvents through soxhlet extraction using fourier transform infrared (FTIR). The IR spectroscopy has been used for analyzing edible oils as a convenient, fast and simple technique [10]. Moreover, the application of FTIR spectroscopy has become a powerful technique in analyzing of food especially in oil and fats industries for formulation of food products [11].

2. MATERIALS AND METHODS

2.1. Extraction of Oil

The perah seed collected from a local farm in Perak, Malaysia. It was ground using a laboratory grinder to fine powder and 5 gram of it was taken to each thimble for soxhlet extraction. Three different solvents were used i.e. Hexane, Methanol and isopropanol. Soxhlet process was carried out by refluxing each for 6 hours on a water bath. After the time elapsed a rotary evaporator was used to evaporate the solvents and the samples were collected. Samples were preserved in sealed bottles at -20 °C for further analysis.

2.2. Oil Yield Calculation

The perah oil extracted by soxhlet extraction was then calculated for each extraction solvent (hexane, isopropanol and methanol). The oil yield was express in term of mass percentage are formulated as equation 1 below [12].

$$Oil\ yeild\,(\%) = \frac{mass\ of\ oil\ extracted\ (g)}{mass\ of\ sample\ (g)} \times 100\% \quad (1)$$

2.3. Colour Determination

Color was determined by using the Konica Minolta Colorimeter (Color Reader CR10, Japan) as L*, a* and b* colour parameters . Where the colour L* indicated the lightness of the colour while a* represents the green and red when negative in value. The parameter b* represents the yellow and blue where the positive value of b* showing the yellowness and negative value showing the blue.

2.4. Compound Determination by Gas Chromatography Mass Spectrometry (GC-MS)

The identification of compound from extracted perah seed was indentifies by using GC-MS (Agilant Technology, USA. One µl of the samples are being injected into the GC-MS. A CG-MS analysis was performed by Agilent gas chromatography equipped with flame ionization detector and capillary column 30m x 0.25mm x 0.25µm. The detector temperature was programme at 80-325^{o}C with flowrate of 1.2 ml/min. For the injector temperature, it was set at 325^{o}C. Helium was used as the carrier gas. The peaks detected by retention time were indentified by matching (>95%) with Wiley reference library.

2.5. Fatty Acids Analysis

Free Fatty acids analysis of the perah seed oil was analyzed by using gas chromatography (GC). 0.2µl of the sample was injected into the machine The CG analysis was performed by Perkin Elmer gas chromatography (GC Autosystem XL, USA) equipped with flame ionization detector and capillary column 15m x 0.53 mm x 0.5 µm. The detector temperature was programmed at 240^{o}C with flowrate of 2 ml/min. For the injector temperature, it was set at 250^{o}C. The column temperature was set at 150 which raised to 240 ^{o}C at different rates from 4 ^{o}C/min to 7 ^{o}C/min at different stages. Nitrogen was used as the carrier gas. The peaks were detected by retention time and were indentified by comparing with standards under the same condition.

2.6. FTIR Analysis

FTIR unit (AVATAR 360, Nicolet, U.S) was equipped with a deuterated triglycine sulphate (DTGS) as a detector and a KBr/ Germanium as a beam splitter, interfaced to a computer operated under windows based and connected to OMNIC operating software system was used for FTIR spectra acquisition. Automatic dehumidifier was used to diminish water vapor interface from the machine. A few drop of each sample were put onto the Attenuated Total reflectance (ATR) multibounce plate of crystal at controlled ambient temperature (25 ºC).

All spectra were recorded from 4000 to 400 cm^{-1} co-adding 32 interferograms with measurement accuracy in vc the frequency data at each measured point of 0.01 cm^{-1}, due to the laser internal reference of the instrument. Each time this spectrum was subtracted from the background air spectrum. A new air spectrum was taken after every scan with sample. The ATR plate was carefully cleaned with ethanol and was dried with a soft tissue before taking the spectra of a new sample.

3. RESULT AND DISCUSSION

3.1. Percent Yield of Perah Oil Extracted by Different Solvents

The extraction of perah seed oil using isopropanal as a solvent results a higher yield percentage (56.1 %) compared to that of hexane (47.3%) and methanol (25.8%). This result is might be due to the polarity of the solvent used. According to Kim *et al*. (2007), the extraction of the organic substances are easier to extract when the polarity is matched with the solvent used for extraction, based on ‘like dissolves like’ [13]. Hexane and iso-propanol both are non-polar solvent where as methanol is a polar solvent. So Hexane and iso-propanol can produce more yield than methanol as solvent.

3.2. Colour Determination

Colour is one of the important parameters for determination of sample’s physical properties. It is sometimes easy to perceive about the products quality through visual observation. The difference in colour will give different perception to the consumers. According to Moyano *et al.* (2008), measurement of colour is very important for food producer to attract the consumers [14]. The aesthetic value of food chiefly depends on the color and smell of it.

Using Conica Minolta Colorimeter, the L* a* b* measurements can be used as colour classification. The higher of the L* value, the lighter of the oil colour. It was found that the colour of the oil extracted by methanol was lighter than hexane and isopropanol based on their L* value (Table **1**). This observation could be because methanol being polar does not extract all components but only selective components. Result also showed that the negative value of a* for all three oil samples, therefore, the more greenness was observed for the extracted perah oil. However, oil extracted using methanol showed more of greeness comparing to oil extracted by isopropanol and hexane. All samples showed a positive value in b* indication and thus are more yellow. The isopropanol extracted yield gives the highest value of b* among all three. Based on Vanesa *et al.* (2011), the intensity of the colour of vegetable oils depends mainly upon the presence of carotenoids [15]. Hence, the darker colour of the extracted perah oil by isopropanol solvent has probably extracted more carotenoids compared to others solvents.

3.3. Compound Detection by Gas Chromatography Mass Spectrometry (GC-MS)

In the GS-MC analysis as shown in Figure **1**, the extraction of oil using hexane detected most compounds (37) followed by methanol (23) and isopropanol (13). This result might be because of the compounds in the perah oil tend to be fully extracted by hexane due to its suitable polarity.

Table **2** shows several beneficial compounds detected and its functions which probably can be used in the cosmetic industries. The amount of palmatic acid, linoleic acid and oleic acids are higher for the methanol extraction while no linoleic acid was detected by isopropanol extraction. The other beneficial compounds such as the squalence, vitamin E, and L.beta.-sitosterol were not detected when methanol solvent was used. But, it can be detected in significant amount when using hexane and isopropanol solvent. However, there was no detection of vitamin E for extraction by isopropanol solvent.

The discovery of the beneficial compound such as vitamin E, squalene and L. beta.-sitosterol in perah oil, would be potentially useful for the skincare wellness especially in cosmetics industries. Essential oils, especially, provide antioxidant and anti-bacterial/fungal [23], however, this kind of seed oil is suitable to use for

Table 1: Colour of the Perah Oil Extracted by Difference Types of Solvent

	Hexane	Isopropanol	Methanol
	Yellow	Dark yellow	Pale yellow
			
L	44.4	44.1	45.9
a-	0.6	0.8	0.5
b +	10.0	12.0	8.6

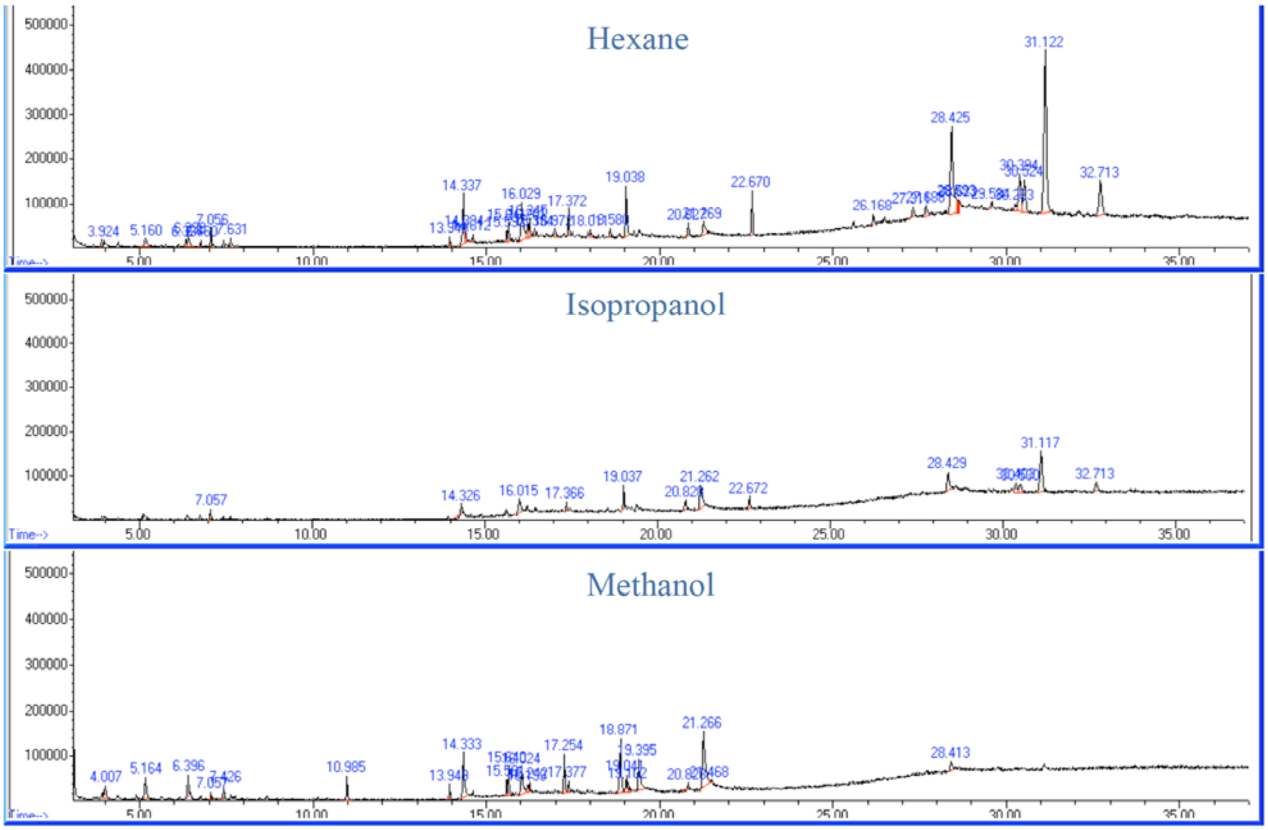

Figure 1: GC-MS analysis of the perah oil for hexane, isopropanol and Methanol solvent.

Table 2: Compound Determination by GC-MS and their Functions

Beneficial compounds in extracts	Hexane (%)	Isopropanol (%)	Methanol (%)	Functions
Palmitic acids	4.09	1.65	9.56	-Promote optimum body function -Diabetic treatment, insulin control
Linoleic acids	0.52	-	1.68	-Healthy growth of human skin [16]
Oleic acids	5.42	7.71	7.63	-Cardiovascular diseases prevention [16]
Squalene	3.37	4.52	-	-Prevents oxidation of body lipids [17] -Against certain type of cancers [18]
Vitamin E	0.90	-	-	-Prevents oxidation of body lipids -Reduction of heart disease -Delay of alzheimer's disease -Prevention of cancer -Antioxidant [17]
L.beta.-sitosterol	15.62	10.97	-	-Treating heart disease & high cholesterol -Boosting immune system, -Preventing colon cancer -Apply to the skin for treating wounds and burns (WebMD.com)

Table 3: Fatty Acid Analysis

	Isopropanol (%)	Hexane (%)	Methanol (%)
C18:0	1.38	1.67	-
C18:1	0.01	3.15	-
C18.2	0.01	0.01	0.29
C18:3	1.85	3.59	-

C18:0- Stearic acid, C18:1- oleic acid, C18:2-linoleic, C18:3-linoleneic.

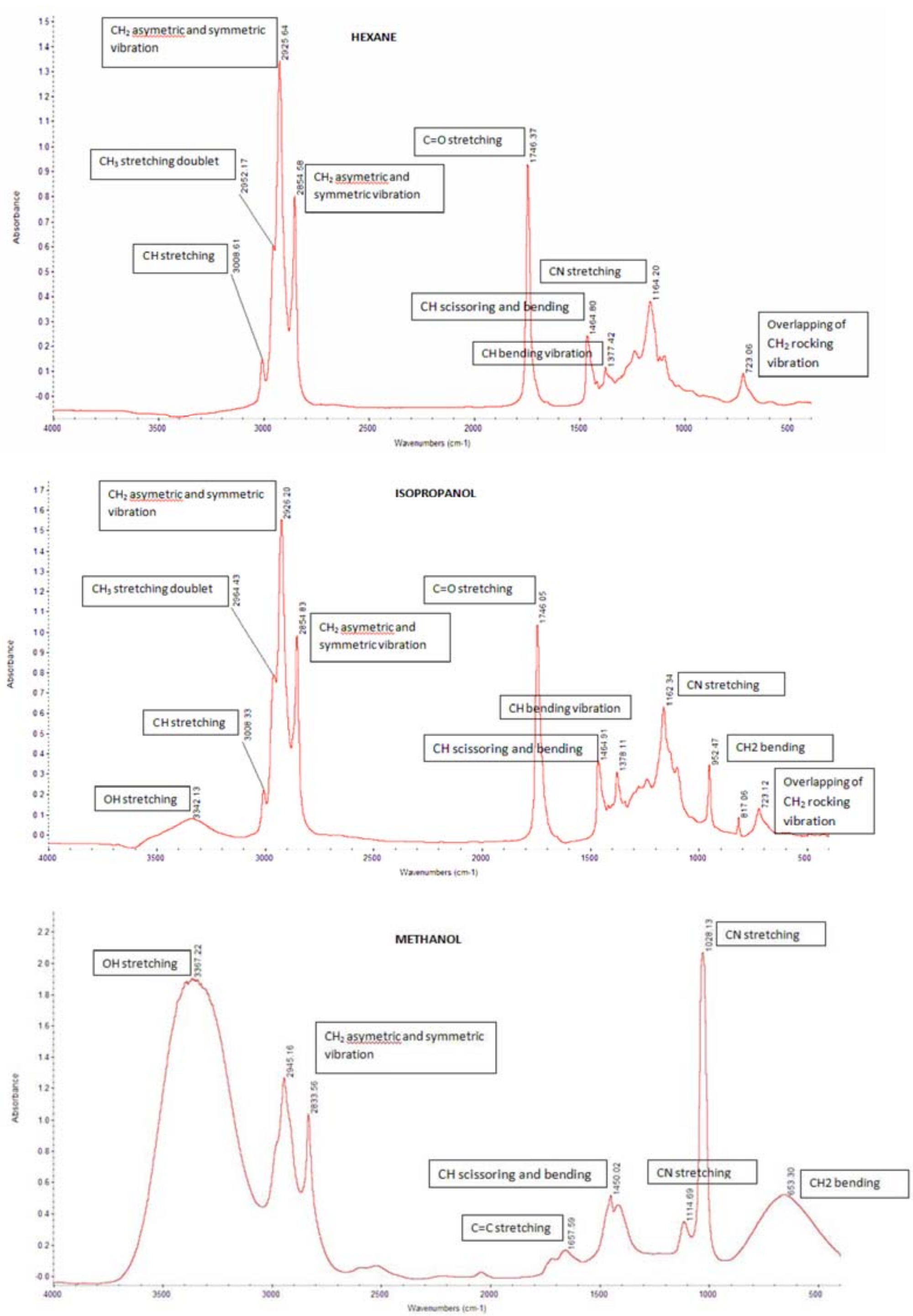

Figure 2: FTIR analysis of the perah oil for hexane, isopropanol and Methanol solvent.

cooking which contains many necessary nutrients and vitamins. Previous studies on squalene and vitamin E reported that vitamin E is the most abundant lipid-soluble antioxidant which is found on epidermis and applied to the skin when critical thus it is one of the popular and necessary cosmetic ingredients [19]. Besides, Its an essential vitamin that human body can not produce so the intake of vegetables, fruits, nuts and vegetable oil chiefly contribute to the demand of it in human body [20]. On the other side, squalene is an antioxidant and an important constituents in skin care products [21]. Olive oil is proved to reduce skin darmatities when applied as such or with emoluent cream containing olive oil. The squalene is believed to play a key role in this very phenomenon of olive oil [22].

3.4. Fatty Acids Analysis

The different solvents extractions methods affect the fatty acids composition. The fatty acids composition of each solvent extraction is tabulated in Table **3**. Comparing among the three solvents, the hexane gave the highest value of fatty acid compare to extraction by isopropanal and methanol. Linoleneic or omega 3 was detected by hexane extraction of 3.59% of yield, while the isopropanol extracted 1.85%. There was no omega 3 detectedby using methanol as solvent. These results proved that methanol is not a suitable solvent to extract the perah seed oil.

The content of essential oil especially omega-3 in perah oil brings huge potential as a new source of health supplement as well as also as a functional ingredient for food. This oil can be used successfully as an alternative for the usage of omega-3 from fish sources in the form renewable source and thus in cheaper and economical aspect to the consumers. The extracted perah oil can be potentially applied in health food application such as salad dressing, cooking/frying, and food additives/supplement.

3.5. FTIR Analysis

The FTIR spectra for hexane and isopropanol extracted oil were found identical (Figure **2**). The both solvents being similar in characteristic extracted similar. The spectra showed typical absorption bands in the 3600-3200, 3300-3000, 2960-2850, 1760-1670, 1650-1580, and 1340-1020 cm^{-1} regions. The absorbence at these spectral regions are generally associated with –OH groups (3600-3200), alkanes groups (2960-2850), Aldehydes, Ketones, Carboxylic acids, Esters (1760-1670), amide bend (1650-1580), and amide stretch (1340-1020), for the methanol extraction, in Figure **2** the structure of the oil is obviously difference. Due to the polar solvent and with extraction high temperature the oil probably had undergone to polymerization. The absorbance from 2700 cm^{-1} to 3700cm^{-1} showed broad picks which probably because of the polymers formed during the extraction process. This reinforces why extraction using methanol give the small amount of yield.

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