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The Effect of Solvents on the Soxhlet Extraction of Omega 3 from Perah Oil

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Graphical abstract

Abstract

This study aims to investigate the influence of solvent on the Soxhlet extraction of omega 3 in perah seed oil. Three types of extraction solvent include hexane, hexane: methanol (90:10) and methanol: ethanol (70:30) were investigated. The concentration of extracted omega 3 was measured using Gas chromatography (GC), the colour of extracted oil was classified using colormeter and the total phenolic content (TPC) by Folin-Ciocalteau colorimetry. Based on the total yield extraction of the oil, the result shows that hexane solvent (57.5%) shows better performance compared to hexane-methanol (53.42%) and ethanol-methanol (34.52%). For the omega 3 concentration, hexane-methanol solvent provided highest yield at 1.41g ω-3/g oil followed by ethanol-methanol and hexane at 1.32 ω-3/g oil and 1.15 ω-3/g oil respectively. On the other hand, the extracted oil using hexane as extraction solvent was appeared to be lighter in colour as compared to other solvents studied. In addition, total phenolics content of perah oil was high with hexane solvent. As conclusion, the polar solvent of methanol and ethanol promoted the extraction of omega 3; however, it may cause a darker appearance of the extract as well as reducing the content of phenolic compounds.

Keywords: Soxhlet extraction; perah seed oil; concentration Omega 3; colour

1.0 INTRODUCTION

The demand on the new and low cost food with high nutritional value, especially from sustainable resources has been increased continuously over the years. Perah (*Elacteriospermum tapos blume*) as shown in Figure 1, a member of *Euphorbiaceae* family and it is a monoecious conopy species situated in Southeast Asian tropical rainforest which includes peninsular Malaysia, peninsular Thailand, Brunei, Sumatra, Java and Borneo [1-5]. It is one of the abundant and under-utilized seeds which has been found to be rich in α-linolenic acid (ALA.18:3n-3), the parent of omega 3 polyunsaturated fatty acid (Figure 2) [2].

Figure 1 Fresh perah seed

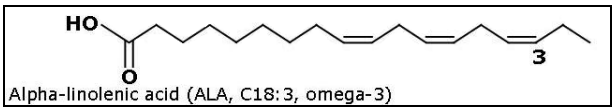

Figure 2 Molecular structure of Omega 3

Omega 3 is one of the essential polyunsaturated fatty acids due to the incapability of human body to synthesis it and sufficient intake must be obtained from the diet [6]. Omega 3 plays important role in prevention of many chronic diseases include coronary heart disease [7] and cancer [8]. Besides, lack of omega 3 may result to adverse clinical symptoms including neurological abnormalities and poor growth [9]. Based on American Health Association, AHA (2002), evidence from prospective secondary prevention studies suggested that 1.5-3g/day ALA supplement is required in order to significantly reduce subsequent cardiac related mortality [10].

Phenolics are secondary metabolites synthesis by plant during normal development as response to the infection, wounding and UV radiation [11]. It is a group of phytochemicals which derived from phenylalanine and tyrosine [11]. Due to literature by Sengul *et al.* (2009), the phenolic compound contributes to the bitterness, astringency, colour, flavor, aroma and oxidative stability of food as well as providing health

beneficial effect which give significant benefit to consumer and producers [11-12].

Soxhlet extraction is one of extraction method that can be considered as references to other extraction techniques which almost used in extraction of plant seed oil [13]. The effectiveness to extract of the interest compounds from plant matrics is highly depends on the extraction solvent used specifically in term of polarity [14]. Therefore, this study aims to investigate the influence of solvent on the Soxhlet extraction of concentration omega 3 in perah seed oil. In addition, the colours and total phenolic content of the extracts yield using different solvents were observed and compared.

■2.0 EXPERIMENTAL

2.1 Seed Preparation

The seed preparation is based on the modification method of Ixtaina (2010) [15]. Fresh perah seeds are collected from a local farm in Kuala Lipis, Pahang, Malaysia and were cleaned manually and flooded with tap water in order to separate the extraneous matters (dust, vain seeds and straw from threshed seeds). The shell was removed afterwards. The cleaned seeds were ground by a laboratory grinder MX-898M (Panasonic, Malaysia) and sieved by Retschsiever (Retsch, Germany) to a particle size of 1.0 mm.

2.2 Soxhlet Extraction

5 gram of grinded perah seed was placed into each thimble for Soxhlet extraction. Three different solvent ratios were used i.e. Hexane, hexane: methanol (90:10) and ethanol: methanol (70:30) and the total amount of the solvent was 150 ml. This process was carried out by refluxing each for 6 hours on a water bath. After elapsed time, a rotary evaporator was used to evaporate the solvents. Then, the samples were collected and preserved in sealed bottles at -20°C for further analysis.

2.3 Oil Yield Calculation

The perah oil extracted by soxhlet extraction was then calculated for each extraction solvent (Hexane, hexane: methanol (90:10) and ethanol: methanol (70:30)). The oil yield was express in term of mass percentage are formulated as Equation 1 below [16].

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

2.4 Omega 3 Fatty Acid Analysis

The fatty acid methyl esters were prepared by esterification of perah seed oil with sodium methoxide according to standard method current protocol in food analytical chemistry (2002). Gas chromatography (GC) was performed to identify the content of omega 3 composition of the perah seed oil. About 0.1µl of the sample are being injected into the GC. A CG analysis was performed on Perkin Elmer gas chromatography equipped with flame ionization detector and capillary column, DB-Wax (15m x 0.53 mm x 0.5 µm). The detector temperature was programmed at 50°C 1 min, 25°C/min to 200^{0}C, 3°C/min to 230, 18 min split ratio. For the injector temperature, it was set at 250^{0}C. Helium was used as the carrier gas. Then the peaks detected by retention time are identified by comparing with standards under the same condition.

2.5 Quantification of Calculated Omega 3

Different concentration (8 ppm, 12 ppm, 20 ppm, and 50 ppm) had been prepared from Omega 3 standard (Sigma Aldrich, Germany). The standards were injected in GC by using same condition of fatty acids analysis. The graph calibration curve absorbance versus concentration was plotted. The concentration of extracted omega 3 was determined in mg of ω3/g of oil by using the Equation 2. The con ω-3 means concentration of the extracted omega 3, mg of ω-3/g of oil; C means concentration of omega 3 obtained from calibration curve, mg/mL; V means volume of omega 3 solution, mL and Woil is weight of the extracted oil, g.

$$con._{\omega-3} = \frac{[C \times V]}{W_{oil}} \qquad (2)$$

2.6 Colour

Colour is one of important physical analysis to determine existent of the Omega 3 in the oil. The sample of the oil colour is measured by using the Konica Minolta Colormeter and the colour parameters L*, a* and b* were determined. Where the colour L* indicated the lightness of the colour sample while a* represents the green and red which negative value of a* measured the greenness and positive value of a* measured the redness. The parameter b* represents the yellow and blue where the positive value of b* showing the yellowness and negative value showing the blue.

2.7 Total Phenolic Compound

Total phenolic content (TPC) in the extracts is determined by using Folin-Ciocalteu (FC) colorimetry method in current protocol of food analytical chemistry (2002) and the results were expressed as gallic acids equivalent (GAE).

■3.0 RESULTS AND DISCUSSION

3.1 Oil Yield

Figure 3 shows the Oil yield using different extraction solvents. Apparently, using hexane alone was more effective to extract the oil from the Perah Seed as higher oil yield of 57.50 % was obtained. The hexane used in combination with methanol has resulted to the decrease of oil yield and the lowest oil yield was observed in ethanol-methanol solvent. This phenomenon might be due to the polarity of the solvent used. According to Tan *et al.* (2013), the extraction of organic substances could be easier if the polarity is matched with the solvent used for extraction, based on 'like dissolves like' [14, 17]. As such, the non-polar hexane was more effective to extract the organic substances of oil than the polar solvents of methanol and ethanol.

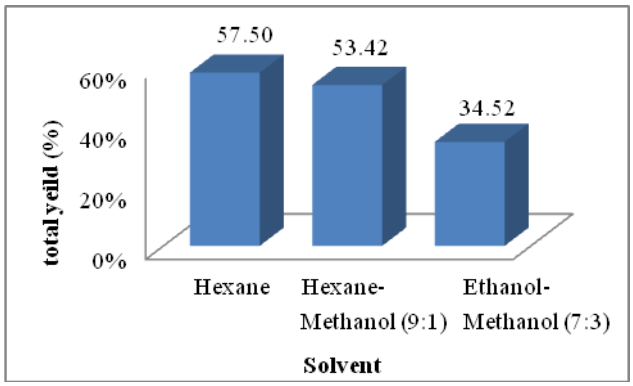

Figure 3 Oil yield by difference solvent extraction

3.2 Omega 3 Fatty Acid Analysis

As shown in Table 1, higher concentration of omega 3 fatty acid was obtained using hexane-methanol solvent and ethanol-methanol solvent which give results 1.41± 0.05 and 1.32± 0.04 mg ω-3/g oil respectively. While, hexane alone as a solvent extraction was give the least amount of the omega 3 concentration (1.15± 0.05 mg ω-3/g oil). The presence of the polar compound such as methanol and ethanol tend to increase the yield of omega 3 during the extraction. According to study done by Sánchez-Camargo *et al.* (2012), the extraction of omega 3 using supercritical fluid extraction which involve co-solvent of ethanol, the yield of omega 3 composition was increased with increase of ethanol composition. Probably, the omega 3 fatty acid is bonded better to more polar molecules. The addition of polar solvent makes the solvent less selective and tends to extract large amount of polar and non-polar substances [18].

From the results, high concentration of omega 3 was found in perah seed and it has high potential to be the new source for the ALA dietary supplement or functional ingredient for food. Besides, the extracted perah oil can be potentially used in health food application such as salad dressing, cooking/frying, and food additives/supplement.

Table 1 Concentration omega 3 by difference solvent extraction

Solvent	Concentration ($con._{\omega-3}$), mg ω-3/g oil
Hexane	1.15± 0.05
Hexane: Methanol	1.41± 0.05
Ethanol: Methanol	1.32± 0.04

3.3 Colour

Using Conica Minolta Colorimeter, the L* a* b* measurements can be used as colour classification. The higher of the L* value, the lighter the colour of the oil. It was found that the colour of the oil extracted by hexane was lighter than hexane-methanol and ethanol- methanol solvents based on their L* value as shown in Table 2. Result also showed that the negative value of a* for all oil samples, therefore, more greenness was observed for the extracted perah oil. Based on a* value, the greenness of the extracted oil was not affected by the solvent used. All samples showed a positive value in b* indication and thus are more yellow. The ethanol: methanol extracted yield gives the highest value of b* among the three solvents studied. Based on Vanesa *et al.* (2011), the intensity of the colour of vegetable oils depends mainly upon the presence of carotenoids [19]. Hence, the darker colour of the extracted perah oil by polar solvent may probably extracted more carotenoids compared to others solvents.

Table 2 Colour of the perah oil extracted by difference solvent extraction

	Hexane	Hexane: Methanol	Ethanol: Methanol
	Yellow	pale yellow	dark yellow
			
L	54.44±3.46	52.64±0.61	52.66±1.65
a-	1.1±0.40	0.9±0.47	1.08±0.74
b +	8.66±0.55	9.3±0.78	9.54±0.44

3.4 Total Phenolic Content

The total phenolic content (TPC) in the extracts was determined using Folin Ciocaltaeu method as shown in Figure 4. The TPC found were 55mg GAE/L, 48mg GAE/L and 42 mg GAE/L in the extracts using the extraction solvents of hexane, hexane-methanol and ethanol-methanol respectively. This indicated that hexane has higher tendency to extract the phenolic compound in perah oil although it is a non-polar solvent. A similar results was reported by Hsouna *et al.* (2011) where the non-polar solvents of hexane and ethyl gives a high extraction yield of TPC from ceratonia siliqua leaf [20]. However, a contary result was found where the TPC content in Horseradish Roots could be best extracted using polar solvents of ethanol and ethanol: water [21]. As such, solvent polarity plays a key role in increasing pheonolic solubility [11].

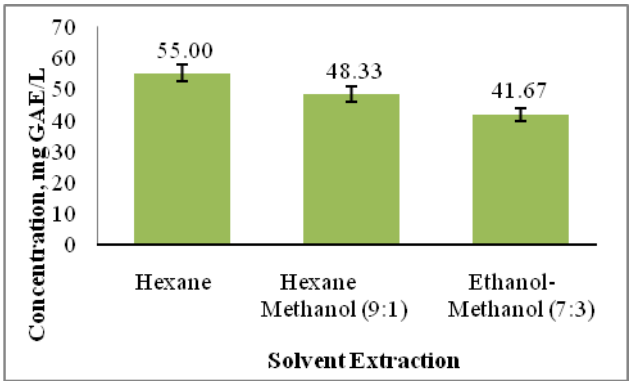

Figure 4 Total phenolic compound of difference solvent

4.0 CONCLUSION

The usage of polar solvents of methanol and ethanol decreased the yield of oil. However, the concentration of the omega 3 was increased with the presence of polar solvent. The highest concentration of omega 3 of 1.41 ω-3/g oil is obtained using hexane-methanol solvent at the ratio of 90:10. The extracted oil was appeared darker with presence of the polar solvent. Existence of polar solvent also contributes in reducing the total phenolic content. As conclusion, the addition small amount of polar solvent methanol is considering the best choice for increasing the

concentration of the omega 3 content due to the omega 3 fatty acid is bonded better to more polar molecules.

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