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PHYSICOCHEMICAL PROPERTIES OF RAMBUTAN SEED FAT

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ABSTRACT

Rambutan (Nephelium lappaceum L.) fruit is abundantly present in Malaysia. It is a seasonal fruit that present twice a year. It has short shelf life at ambient temperature has contributed to fruit wastage. Thus, the initiative of producing canned rambutan is an innovation that makes rambutan fruit available throughout the year. The canned rambutan industry leaves large amount of rambutan seed. This study focused on utililization of rambutan seed as valuable product which is Rambutan Seed Fat (RSF). The RSF was extracted using Soxhlet Extraction method for 8 hours. The objective of this study was to determine the physicochemical properties of RSF: melting point (°C), Refractive Index (RI), Total Carotene Content (TCC), water activity (Aw), acid value (AV), peroxide value (PV) and saponification value (SV). The results showed that: 38.00±1.00 – 48.83±1.61°C melting point, 1.46±0.00 RI, 1.18±0.06 mg/kg TCC, 0.4721±0.0176 Aw, 1.2162±0.1520 mg KOH/g AV, 9.6000±0.4000 g/g PV and 146.8040±18.0182 mg KOH/g SV, respectively. According to the results, RSF showed high industrial potential as cocoa butter replacement in chocolates and cosmetics production as the physicochemical properties of RSF is quite similar to cocoa butter.

Keywords: *Rambutan, Rambutan seed, Rambutan seed fat*

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1. INTRODUCTION

Rambutan (*Nephelium lappaceum L.)* is an abundant seasonal fruit in Malaysia. Rambutan is an exotic fruit originated from Southeast Asia which belongs to the family of Sapindaceae (Lasekan et al., 2015). It made up of hairy body, white flesh and light brown seed. Due to abundantly present over a short period of time, it leads to overproduction of the fruit without fully utilization of the fruit. When it overripe, it undergoes a rapidly loss of moisture and darkening of the rind. The dehydration and darkening process of the fruit are followed by rind cracking and exposure of the juicy part, which are pathological damage. Fresh rambutan can be stored up to 4 to 5 days at ambient temperature and relative humidity (Syarifah et al., 2015). Usually, this fruit is

consumed fresh, but the rambutan flesh is industrially processed to produce juice, jams, jellies and marmalades (Klinkesorn et al., 2011).

The application of rambutan flesh leave a by-product which are the skin and light brown seed. The rambutan fruits are deseeding and these seeds (~4-9g/100g) are a wastage in the canning industry (Lourith et al., 2016). Roasted rambutan seeds are considered edible in some Asian countries. The light brown oval seed is slightly bitter. According to Lasekan et al. (2015), rambutan seeds have a bitter taste due to traces of alkaloid, tannin, saponin, and phenolic compounds, such as: ellagic acid, corilagin, and geraniin. The seed also contain relatively higher amount of fat (between 14% and 41%). The increasing feedstock of rambutan for industry means rambutan seed wastage is also increasing (Mahisanunt, et al., 2016).

Rambutan seed has a great potential in food and cosmetic industries due to it relatively high amount of edible fat. It could be suitable as a cocoa butter substitute in the food industry and lip balm for cosmetic industry. Studies of potential rambutan seed fat applications in the food and bioenergy industries have been undertaken that can give the demand for vegetable fat and oils for industrial proposes in medicinal and cosmetics products, processing of rambutan seed residue from the processed food industry for certain industrial applications is worth of attention (Lourith et al., 2016). Rambutan seeds used as a natural sustainable source of fat and oil (Yoswathana et al., 2013). The physical and chemical properties of fat and oil is key factor to know the usage and its applications in industry. Thus, this research was done to determine the physicochemical properties of rambutan seed fat.

2. MATERIALS AND METHODS

2.1 Raw Materials

Rambutan fruits were obtained from a local market in Kuala Pilah, Negeri Sembilan. The skin were peeled off and flesh was removed from seeds. The seeds were washed and wrapped with aluminium foil before stored at -20°C for further experiments. The weight of rambutan fruit and rambutan seeds were recorded.

2.2 Preparation of Rambutan Seed Fat

The dried Rambutan seeds were ground to a fine powder. The extraction method that had been used was Soxhlet extraction method. The dried rambutan seed powder were weighed accurately 5g each into an extraction thimble. The opening of the thimble was loosely plugged with cotton and the thimble was placed into a Soxhlet extractor. Dried round bottom flask was weighed accurately and 150ml of petroleum ether was inserted. The apparatus was

connected to the condenser. The tap water was connected and the extraction started carried out for 8 hours on an electrothermal extraction unit. The boiling point of petroleum ether was set to 50°C. The flask containing the petroleum ether extract was removed after the extraction complete. Then, the petroleum ether extract was evaporated on a boiling water bath. The flask was transferred into an oven at 105°C for one hour to dry the extract. The flask was transferred immediately into a desiccator to cool and weigh. Three replicate extractions were performed.

% Fat in sample = (weight of fat in rambutan seed fat (g))/(weight of rambutan seed powder (g)) x 100

Weight of fat in sample = (weight of flask + fat) – weight of flask

2.3 Physicochemical Analysis of Rambutan Seed Fat

2.3.1 Determination of Melting Point

RSF was filled into a capillary tube by 1cm capacity using a piece of filter paper. The capillary tube was cooled at a temperature of less than 0°C for 2 hours. The tube was submerged in 700ml of water in position of the top end of the RSF is 1cm below the water level. The thermometer was hanged in the middle of the beaker. The water was heated at 100°C for 1 minute. The temperature was recorded when the fat starts to slip out of the tube and the final temperature is when all the fat has slipped out. This temperature is referred to as the slip point (or melting point).

2.3.2 Determination of Refractive Index (RI)

RI was obtained using a refractometer and a sodium vapour lamp. Temperature used was: 20°C for oils, 40-60°C for hydrogenated fats and 80°C for waxes. The adjustment scale procedure: 2-3 drops of distilled water was placed on the main prism surface using a syringe, cover with the secondary prism and look through the eyepiece. The thermometer scale was 20°C and the refractometer was set at 1.3330 (Brix 0%). The secondary prism was open and 2-3 drops of oil at 40°C was placed at the centre of the main prism. The RI was recorded.

2.3.3 Determination of Total Carotene Content (TCC)

Carotene content in the sample was analysed by Ultraviolet-Visible (UV-Vis) spectrophotometer at 446nm using MPOB test method (MPOB Test Method, 2004). The RSF was homogenized and weighed accurately to 0.1g in the 25ml volumetric flask and diluted with n-hexane solution to the mark. The solution was transferred into a 1cm quart cuvette and the absorbance was measured at

446nm against n-hexane. The carotene content of RSF is defined and calculated as β-carotene in parts per million (ppm).

The calculation was as follows:

Carotenoids content = [V x 383 x (As-Ab)]/(100 x W)

Where:
V = The volume used for analysis
383 = The extinction coefficient for carotenoids
As = The absorbance of the sample
Ab = The cuvette error
W = The weight of the sample in g

2.3.4 Determination of Water Activity (W_A)

Water activity of RSF was determined using AQUA-LAB water activity system. Water activity is the amount of water in a substance relative to the total amount of water it can hold (Vaisala, 2009). It is defined as:

Water activity, $a_w = p/p_0$

Where;
p = the partial pressure of water in substance above the material
p_0 = the saturated vapour pressure of pure water at the same temperature

2.3.5 Determination of Acid Value (AV)

The AV was determined by mixed equal volumes of 25ml diethyl ether and 25ml of alcohol and 1ml of 1% phenolphthalein indicator in a conical flask. This solution was neutralized with 0.1M NaOH. 1 to 10g of oil was dissolved in the mixed neutral solvent. 0.1M NaOH was titrated until a pink colour persists for 15 seconds was obtained (Micheal et al., 2014).

Acid value = Titre value (ml) x 5.61/weight of sample (g)

Free fatty acid value is usually calculated as oleic acid (1ml 0.1M NaOH = 0.0282g oleic acid)

2.3.6 Determination of Peroxide Value (PV)

1g of RSF was weighed into dry and clean test tube. 1g of potassium iodide powder and 20ml of solvent mixture (glacial acetic acid:chloroform in 2:1 (v/v)) was added while sample is still a liquid. The test tube was placed in boiling

water to boil vigorously for 30 seconds. The oil was poured into a conical flask containing 20ml of 5% potassium iodide solution. The test tube was rinsed twice with 25ml distilled water and poured back into the conical flask. The mixture was titrated with 0.002M sodium thiosulphate until yellow colour of the reactant has disappeared. 5 drops of starch solution (1%) was added, which gave a light blue colour. The mixture was titrated again until the light blue colour discharged (MPOB Test Method, 2004).

The calculation was as follows:

Peroxide value = (Vs -Vb)/weight of sample x T x 10^3

Where;
T = Molarity of sodium thiosulphate
Vs = Volume in ml titration for sample
Vb = Volume of ml titration for blank

2.3.7 Determination of Saponification Value (SV)

The sample was melted if it is not in liquid turn and was filtered through filter paper to removed solid impurities and traces of moisture. 2g of RSF was weighed and added to a flask with 30ml of ethanoic potassium hydroxide (KOH) solution and was attached to a reflux condenser and heated on a water bath for 1 hour with occasional shaking to ensure the sample are fully dissolved. After the sample was cooled, 1ml of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until pink endpoint is reached. A blank determination was also carried out omitting the oil and saponification value was calculated using equation (Micheal et al., 2014).

The calculation was as follows:

Saponification value = [(b-a) x M x 56.1]/sample weight (g)

Where;
a = sample titre value
b = blank titre value
M = molarity of the HCl
56.1 = Molecular weight of KOH

2.4 Statistical Analysis

All tests were performed in triplicates. Data was presented as means ± standard deviation. Differences among means were evaluated using one-way ANOVA,

SPSS version 15.0. The level of significance applied was $p < 0.05$.

3. RESULTS AND DISCUSSION

In this research, rambutan seed was extracted using soxhlet extraction method. Extraction of rambutan seed fat using different extraction methods and solvents yield different fatty acids and physicochemical properties. Extraction process can be prepared by several methods; reflux, soxhlet and maceration (Lourith et al., 2016). The results have been presented and discussed. The possible interpretations have been given under the following headings:

Analysis	***Value***
Melting point	38.00±1.00 – 48.83±1.61°C
Refractive index	1.46±0.00
Total carotene content	1.18±0.06mg/kg
Water activity	0.4721±0.0176
Acid value	1.2162±0.1520mg KOH/g
Peroxide value	9.6000±0.4000g/g
Saponification value	146.8040±18.0182mg KOH/g

Table 1: Physicochemical analysis of Rambutan Seed Fat (RSF)

3.1 Determination of Melting Point

Based on the results obtained, the RSF started to slip out from the capillary tube at 38.00±1.00 and completely slipped out at 48.83±1.61°C. Yanty et al. (2013) reported that the melting point of RSO was 39.2°C. Akhtar et al. (2016) reported that melting point of cocoa butter was in between 27 to 35°C. That means, RSF contain higher saturated fat compared to cocoa butter. As the chain's length of the saturated fat increase, the melting point also increases. This may provide as a reliable source of cocoa butter substitute. The melting point of the fats is used to characterize oils and fats and it relation to their physical properties, such as hardness and thermal behaviour (Nassu and Goncalves, 1999).

3.2 Determination of Refractive Index (RI)

RI value of RSF was 1.46±0.00. Sirisompong (2011) reported that the RI was 1.469±0.001. The RI gives information about the intermolecular interaction of the system. It had been established that the density and RI are strongly correlated. The molar refractivity is approximately proportional to the molecular weight of the hydrocarbon molecule. RI was found to decrease linearly with increasing temperature (George and Singh, 2016). The RI value increase with the increment in the length of the carbon chain and number of double bond present.

3.3 Determination of Total Carotene Content (TCC)

Carotenes are minor components which are responsible for the orange-red characteristic of crude palm oil. In humans, carotene such as β-carotene is a precursor to vitamin A, a pigment essential for good vision and eye health. β-carotene is known as a powerful antioxidant because it destroys toxic free radicals. Therefore, it is widely used for vitamin-enriched margarine, nutrient preparation and pharmaceuticals (Noor and Roji, 2011). β-carotene is a powerful antioxidant which protects against cardiovascular disease as it inhibit the oxidation process of low-density lipoprotein (LDL) (Santos et al., 2015). It is currently incorporated in a variety of dietary supplements, including multivitamin, vitamin A and antioxidant formulations. It was reported that β-carotene prevent or delay carcinogenesis induced by virus and chemicals (Moh et al., 1999). Thus, it was found that RSF has 1.18±0.06 mg/kg of TCC. However, carotenes are susceptible to degradation by oxidation and thermal process, especially under severe processing and storage condition due to their unsaturated nature. It has been reported that normal room temperature was found to decrease carotenes concentration due to naturally occurring bioactive compounds which has high affinity towards heat and light (Noor and Roji, 2011).

3.4 Determination of Water Activity

The water activity of RSF was 0.4721±0.0176. Every fluid can hold a certain amount of dissolved water. The maximum amount of water that a particular fluid can contain in solution is referred to as its saturation point. The free water is a destructive contaminant to almost all oil applications. Water activity is dependent of the liquid being measured. Since water activity applies to all fluids and solids, it can be used widely for all substances regardless of chemical composition or physical characteristics (Vaisala, 2009). Moisture content was determined to be low especially in oil, suggesting that there is minimal possibility of microbial deterioration.

3.5 Determination of Acid Value

AV is an important index of physicochemical property of oil which is used to indicate the quality, age, edibility and suitability of oil in industrial usage. The presence of free fatty acid in oil or fat is an indication of previous lipase activity, either hydrolytic action or oxidation. AV are used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat (Michael et al., 2014). AV for RSF was 1.2162±0.152 mg KOH/g. Definition of AV of oil is the quantity of KOH in mg which is necessary

for neutralization of free fat acids in 1g of oil. As the increase of AV, the rancidity also increases. Lourith et al. (2016) reported that AV of rambutan fat was 4.35±0.00 mg KOH/g using maceration in n-hexane method. RSF was seen as a suitable ingredient in confectionary industry as the AV is low.

3.6 Determination of Peroxide Value

The quality of the oils is dependent on their chemical compositions, such as the percentage of unsaturation fatty acid. The PV, which depends on temperature, time and light, measures the extent of primary oxidation of oils (rancidification). As the PV decrease, the rancidity measurement also decreases. Rancidity of oils can produce potentially toxic compounds associated with long term health effects such as neurological disorders, heart and cancer. Oils with a high degree of unsaturation are highly susceptible to oxidation as compared to saturated oils. Oils also become susceptible to microbial rancidity, in which microorganisms such as bacteria and yeast use their enzymes to break down chemical structures in the oil, leading to the production of unwanted odours and flavours (Kaleem et al., 2015). The PV of oil represent the quantity of peroxide which is found in such food structure and who have the capacity to liberate in one oxidative processes iodine I_2 by potassium iodide (KI). PV is usually reported as the ml of 0.002 M sodium thiosulphate per gram of sample. If this value is multiplied by 2, the figure equals milliequivalents of peroxide oxygen per kilogram of sample (mEq/kg), which has greater international recognition. Fresh oil usually has PV below 10 mEq/kg. A rancid taste often begins to be noticeable when the PV is between 20-40 mEq/kg. Rambutan seed fat possessed a lower PV than other fats, which is consistent with the low iodine value and confirms a low degree of hydrogenation. PV for RSF was 9.6000±0.4000g/g. Lourith et al. (2016) reported that the PV for RSF was 1.00±0.00 g/g. It is important to add antioxidants such as vitamin E or C as preservatives in vegetable oils to delay or slow down the development of rancidity. Oxidation of lipids is another common and often undesirable chemical change that may influence flavour, aroma, nutritional quality and in some cases even the fineness of the product. It also causes darkening of the oil colour, formation of foam on the oil surface and increases the viscosity of the oil. Oxidation can be inhibited or retarded by some methods such as vacuum packaging, modified atmosphere packaging and refrigeration/freezing (Kaleem et al., 2015).

3.7 Determination of Saponification Value

The SV for RSF was 146.8040±18.0182mg KOH/g. The SV of rambutan fat was 182.1±0.16 mg KOH/oil g as reported by Yanty et al. (2013). Meanwhile, Lourith et al. (2016) reported that 246.73±0.10 mg KOH/oil g. Sirisompong (2011) reported that 166±3.00 mg KOH/oil g. The SV of rambutan's fat is also similar like those of cocoa butter (194.4 mg KOH/g) and has suggested that such oils/fats may be utilized in the production of liquid soap and shampoos (Yanty et al., 2013). The SV indicate the proportion of short chain or low molecular weight fatty acid constituents of the

rambutan seed fat (Lourith et al., 2016).

4. CONCLUSION

Based on the result of melting point, it indicates that RSF contain high saturated fatty acid as the melting point was high. Also with RI, the low of RI value, the higher of saturated fat or single bond present. Carotene indicates the present of Vitamin A and a powerful antioxidant. Water activity of RSF is low which contribute to low possibility of microbial deterioration. AV and PV is low means the rancidity of oil low. The low SV, the longer of fatty acid chain present in RSF and higher number of its molecular weight.

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