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Physiochemical Analysis of Cocoa Powder-Like from Roasted Seeds of Fermented *Nephelium lappaceum* L. (Rambutan) and *Nephelium mutabile* Bl. (Pulasan) Fruits

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The present study demonstrated the physiochemical analysis of cocoa powder-like from roasted seeds of fermented rambutan and pulasan fruits in comparison to commercial cocoa powder. The crude fat content of cocoa powder (25.70%) was lower compared to both rambutan and pulasan seeds (35.00%). The major fatty acids in pulasan seed were similar to cocoa fat, which were stearic acid and petroselinic acid. The major cocoa-like flavour component~~s~~, pyrazine was found in both rambutan and pulasan seeds. Lower saponin content was obtained in pulasan seed compared to rambutan seed. Meanwhile, pulasan seed contained higher TPC concentration than rambutan seed. Both fruit seeds were concluded to have high potential to be utilized as cocoa powder substitute in the future and this could open up a new direction in the flavour and fragrance sector~~,~~ and in the ~~s~~ manufacture of cocoa-like food products.

Key words: Fat properties; polyphenol; volatile compounds; *Nephelium lappaceum* L.; *Nephelium mutabile*

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Cocoa bean is an important raw material source for the chocolate and confectionery manufacturing industry. In cocoa powder processing, cocoa fat is normally removed followed by grinding the leftover bean. Nowadays, the price of cocoa fruit is getting higher due to limited plantation supplies [1]. This condition causes many researchers to put their efforts ~~on~~ into finding substitutive sources ~~of~~ for cocoa powder [2].

Generally, rambutan seeds are discarded after processing and can cause serious environmental issue if improperly disposed of [3]. Hence, various properties of rambutan seeds were studied by researchers in order to fully utilize them. However, most of the studies are on the potential of rambutan seed fat as a cocoa butter fat replacer and less information was available on cocoa powder. A ~~r~~recent research reported that rambutan seed can be processed into a cocoa-like powder by simulating the processing of cocoa powder, especially the fermentation and roasting stages [4]. They found that rambutan seed powder shared some similar physicochemical properties as cocoa powder when optimum condition was applied in the processing. The optimum roasting conditions of 25min and 131°C produced rambutan seed powder with physiochemical properties and color closest ~~d~~ to cocoa powder [4]. Fermentation and roasting treatments are important to generate unique flavor compounds for quality enhancement of food product [5]. Fermentation plays a significant role in generating the flavor of rambutan seed powder similar to that of cocoa powder [6]. Flavor precursors are produced during fermentation and converted to special flavor after roasting. Pyrazine compounds that are produced from fermented and roasted rambutan seeds give rise to cocoa-like flavor [4]. Thus, fermentation and roasting are significant in order to transform rambutan seed powder into cocoa powder-like.

Pulasan fruit is a type of tropical fruit, which is under the same family as rambutan. The boiled and roasted seeds of pulasan can be made ~~as~~ into a cocoa-like beverages due to the presence of edible oil [7]. Thus, it has the potential to be another alternative to produce cocoa powder-like. However, so far there is no study on the potential of pulasan seed as a replacement for cocoa products. Only limited information on the physical appearance, origin and uses are available [4]. This is study will enrich the knowledge on the physicochemical properties of pulasan seed and its possibility to be made ~~as~~ into a cocoa powder-like. In this research, the physicochemical properties of fermented and roasted seeds of rambutan and pulasan including crude fat content, fatty acid composition of fat, triacylglycerol profile of fat, thermal profile of fat, the saponin content (anti-nutrient), the total phenolic content (antioxidant) and the volatile compounds profile were studied. The properties of both fruit seeds were also compared with commercial cocoa powder.

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MATERIALS AND METHODS

Preparation of materials

The cocoa powder was collected from a commercial plantation at Jengka, Pahang, and 1 kg of both fresh rambutan and pulasan fruits were collected from a commercial plantation at Jaya Gading, Pahang in Malaysia. The fruits used in this work were unflawed, fully grown, and ready for consumption, with consistent size and red in colour. The skins of the fruit samples were removed. Subsequently, the fruits were vacuum packed and placed in a chest freezer at -20 °C.

Fermentation and drying of rambutan and pulasan seeds

Layer by layer concept was used to arrange the seeds evenly in the plastic container with banana leaf to create anaerobic condition. The sample containers were covered by a cotton cloth and eight days of fermentation was carried out at room temperature. After fermentation, all the seed samples were transferred to similarly ~~e~~ labelled aluminium plates and dried at 60 °C for 48 h in a convection oven (UNE 400, Memmert, Germany). This method was adopted from Chai *et al.* [4] with slight modification.

Roasting and grinding of rambutan and pulasan seeds

The same convection oven was used to roast the seeds at 131 °C for 25 min. After roasting, all the samples were cooled for 10 minutes. The skins of the seeds were then removed and the remaining core seeds were grounded using a mortar and pestle. The rambutan and pulasan grounded seed sample was then transferred into individual sealable plastic bag and stored in a desiccator containing silica gels (blue bead, 2-4 mm, Bendosen, Malaysia) prior to analysis. This method was adopted from Chai *et al.* [4] with slight modification.

Determination of crude fat content

The crude fat content of the seed powder~~s~~ was extracted using petroleum ether (40-60 °C, Bendosen, Malaysia) in a Soxhlet thimble for 8 hours and then purified *via* vacuum rotary evaporation ~~or~~ (IKA digital evaporator, IKA, China).

Determination of fatty acid composition

1 mg of each seed fat was ~~ere~~ dissolved in 1 mL of *n*-hexane (Friendemann Schmidt, Australia) and vortexed for 30s before filtering. 1 µL of the mixture was injected into a gas chromatography system (7890A, Agilent Technologies, USA) with an automatic injector (G4513A, Agilent Technologies, China) at 250 °C and 7.0699 psi pressure used in the splitless mode. A HP-5 capillary column (30 m×250 µm id×0.25 µm film thickness) (19091J-433, Agilent Technologies, USA) (equivalent to 5% phenyl methyl siloxane) was used. The initial temperature of the column was 40 °C hold for 2 min and increased to 310 °C at 10 °C/min for 5 min. The detector used was a quadrupole mass spectrometer (5975C, Agilent Technologies, USA) together with an electronic impact ionization system. Helium was used as the carrier gas, with the septum purge gas flow at 3 mL/min. Compound identification was done by comparing the mass spectra of each compound with the NIST MS Search 11.0 Library of Mass Spectra in GC-MS Data Analysis software.

Determination of triacylglycerol (TAG) profile

1 mg of each seed fat was ~~ere~~ dissolved in 1mL UPLC-grade methanol (A458-1, Fisher Scientific, USA) and subjected to UPLC-QTOF/MS analysis, which was adopted from Abd Hamid *et al.* [8] with slight modification.

Determination of volatile compounds profile

Solid phase micro-extraction in the headspace (SPME-HS) was used to trap the volatile compounds of the seed powders. 1 g of each sample powder was placed in a 20 mL screw top headspace vial separately and heated at 60 °C above a heater with the fiber placed in the headspace for 30 min exposition. A gas chromatography system (7890A, Agilent Technologies, USA) with a manual sampler and a connected mass spectrometer as detector (5975C, Agilent Technologies, USA) was used to analyze the SPME-HS fiber with trapped volatile compounds. A HP-5 capillary column (30 m×250 µm id×0.25 µm film thickness) (19091J-433, Agilent Technologies, USA) (equivalent to 5% phenyl methyl siloxane) was used for separation of the compounds. The oven temperature program and injection conditions were adopted from Rodriguez-Campos *et al.* [9]

Determination of saponin content

The seed powders were defatted through extraction in *n*-hexane. 1 g of each sample powder was added with 10 mL of n-hexane in a beaker and placed in~~to~~ an ultrasonic cleaner (8510, Branson, USA) for 30 min. The solution was then filtered through vacuum filtration. Saponin content was extracted from 0.3 g of residues with 5 mL of 80% aqueous methanol (Systerm, Malaysia) in an ultrasonic cleaner. The extract was then filtered. A total of three times defatting approach and saponin extraction-filtration steps were carried out for each sample powder separately. The method of analysis for saponin content was adopted from Mehdizadeh *et al.* [6] with some modifications, where a microplate reader (Infinite M200 PRO, Tecan, Switzerland) was used and the concentrations of crude soybean were prepared as 5000 ppm, 10000 ppm, 15000 ppm, 20000 ppm and 25000 ppm in 80% aqueous methanol. The final saponin content (mg of soya saponin/g sample) was

calculated using the formula C = c(V/M), where c was the concentration of soybean saponin obtained from the calibration curve and V and M were the volume of extractive solvent and mass of sample respectively.

Determination of total phenolic content (TPC)

The same defatting approach used in saponin determination was used for all the sample powders in TPC determination. However, the whole TPC determination was carried out in the dark. TPC was extracted from 0.5 g of residues with 5 mL of ethanol (Systerm, Malaysia) using the same extraction procedure as that for saponin extraction. The Folin-Ciocalteu method for TPC analysis was adopted from Sembiring *et al.* [10] with some modifications, where the concentration of diluted gallic acid (anhydrous, Merck, German) was prepared as 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm in ethanol. The final TPC (mg of GAE/g sample) was calculated using the same formula of C = c(V/M) as in saponin content calculation.

RESULTS AND DISCUSSION

Crude Fat Content

The crude fat content of each seed powder is as tabulated in Table 1. The average crude fat content in cocoa powder was 2.57 g while that for each rambutan and pulasan seed powder was 3.5 g. This indicates that the crude fat contents of both fruit seeds were higher than that of cocoa powder by 9.30%. The low value of crude fat content in cocoa powder may be due to its processing, where a large amount of cocoa fat was removed [11]. Data from previous study conducted by Benković *et al*. showed that 10-18 g fat content was found in 100 g of cocoa powder [12]. Regardless of fermentation, cocoa bean has crude fat content at around 50%-55% [13]. This revealed that processed cocoa powder normally has a lower fat content.

Fatty Acid Composition

The fatty acid compositions of the three seed fats with over 80% quality of matched mass spectra are tabulated in Table 2. The major fatty acid peaks of cocoa, rambutan and pulasan seed fats appeared 19-25 min after sample injection in varying amounts. All the detected fatty acids in cocoa fat were found in both rambutan and pulasan seed fats albeit in different concentrations. The results showed that cocoa fat contained higher amounts of saturated fatty acids (62.65%) than unsaturated fatty acids (37.35%) but the reverse is true for rambutan and pulasan seed fats., The major fatty acids of cocoa fat are stearic acid (40.19%) and oleic acid (37.35%) but for rambutan seed fat, the major fatty acids are petroselinic acid (51.71%) and arachidic acid (37.45%). In fact, petroselinic acid, oleic acid, and cis-vaccenic acid are isomers ($C_{18}H_{34}O_2$). (redundant)., Pulasan seed fat contained two major fatty acids which are similar to cocoa fat, namely stearic acid (35.72%) and petroselinic acid (50.38%).

Table 1. Crude fat content of cocoa, rambutan and pulasan seeds

Samples	Crude fat content (g/10 g)	Fat yield (%)
Cocoa	2.57 ± 0.33	25.70
Rambutan	3.50 ± 0.30	35.00
Pulasan	3.50 ± 0.25	35.00

Table 2. Fatty acid composition of cocoa butter, rambutan and pulasan seed fat

Fatty acid	Cocoa (%)	Rambutan (%)	Pulasan (%)
Palmitic acid	20.77 ± 0.03	*1.17* ± 0.078	1.07 ± 0.040
Stearic acid	40.19 ± 0.870	*7.49* ± 0.590	35.72 ± 0.505
Petroselinic acid	-	*51.71* ± 0.02	50.38 ± 0.100
Oleic acid	37.35 ± 0.910	*0.51* ± 0.005	2.39 ± 0.150
cis-Vaccenic acid	-	*0.94* ± 0.320	-
Arachidic acid	1.69 ± 0.635	*37.45* ± 3.195	10.41 ± 0.505
Behenic acid	-	*0.50* ± 0.03	0.03 ± 0.015
Erucic acid	-	*0.24* ± 0.000	-

Triacylglycerol (TAG) Profile

The TAG analysis results of ~~each~~ the three seed fats are shown in Table 3. There was a total of 15 TAG compounds found in cocoa fat. Based on the results in Table 3, it can be seen that both rambutan and pulasan seed fats contain~~ed~~ most of the TAG compounds ~~as~~ found in cocoa fat. The highest ion response in cocoa fat and rambutan seed fat was triglycerol monostearate while for pulasan the highest ion response was diglycerol tripalmitate. Comparing the compounds detected in cocoa fat, rambutan seed fat had 14 compounds similar to those in cocoa fat, while pulasan seed fat had 12 compounds similar to those of cocoa fat. However, diglycerol monostearate is present in rambutan seed fat while glycerol monostearate, triglycerol distearate and diglycerol tristearate are present in pulasan seed fat. These compounds are not present in cocoa fat. Rambutan seed fat was observed to contain~~s~~ more TAG compared to pulasan seed fat.

Volatile Compounds Profile

Both rambutan and pulasan seeds released concentrated smells very similar to that of cocoa powder after being roasted. Hence, the volatile compounds profile of cocoa powder was compared to the volatile compounds mass spectra of rambutan and pulasan seed powders. Similar significant peaks appeared at 1.8 min, 8.4-8.5 min, 11.0-11.6 min, 12.0-12.5 min and 14-14.5 min albeit in different concentrations. From Table 4, it can be seen that there were11 volatile compounds in cocoa powder with over 80% quality of matched mass spectra. Of these volatile compounds, two similar components were detected in rambutan seeds while 3 similar components were detected in pulasan seeds. The significant result was the presence of pyrazine groups such as 2,5-dimethyl-pyrazine and tetramethyl-pyrazine in all samples of rambutan and pulasan seed powders. As reported by Chai *et al*. [4], pyrazines were the key compounds used to indicate the quality and amount of cocoa flavor after roasting. These pyrazine groups contribute to the flavors of roasted (?)hazelnut, milk coffee and mocha [14]. Benzaldehyde was also found in both fruit seeds as well. The benzaldehyde compounds tend to give an almond-like flavor since ~~the~~ almond extracts contained a known amount of benzaldehyde [15]. In short, the major cocoa-like flavor components were developed in both rambutan and pulasan seed powders after seed roasting and this can be their potential ~~to~~ as replacements for cocoa beans.

Table 3. The TAG profile of cocoa, rambutan and pulasan seed fats

Component name	Ion response		
	Cocoa	Rambutan	Pulasan
Diglycerol monostearate	287	-	196
Triglycerol monostearate	17762	35871	16112
Glycerol monostearate	386	1196	-
Triglycerol distearate	1050	1518	-
Diglycerol distearate	392	1892	944
Glycerol-1,3-distearate	10130	245	76
Diglycerol tri~~i~~stearate	10329	874	-
Triglycerol monopalmitate	-	337	120
Diglycerol monopalmitate	81	407	86
Glycerol monopalmitate	87	310	235
Triglycerol dipalmitate	2137	558	1297
Diglycerol dipalmitate	2893	1625	2504
Glycerol-1,3-dipalmitate	218	224	-
Triglycerol tripalmitate	5162	1304	115
Diglycerol tri~~i~~palmitate	761	22516	21185
Glycerol 1-palmitate-3-stearate	139	655	1045

Table 4. Volatile compounds of rambutan and pulasan seeds

Detected volatile compounds (cocoa powder B)	Rambutan	Pulasan
Pyrazine, 2,5-dimethyl-	13.16 ± 0.195	11.57 ± 0.516
Benzaldehyde	39.77 ± 3.204	48.87 ± 1.696
1-Aminobenzotriazole	12.56 ± 0.000	NA
Furan, 2-pentyl-	7.89 ± 0.345	NA
1,5-Cyclooctadiene,1,5-dimethyl-	NA	NA
Benzeneacetaldehyde	1.19 ± 0.000	2.23 ± 0.024
Pyrazine, tetramethyl-	25.43 ± 0.961	37.33 ± 0.991
Dodecane	NA	NA
Diethyl Phthalate	NA	NA
Bis(2-ethylhexyl) phthalate	NA	NA

NA- Not Available

Table 5. Saponin and total phenolic content of cocoa, rambutan and pulasan seed powders

Sample	Concentration of saponin (mg soybean/ g sample)	Concentration of gallic acid (mg GAE/ g sample)
Cocoa	380.5556 ± 0.2222	8.6977 ± 0.0003
Rambutan	147.8175 ± 0.1240	2.8640 ± 0.0005
Pulasan	119.0476 ± 0.3072	4.4163 ± 0.0003

Saponin Content and Total Phenolic Content (TPC)

The saponin contents of all defatted seed powders are shown in Table 5. The concentration of saponin was determined from the standard curve of soybean saponin. The results indicate that cocoa powder contained the highest amount of saponin at 380.5556 mg soybean saponin/g sample compared to rambutan and pulasan seed powders with a difference of 35.95% and 40.39% respectively. Pulasan seed with a lower saponin content than rambutan seed may be slightly superior in terms of a lower bitterness in taste. Pulasan seeds are edible in the raw since the toxic hydrocyanic acid is not presente~~d~~ in the seed [7]. The lower the saponin content, the better the nutritional value since high saponin content negatively affect human growth i.e. growth depression [3].

The TPC of all defatted seed powders are also shown in Table 5. Dark condition was applied due to the significant light effect toward antioxidant properties. From the result, cocoa powder showed the highest TPC value at a concentration of 8.6977 ± 0.0003 mg GAE/g sample compared to the TPC values of both rambutan and pulasan seed powders with a difference of 36.52% and 26.80% respectively. Comparing the rambutan and pulasan seed powders, rambutan which showed a lower TPC level. This will further reduce its nutritional value. Pulasan seed, with a slightly higher TPC value and lower saponin content is a better potential to replace cocoa powder as compared to rambutan seed even though its TPC value was less than that of cocoa powder.

CONCLUSION

The fat properties, polyphenols and volatile compounds of rambutan and pulasan seeds after fermentation—and roasting were determined and compared to commercial cocoa powder. The crude fat content of cocoa was lower than that of rambutan and pulasan seeds, which may due to the removal of a large amount cocoa fat during processing. Both stearic acid and petroselinic acid which were major fatty acids in pulasan seed fat were similar to cocoa fat, but for rambutan seed fat the major fatty acids were petroselinic acid and arachidic acid. In term of TAG profile, rambutan seed fat was almost similar to cocoa fat excpt for the absence of diglycerol monostearate. The melting point of pulasan seed fat which was at 6.3 °C, was closer to that of cocoa fat (6.6 °C) rather than that of rambutan seed fat (5.8 °C). The~~is~~ same goes for crystallization point, where pulasan seed fat crystallized at -21.3 °C which was closer to that of cocoa fat (-20.0 °C) as compared to that for rambutan seed fat (-18.6 °C). Major cocoa-like flavor components were found in both rambutan and pulasan seeds, especially the vital pyrazine groups. Lower saponin content in pulasan seed means lower bitterness than that of rambutan seed. Higher TPC values in pulasan seed made it slightly superior in term of antioxidant property, compared to that of rambutan

seed. In terms of the closest similarity to cocoa powder and its fat, four out of seven properties studied were shown by pulasan seed. However, both rambutan and pulasan seeds in this study are concluded to have the potentials to be cocoa powder replacers.

(Need a table and methods writeup for melting and crystallization points which were brought out in the conclusion but not in the main text otherwise omit this sentence on mp and cry pt in conclusion)

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REFERENCES

1. Saadoon, F., Varastegani, B., Yang, T. A. & Zzaman, W. (2017) Physical chemical properties of fermented and roasted rambutan seed fat (RSF) as a potential source of cocoa butter replacer. *International Journal on Advanced Science, Engineering and Information Technology*, **7(1)**, 57–62.

2. Issara, U., Zzaman, W. & Yang, T. A. (2014) Rambutan seed fat as a potential source of cocoa butter substitute in confectionary product. *International Food Research Journal*, **21(1)**.

3. Chai, K. F., Mohd Adzahan, N., Karim, R., Rukayadi, Y. & Ghazali, H. M. (2018) Characteristics of fat, and saponin and tannin contents of 11 varieties of rambutan (Nephelium lappaceum L.) seed. *International Journal of Food Properties*, **21(1)**, 1091–1106.

4. Chai, K. F., Chang, L. S., Adzahan, N. M., Karim, R., Rukayadi, Y. & Ghazali, H. M. (2019) Physicochemical properties and toxicity of cocoa powder-like product from roasted seeds of fermented rambutan (Nephelium lappaceum L.) fruit. *Food chemistry*, **271**, 298–308.

5. Febrianto, N. A., Yang, T. A. & Wan Abdullah, W. A. (2016) Cocoa-like flavor compound development of rambutan seed fat as the effect of fermentation and roasting. *International Food Research Journal*, **23(5)**.

6. Mehdizadeh, S., Lasekan, O., Muhammad, K. & Baharin, B. (2015) Variability in the fermentation index, polyphenols and amino acids of seeds of rambutan (Nephelium lappaceum L.) during fermentation. *Journal of Food Composition and Analysis*, **37**, 128–135.

7. Morton, J. F. (1987) *Fruits of warm climates*. JF Morton.

8. Abd Hamid, H., Ramli, A. N. M., Zamri, N. & Yusoff, M. M. (2018) UPLC-QTOF/MS-based phenolic profiling of Melastomaceae, their antioxidant activity and cytotoxic effects against human breast cancer cell MDA-MB-231. *Food chemistry*, **265**, 253–259.

9. Rodriguez-Campos, J., Escalona-Buendía, H. B., Orozco-Avila, I., Lugo-Cervantes, E. & Jaramillo-Flores, M. E. (2011) Dynamics of volatile and non-volatile compounds in cocoa (Theobroma cacao L.) during fermentation and drying processes using principal components analysis. *Food Research International*, **44(1)**, 250–258.

10. Sembiring, E. N., Elya, B. & Sauriasari, R. (2018) Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of Caesalpinia bonduc (L.) Roxb. *Pharmacognosy journal*, **10(1)**.

11. Cheng, H., Wei, K. & Wang, L. (2015) The impact of variety, environment and agricultural practices on catechins and caffeine in plucked tea leaves. In *Processing and Impact on Active Components in Food* , 597–603.

12. Benković, M., Tušek, A. J., Belščak-Cvitanović, A., Lenart, A., Domian, E., Komes, D. & Bauman, I. (2015) Artificial neural network modelling of changes in physical and chemical properties of cocoa powder mixtures during agglomeration. *LWT-Food Science and Technology*, **64(1**), 140–148.

13. Afoakwa, E. O., Quao, J., Takrama, J., Budu, A. S. & Saalia, F. K. (2013) Chemical composition and physical quality characteristics of Ghanaian cocoa beans as affected by pulp pre-conditioning and fermentation. *Journal of food science and technology*, **50(6)**, 1097–1105.

14. Afoakwa, E. O., Paterson, A., Fowler, M. & Ryan, A. (2008) Flavor formation and character in cocoa and chocolate: a critical review. *Critical reviews in food science and nutrition*, **48(9)**, 840–857.

15. Denis, W. & Dunbar, P. B. (1909) The Determination of Benzaldehyde in Almond Flavoring Extract. *Industrial & Engineering Chemistry*, **1(4)**, 256–257.