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Comparative study of fat properties and phenolic contents of fermented rambutan (*Nephelium lappaceum* L.) and pulasan (*Nephelium mutabile* Blume) seeds

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Abstract

Rambutan and pulasan seeds are usually discarded as waste. However, the seeds contain a significant quantity of quality crude fat. Therefore, the present work was conducted to establish and compare the fat properties, and saponin and total phenolic contents of fermented rambutan and pulasan seeds. Results showed that the crude fat yields for rambutan and pulasan seeds were 3.98 and 7.41 g/10 g, respectively. Results also showed decreases in crude fat by 41% for rambutan seeds, and 23% for pulasan seeds after fermentation. The yields of the main fatty acid in rambutan and pulasan seeds, which was oleic acid, were 53.11 and 58.27%, respectively. Only 0.81 and 37.25% of triacylglycerols remained in rambutan and pulasan seed fats, respectively after fermentation. In addition, the melting temperature for both seed fats increased, while the saponin and total phenolic contents in rambutan and pulasan seeds decreased with increasing fermentation time.

Introduction

In Asian countries, there is a large diversity of nutritious tropical fruits such as rambutan (*Nephelium lappaceum* L.) and pulasan (*N. mutabile* Blume). Both rambutan and pulasan are from the family Sapindaceae, and their fruits are abundantly available during the harvest seasons of June to August, and November to January. Generally, the fruits are freshly consumed, or industrially processed into cans with cut pineapple fruit in syrup, and preserves such as jams and jellies, and also into juices and chips. The seeds are usually discarded, thus leading to environmental impacts as the discarded seeds will ferment and release off-odours (Chai *et al.,* 2019). During harvest seasons, there could be too much waste when supply is greater than demand. Therefore, it is necessary to find an alternative to convert the waste into other valuable food products. In the present work, we imitated cocoa bean fermentation in order to develop the appropriate precursors for the flavour, and to reduce the bitterness and astringency of the seeds. This process will lead to beneficial changes in the physicochemical properties of fruit seeds into valuable food products (Chai *et al.*, 2019).

In order to utilise the rambutan and pulasan seeds for different applications, the understanding of their chemical contents is important. The fruit pulp contains high sugars and organic acids, while its seed contains high crude fats (Harahap *et al.*, 2012). Major fatty acids in rambutan seeds are oleic acid (42.0%) and arachidic acid (34.3%). The presence of uncommon saturated fatty acid, such as arachidic acid which is a C20 saturated fatty acid, leads to the existence of semi-solid fat at room temperature. The crude fat in rambutan seeds can be extracted and used to manufacture soaps, candles, fuels, and cosmetic products (Issara *et al.*, 2014). However, there is a limited study on pulasan seeds. Pulasan is closely related to rambutan, and often gets mistaken with it. Pulasan seeds and rind are often traditionally used as medicines (Chan *et al.*, 2012). This shows that pulasan seeds have the potential to be scientifically explored through in-depth investigation and analyses.

Fermentation is one of the common processes to improve the physicochemical properties of rambutan seeds. In the fermentation of cocoa beans, the quantity of flavan-3-ol will affect the bitterness and astringency of the final product (Camu *et al.*, 2008). Fermentation of cocoa beans also influences

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the microbial activities and polyphenol concentrations, thus affecting the flavour of chocolate (Mehdizadeh *et al.,* 2015). As there are scarce information on the utilisation of rambutan and pulasan seeds, the objective of the present work was to create high value food product of both seeds through fermentation. Therefore, it is good to consider fermenting the fruit seeds in a manner similar to cocoa beans in order to obtain a cocoa butter-like product. In addition, this conversion process will also greatly reduce the amount of rambutan and pulasan waste being discarded to the environment.

Materials and methods

Sample collection and preparation

Rambutan and pulasan fruits were collected from a commercial plantation at Jaya Gading, Pahang, Malaysia. The fruits selected were fully grown and ready for consumption, free from damages, and had consistent size and colour. The peels of the fruit samples were removed, and the fruits were vacuum-packed and placed in a chest freezer at -20°C.

Fermentation of rambutan and pulasan seeds

Frozen peeled fruits were thawed at room temperature. Next, rambutan and pulasan seeds weighing 150 g were placed in a perforated plastic container. A non-perforated plastic container was placed below to collect sweating produced during fermentation. To construct a relatively anaerobic condition, banana leaves were used to fully cover the rambutan and pulasan seeds prior to transferring the set up to an incubator for fermentation. The seeds were fermented for 0 (control), 1, 3, and 5 d. The fermentation process was conducted in triplicates. Fermented seeds were collected after each fermentation period, and oven-dried for 48 h at 60°C. Then, the dried seeds were subjected to physicochemical analyses.

Crude fat content of fermented rambutan and pulasan seeds

The crude fat of fermented rambutan and pulasan seeds was extracted using the Soxhlet extraction method with petroleum ether as solvent at 40 to 60°C for 8 h. The fat was stored in a freezer at -20°C for further analysis.

Fatty acid composition of fermented rambutan and pulasan seeds

The fatty acid composition of fermented rambutan and pulasan seeds was analysed by gas chromatography coupled to mass spectrometry (GC-MS). The seed fat was first converted into methyl esters (FAME) by reacting 10 mg of fat with 0.01 mL of 1 mol/L sodium methoxide in 0.15 mL of *n*-hexane. The mixture was vortexed for 5 min. The top layer (1 µL) was then injected into GC-MS equipped with HP-5 column (30 m × 0.25 mm, 0.25 µm i.d.) (equivalent to (5%-phenyl)-methylpolysiloxane). The column was set at an initial temperature of 40°C, and retained for 2 min. The temperature was elevated to 310°C at a rate of 10°C/min for 5 min. Helium was used as the carrier gas, and the total flow rate was 24 mL/min, with the septum purge flow rate of 3 mL/min. The temperatures of the injector and detector were maintained at 250°C. Data were obtained using the GC-MS data analysis software. The confirmation of fatty acids was done manually based on NIST database. The components were quantified by the peak areas produced via the data integrator based on relative percentages.

Triacylglycerol (TAG) profile of fermented rambutan and pulasan seed fats

The triacylglycerol (TAG) profiles of fermented rambutan and pulasan seed fats were analysed using Waters ACQUITY UPLC I-Class/Xevo in line with Waters Xevo G2 Q-TOF mass spectrometer (Milford, MA, USA) following the methods described by Abd Hamid *et al.* (2018) with slight modifications. Briefly, 1 mg of each seed fat sample was dissolved in 1 mL of UHPLC-MS grade methanol. Next, 1 µL of aliquot was injected into UPLC-QTOF/MS at a flow rate of 0.50 mL/min. A linear gradient elution of A (0.1% formic acid in water) and B (acetonitrile) was applied, with the initial percentage of B being 20%, and the percentage was increased until 95%. The TAG peaks were identified by matching with the built-in library in UNIFI software.

Thermal behaviour of fermented rambutan and pulasan seed fats

The thermal behaviour of fermented rambutan and pulasan seed fats was analysed via the differential scanning calorimeter (DSC; NETZSCH Differential Scanning Calorimeter 214 Polyma, Selb, Germany)

following the methods described by Chai *et al.* (2019).

Saponin content of fermented rambutan and pulasan seeds

The saponin content of fermented rambutan and pulasan seed samples was determined by extracting 0.3 g of ground dried samples with 5 mL of 80% aqueous methanol, and sonication for 90 min using an ultrasonic cleaner (Branson 8510 Ultrasonic Cleaner, USA). The extract was then vacuum-filtered and retained in the dark at 4°C. The standard curve was then constructed using different concentrations of crude soy saponin in 80% aqueous methanol, where it was expressed as mg soy saponin per g sample.

Total phenolic content of fermented rambutan and pulasan seeds

The extraction of phenolic compounds of fermented rambutan and pulasan seeds was conducted with 0.5 g of seed powder in 5 mL of ethanol, and sonication for 90 min using an ultrasonic cleaner (Branson 8510 Ultrasonic Cleaner, USA). The extract was then obtained by a vacuum filtration, and 1 mL of extract was diluted in 10 mL volumetric flask with ethanol. The determination of the total phenolic content using the microplate method was based on the 96-well Folin-Ciocalteu technique. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per g of seed powder.

Statistical analysis

All data were presented as mean ± standard deviation (SD). Student *t*-test was used to compare the experimental results, with $p < 0.05$ considered a significant difference.

Results and discussion

Crude fat content of fermented rambutan and pulasan seeds

Figure 1 shows the crude fat contents of fermented rambutan and pulasan seeds. Initially, the rambutan and pulasan seeds' crude fat contents were 3.98 and 7.41 g/10 g, respectively. Following 5-day fermentation, the crude fat contents of rambutan and pulasan seeds decreased by 41 and 23%, respectively. Nevertheless, statistical analysis showed that fermentation time had no significant effect ($p > 0.01$) on the crude fat contents. Other study also showed decreasing trend as fermentation time increased (Asep *et al.*, 2008). This could be due to the action of lipase enzyme in breaking down the TAGs in seeds into free fatty acids (Afoakwa *et al.*, 2011). However, Mehdizadeh *et al.* (2015) reported an increase in the fat content of rambutan seeds following 10-day fermentation, which might be caused by the microorganisms utilising the carbohydrates in seeds. This converts them into fatty acids, which might contribute to the increased crude fat content.

Fatty acid composition of fermented rambutan and pulasan seeds

Based on Table 1, oleic acid (C18:1) (60.37%) and arachidic acid (C20:0) (20.32%) were the main fatty acids in fermented rambutan seeds. A similar observation was reported by Chai *et al.* (2019). Apart from that, erucic acid (C22:1) was also detected in rambutan seed fat subsequent to the 3-day fermentation which might be due to the elongation of

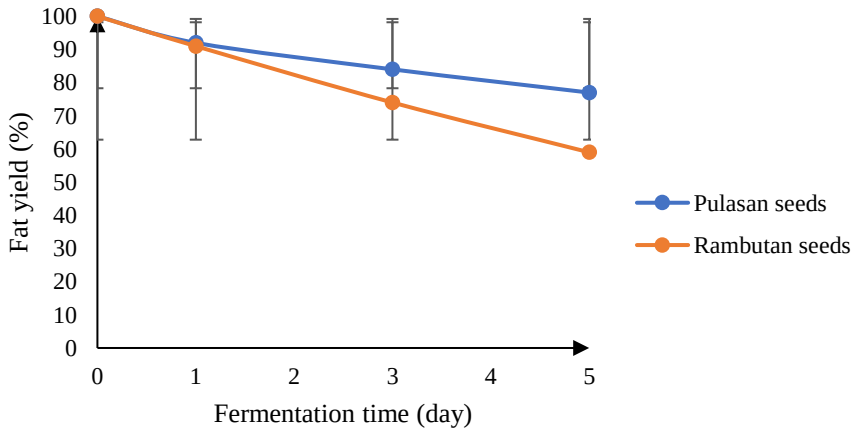


Figure 1. Crude fat contents of fermented rambutan and pulasan seeds.

Table 1. Fatty acid compositions of fermented rambutan and pulasan seeds.

Fatty acid	Rambutan seed Relative percentage (%)				Pulasan seed Relative percentage (%)			
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 0**	**Day 1**	**Day 3**	**Day 5**
Palmitic/hexadecanoic acid (C16:0)	2.71 ± 0.01	2.49 ± 0.00	1.18 ± 0.06	1.12 ± 0.04	3.26 ± 0.36	3.04 ± 0.12	1.35 ± 0.02	1.20 ± 0.04
Palmitoleic/hexadecenoic acid (C16:1)	0.41 ± 0.05	0.16 ± 0.06	0.06 ± 0.00	0.04 ± 0.01	0.07 ± 0.07	-	-	-
Stearic/octadecanoic acid (C18:0)	5.18 ± 0.01	4.87 ± 0.48	5.50 ± 0.41	2.91 ± 2.91	24.22 ± 0.86	32.14 ± 0.79	31.63 ± 1.30	27.40 ± 0.58
Oleic/octadecenoic acid (C18:1)	60.37 ± 1.10	66.00 ± 0.48	55.87 ± 0.93	57.48 ± 3.09	59.85 ± 0.57	50.47 ± 1.61	52.15 ± 2.48	54.77 ± 0.45
Arachidic/eicosanoic acid (C20:0)	20.32 ± 0.82	17.56 ± 0.43	36.88 ± 1.42	37.71 ± 0.29	10.47 ± 0.49	12.95 ± 0.68	14.51 ± 1.20	16.57 ± 0.19
Gondoic/eicosenoic acid (C20:1)	10.47 ± 0.29	8.92 ± 0.38	0.07 ± 0.00	0.11 ± 0.01	2.13 ± 0.51	1.41 ± 0.02	-	-
Behenic/docosanoic acid (C22:0)	0.54 ± 0.02	-	0.34 ± 0.01	0.45 ± 0.06	-	-	-	0.06 ± 0.00
Erucic/docosenoic acid (C22:1)	-	-	0.10 ± 0.01	0.19 ± 0.02	-	-	-	-
Total unsaturated fatty acids	71.24	75.08	56.09	57.81	62.05	51.87	52.51	54.77
Total saturated fatty acids	28.76	24.92	43.91	42.19	37.95	48.13	47.49	45.23

C18 fatty acids through sequential addition of acetate groups to the carboxyl by elongase enzyme (Chai *et al.*, 2019). On the other hand, the main fatty acids in pulasan seeds were oleic acid (C18:1) (59.85%) and stearic acid (C18:0) (24.22%). Although rambutan and pulasan belong to the same genus, they are still different species with different fatty acid compositions.

Based on these results, the fatty acid compositions had changed following fermentation, which might be due to the different metabolic processes that had occurred during fat extraction and fermentation (Abu *et al.*, 2000). The total unsaturated fatty acids decreased, and the total saturated fatty acids increased in both rambutan and pulasan seed fats following fermentation. During fermentation, the microorganisms required unsaturated fatty acids as nutrients to grow and maintain their membrane integrity, as well as to allow them to adapt well with the fermentation stresses. This thus contributed to lower total unsaturated fatty acids in seeds following fermentation (Parkouda *et al.*, 2015).

Triacylglycerol (TAG) profile of fermented rambutan and pulasan seed fats

The TAG compounds in fermented rambutan and pulasan seed fats identified by UPLC-QTOF/MS are tabulated in Table 2. The main TAG detected in rambutan seed fat were glycerol 1-palmitate-3-stearate and diglycerol distearate. However, the concentrations of both decreased following fermentation, with the total TAG in fermented rambutan seed significantly decreased following 5-day fermentation. The main TAG in pulasan seed fat were diglycerol tripalmitate and diglycerol distearate. Similarly, the concentrations of both also decreased following fermentation, with the total TAG in pulasan seeds decreased to only 56.78 and 37.25% following 1- and 5-day fermentation, respectively.

Variations were observed in TAG compositions of rambutan and pulasan seed fats. This might be due to the variation in fatty acid compositions in the seed fats which included stearic acid (C18:0) and palmitic acid (C16:0). The lipase enzyme contributed to higher lipolytic action where TAG were hydrolysed into one glycerol and three free fatty acids. Therefore, the TAG compositions in rambutan and pulasan seed fats decreased following fermentation.

Thermal behaviour of fermented rambutan and pulasan seed fats

The peak for each phase transition of fermented rambutan and pulasan seeds are shown in Figure 2. From the endothermic peaks obtained in the melting profile of rambutan seed fats (Figure 2a), the melting temperature was determined to be 40.7 and 23.7°C during the first and second heating processes, respectively. Manaf *et al.* (2013) found that fresh rambutan seed fat had a melting point of 40°C, which is quite similar to the result obtained in the present work. For pulasan seed fats, the melting temperature was 36.8 and 25.7°C during the first and second heating processes, respectively (Figure 2b). There was a higher melting temperature of rambutan seed fat during the first heating process, as it contained behenic acid (C22:0), a long chain of fatty acids. Since the rambutan and pulasan seed fats showed an increasing trend in melting temperature, it can be concluded that the melting point of seed fat would also increase following fermentation. Generally, the fermented seed fats had higher melting point as the hydrolysis of fat by lipase released high melting glycerides, which might contain long chain saturated fatty acids, such as arachidic acid which melted at 75.5°C (Aslam *et al.*, 2015). However, there were also seed fats with lower melting temperature with increasing fermentation duration due to the desaturation of fatty acids, thus increasing the amount of unsaturated fatty acid (Guerzoni *et al.*, 2001) and lowering the melting temperature.

From the cooling profile of seed fats, it was observed that the crystallisation temperature of rambutan seed fats had peaked at 10.4°C; whereas for pulasan seed fat, the peak was shown at 13.6°C. Based on the results obtained by Febrianto *et al.* (2014), the crystallisation of the rambutan seed had occurred at around -10°C following 6-day fermentation. This is quite close to the result obtained in the present work of -17.7°C following 5-day fermentation. The fluctuation in crystallisation temperature following fermentation might be attributed to variation in fatty acid compositions in the TAG of seed fat following fermentation.

Saponin and total phenolic contents of fermented rambutan and pulasan seeds

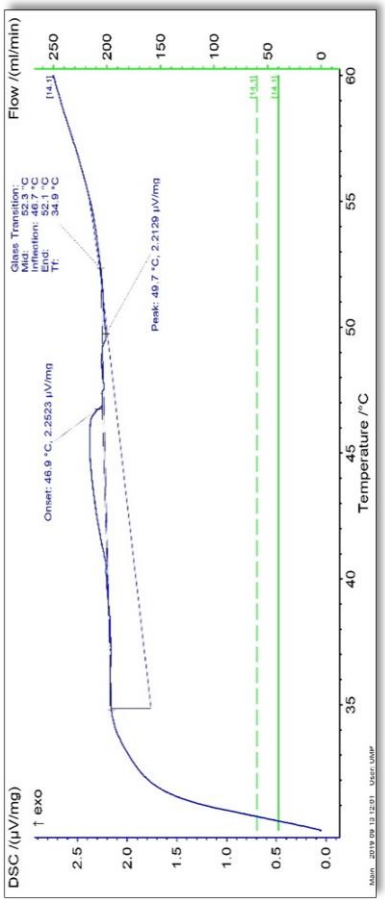
Rambutan and pulasan seeds are slightly bitter due to the alkaloid contents in the seeds and testa which contain saponin and tannin. Since fermentation

Table 2. Ion response of UPLC/QTOF MS on the identified compounds in rambutan and pulasan seeds on day 0, 1, 3, and 5.

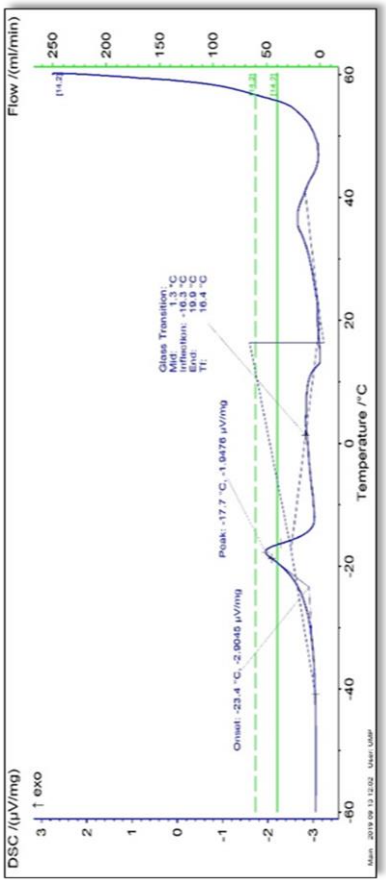
No.	Compound	Ion response			
		Day 0	Day 1	Day 3	Day 5
Rambutan					
1	Diglycerol dipalmitate	2155	27131	-	6195
2	Diglycerol distearate	14940	223712	24725	5202
3	Diglycerol monopalmitate	-	-	-	548
4	Diglycerol monostearate	-	-	-	745
5	Diglycerol triipalmitate	-	-	222928	13723
6	Glycerol 1-palmitate-3-stearate	6552426	29607	9697	5073
7	Glycerol monostearate	-	-	-	1262
8	Glycerol-1,3-dipalmitate	-	2988	-	1970
9	Glycerol-1,3-distearate	-	7147	-	1516
10	Triglycerol monopalmitate	4554	-	-	-
11	Triglycerol distearate	-	282743	19308	15372
12	Triglycerol monostearate	-	-	-	1751
Pulasan					
1	Diglycerol dipalmitate	9891	21170	15623	6508
2	Diglycerol distearate	12273	3471	3646	2903
3	Diglycerol monostearate	1562	276	-	-
4	Diglycerol triipalmitate	33399	1002	42280	4354
5	Glycerol 1-palmitate-3-stearate	7376	9830	36106	7411
6	Glycerol monopalmitate	884	178	-	75
7	Glycerol monostearate	1181	303	-	228
8	Glycerol-1,3-dipalmitate	2389	1211	-	1581
9	Glycerol-1,3-distearate	2287	-	-	266
10	Triglycerol dipalmitate	2316	-	-	1437
11	Triglycerol distearate	10977	9757	9025	717
12	Triglycerol monopalmitate	411	-	-	109
13	Triglycerol monostearate	4364	4012	9117	3868
14	Triglycerol tripalmitate	876	-	-	4133

is one of the preliminary methods to reduce saponin content, the influence of fermentation on saponin content was thus studied and compared between rambutan and pulasan seeds. Based on Table 3, the saponin content in rambutan and pulasan seeds decreased by about 74 and 38%, respectively, following 5-day fermentation. This could be due to the actions of fermentation microorganisms involved in degrading the antinutritive compounds (Tamang *et al.*, 2016). The concentration of saponin in pulasan seeds was higher than that in rambutan seeds. This could be due to difference in climate, season, altitude, plant organs, and species in the same genus (Negi *et al.*, 2011).

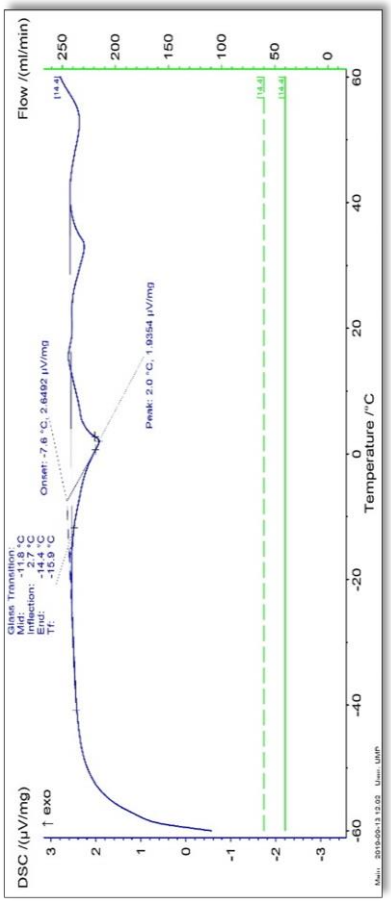
(a) (i) Heating from 30 to 60°C



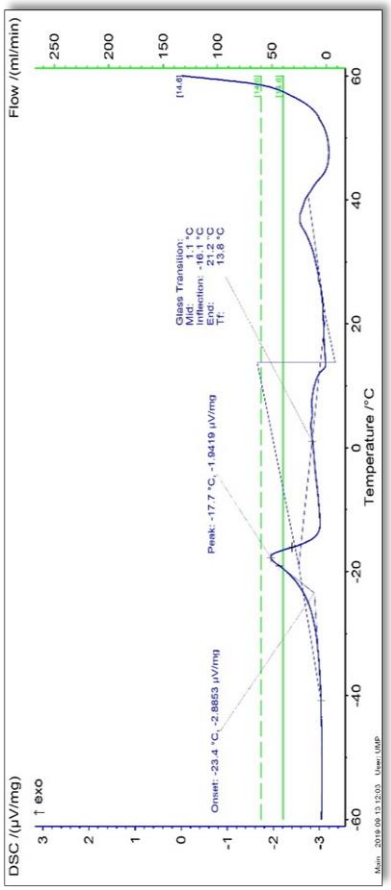
(ii) Cooling from 60 to -60°C



(iii) Heating from -60 to 60°C



(iv) Cooling from 60 to -60°C



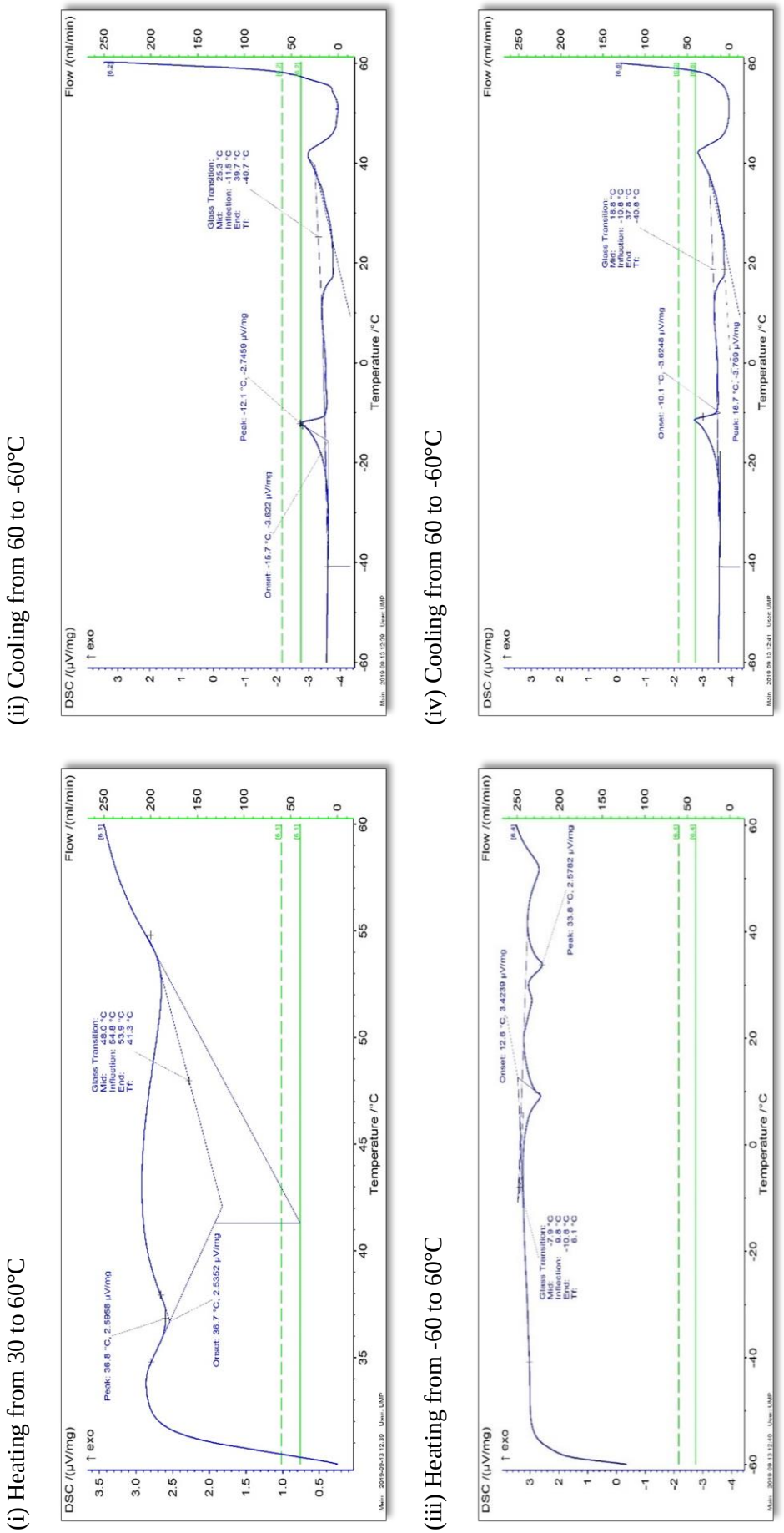


Figure 2. Heating and cooling curves of **(a)** rambutan and **(b)** pulasan seeds' fat following five days of fermentation.

The total phenolic content in rambutan seeds was nearly two-fold higher than that of pulasan seeds. Both rambutan and pulasan seeds showed similar trend, where the total phenolic content decreased by almost 74 and 44%, respectively, following 5-day fermentation. Naturally, phenolic compounds will bind with sugars, thus reducing their availability in organisms. During fermentation, proteolytic enzymes from the organisms hydrolyse the phenolic and sugar complex into high soluble phenols and other active compounds that are readily absorbed by the organisms (Shrestha *et al.*, 2010). Liquid reduction during fermentation gave lower phenolic concentration (Wollgast and Anklam, 2000). Therefore, the total phenolic content in both rambutan and pulasan seeds decreased following fermentation.

Table 3. Saponin and total phenolic contents of fermented rambutan and pulasan seeds.

Fermentation time (day)	Concentration of saponin (mg soybean saponin/g sample)		Concentration of gallic acid (mg GAE/g sample)	
	Rambutan	Pulasan	Rambutan	Pulasan
0	850.17 ± 16.52	1229.67 ± 33.32	7.23 ± 0.27	3.31 ± 0.19
1	461.17 ± 35.49	1095.00 ± 59.35	4.29 ± 0.37	2.98 ± 0.07
3	312.67 ± 24.85	1002.33 ± 26.41	3.03 ± 0.14	2.44 ± 0.19
5	219.50 ± 12.46	760.33 ± 12.45	1.87 ± 0.16	1.85 ± 0.09

Conclusion

Fermentation affected the fat properties, saponin, and total phenolic contents of rambutan and pulasan seeds. The main fatty acid in both rambutan and pulasan seeds was oleic acid. Results demonstrated that oleic acid was gradually replaced by arachidic acid through the elongation of C18 fatty acids after 5 d of fermentation. The crude fats and TAG compounds decreased after 5 d fermentation due to hydrolysis by lipase enzyme. The present work also demonstrated that the antinutrient (saponin and tannin) content of the seeds decreased with fermentation time. Although the results demonstrated that the properties of fermented rambutan and pulasan seeds' fat were unable to completely substitute cocoa butter, it could still be paired with cocoa butter to make it more cost-effective, much like a cocoa butter extender. Consequently, this may help in reducing fruit wastage.

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