FOOD RESEARCH

FULL PAPER

Characteristics of rambutan (*Nephelium lappaceum L*.) seed fat fractions and their potential application as cocoa butter improver

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Abstract

The utilization of rambutan seed (*Nephelium Lappaceum* L.) to produce fat (RSF) and its fractionation could be one of the solutions for better waste management and for ensuring its sustainable utilization. In this study, RSF was fractionated by two-stage acetone fractionation and their physicochemical properties such as fatty acid compositions, iodine value (IV), free fatty acid (FFA), slip melting point (SMP), and solid fat content (SFC) were investigated. The solid fraction-III (F_2-S) exhibited the highest SMP (49.03°C) and lowest IV (27.57 g I_2/100 g). The major fatty acids in all solid fractions were stearic (15.1-21.6%), oleic (25.0-35.5%), and arachidic (42.7-46.9%) acids. The SFC of F_2-S at 20°C (78.57%) and 35°C (22.95%) were found to be higher than solid fraction-I (F_1-S), indicating a harder solid fraction. This study revealed that by performing fractionation of RSF, a cocoa butter improvers (CBI) could be prepared by blending them with other fats that have the potential to be utilized in chocolate manufacturing in tropical countries.

1. Introduction

The modification of fats and oils is an important and useful technique to improve their physicochemical and thermal properties. This technique including blending, hydrogenation, fractionation, and chemical and enzymatic interesterification are used to produce fats for margarine, confectionery, cosmetic, and chemical industries (Timms, 2005; Gibon, 2006; Momeny *et al.,* 2013; Kang *et al.,* 2013; Jin *et al.,* 2016; Kadivar *et al.,* 2016; Lakum and Sonwai, 2018). Solvent fractionation is applied to produce structural lipids such as hard fats (high-melting-point triacylglycerols), polyunsaturated fatty acid-rich oil, creams, and margarines due to the high separation efficiency and yield of aimed fractions with sharper melting properties (Gibon, 2006; Kellens *et al.,* 2007; Mu *et al.,* 2016; Jin *et al.,* 2016). For example, palm oil is fractionated into palm stearin and liquid and is the most widely fractionated oil worldwide (Senanayake and Shahidi, 2005; Gibon, 2006). Meanwhile, these palm oil fractions have been used to prepare wide variety of food products, especially confectionery products (Jahurul, Zaidul, Norulaini, Sahena, Kamaruzzaman *et al.,* 2014; Jahurul, Zaidul, Norulaini, Sahena, Abedin *et al.,* 2014; Lakum and Sonwai, 2018).

Cocoa butter (CB) for instance, is a high-priced constituent and crucial ingredient in chocolate formulations, and responsible for the melting behavior and glossy texture of chocolate products (Jin *et al.,* 2016; Kadivar *et al.,* 2016; Jia *et al.,* 2019). Despite being the most ideal constituent and ingredients in chocolate and other confectionery products, the low melting point of CB causes the products, especially chocolate to melt easily in subtropical and tropical areas (Shahidi, 2005). Furthermore, demand for chocolate products keeps increasing despite the high-cost and uncertainty in the supply of CB increasing the interests among manufacturers and researchers to find for alternatives such as cocoa butter equivalents (CBE) (Tchobo *et al.,* 2009). Cocoa butter equivalents (CBEs) are vegetable fats that possess similar physical and chemical properties with CB (Smith, 2001). CBE is specifically designed to include a structure of glyceride identical to that of CB. In mixtures for the manufacturing of chocolate, their properties are supposed to be identical and consistent with CB. CBEs are classified into two subgroups, which are cocoa butter extenders (CBEXs) and cocoa butter improvers CBIs (Lipp and Anklam, 1998). CBEXs usually is not compatible to be mixed in any proportion with CB, whereas CBIs are more comparable to CBEs, which contain higher amounts of solid triglycerides and are commonly used to improve soft cocoa butter due to this characteristic (Naik and Kumar, 2014).

Rambutan (*Nephelium lappaceum* L.) is one of the

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most known seasonal fruit in Southeast Asia for its sweet-sensational taste and noticeable appearance (Sirisompong *et al.,* 2011; Issara *et al.,* 2014). It can be freshly consumed and processed for industrial commercialization as jams, juices, spreads, jellies, canned fruit, syrup, and marmalades (Sonwai and Ponprachanuvut, 2012; Lourith *et al.,* 2016). Rambutan seed is considered as a waste in rambutan canning manufactures with a noteworthy value approximately 94,500 tons/year in Thailand, Indonesia, and Malaysia (Norlia *et al.,* 2011). After direct consumption or industrial processing, the seeds and peels are discarded as a co-product that can raise severe environmental issues and cause economic losses if not managed effectively (Solís-Fuentes *et al.,* 2010; Chai *et al.,* 2018). This extensive value has become an issue that needs to be solved. Meanwhile, RSF is used for making candles, soaps, and fuel manufacturing. Moreover, it is also a possible source of natural edible fat such as confectionery products that would be beneficial in the food industry (Solís-Fuentes *et al.,* 2010). However, modification of the physicochemical and thermal properties of RSF to become the new alternative to be used in the food industry, can contribute to industrial economic gains and become one of the solutions to the fruit wastes management. Therefore, this study was aimed to provide new information about two-stage acetone fractionation of RSF. The physicochemical properties such as fatty acids, iodine value (IV), free fatty acid (FFA), slip melting point (SMP), and solid fat content (SFC) of each RSF fractions were also determined.

2. Material and methods

2.1 Preparation of rambutan seed powder

Rambutan fruit (mature, red in colour) was purchased from local market Papar, Sabah. The peels and flesh were removed using a household knife and then were manually washed and cleaned. The seeds were cut into smaller pieces and then air-dried in an oven at 50°C for 18 h. The dried seeds were then finely ground into powder using a warring blender (Pensonic, Malaysia), stored in zip-lock bags. The powder was stored in a desiccator for further analysis.

2.2 Extraction of RSF using Soxhlet

The extraction of RSF was performed using Soxhlet extractor. Approximately, 100 g of rambutan seed powder was transferred into 2 L Soxhlet extractor (Favorit, Malaysia) and the extraction was performed using *n*-hexane as a solvent for 8 h. The fat was recovered using rotary vacuum evaporator (Heidolph, Germany) and then dried in an oven at 60°C for 2 hrs.

The extracted fat was then weighed and stored at -20°C for further analysis. The total RSF fat yield was obtained as a percentage.

2.3 Fractionation of RSF

Fractionation of RSF was performed as two-stage acetone based on the method developed by Jin *et al.* (2016) with slight modification. The fractionation process is shown in Figure 1. Approximately, 100 g of RSF was melted uniformly and mixed with acetone at a ratio of 1:5 (w/v) and left for 8 h to crystalize (at 18°C). The crystalized (fraction-I) and liquid (fraction-II) fractions then separated using filter paper under vacuum and dried by rotary evaporator (Heidolph, Germany). The crystallized fractions were further cooled to 12°C and kept for 40 mins. The second solid fraction (fraction-III) was obtained after separation with the second liquid fraction (fraction-IV) by filtering the fraction (cool at 5°C and keep for 8 hrs). All fractions were separated and dried by the same method.
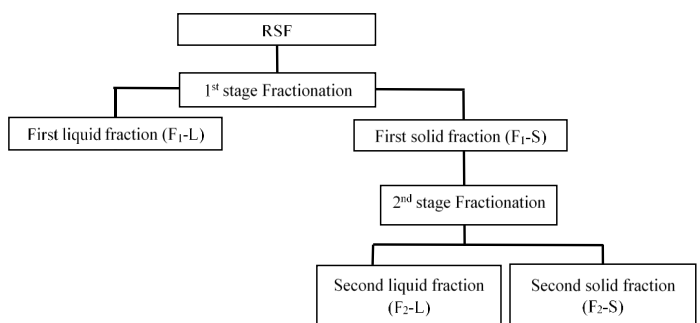

Figure 1. Rambutan seed fat fractions produced by two-stage acetone fractionation.

2.4 Determination of physicochemical properties of RSF and its fractions

The physicochemical properties of RSF and its fractions (fatty acids, SMP, IV, and FFA) were analyzed according to the official method established by American Oil Chemists' Society (2003).

2.5 Determination of fatty acids by GC-FID

Fatty acids were determined by Gas-Chromatography with flame ionization (GC-FID) detector according to the previous study (Jahurul, Zaidul, Norulaini, Saleha, Kamaruzzaman *et al.,* 2014). The GC-FID (Agilent, USA) with a BPX 70 capillary column was used with a DB-23 (J&W Scientific) capillary column. The carrier gas used was helium that has a flow rate of 1.5 ml/minute. The operation of the split/splitless injector was set at 25°C with the split rate of 100:1. The interface temperature of GC-FID was adjusted to 220°C with ion source temperature at 200°C. The oven temperature of GC was set at 80°C with holding time of

2 mins, ramped to 230°C at the rate of 2.5°C/min with holding time up to 5 minutes. Fatty acid compositions calculated were based on percentage peak area of the GC chromatogram.

. 2.6 Determination of solid fat content (SFC)

The SFC of crude RSF and its fractions was determined by pNMR (Bruker minispec mq20 NMR analyzer) according to Fiebig and Lüttke (2003). RSF and its fractions were tempered at 60°C for 5 mins (or longer), followed by chilling at 0°C for 90 mins and kept at the desired temperatures for 60 to 65 mins prior to measurements. The melting, chilling and holding of the test samples were carried out in the pre-equilibrated thermostat bath. The temperature ranges used for the determination of SFC were 10–40°C.

2.7 Statistical analysis

All the experiments were conducted in triplicates and duplicates reported as mean ± standard deviation. Two-way analysis of variance (ANOVA) and Tukey Honest Significance Difference (HSD) test was applied at a 95% confidence interval using the SPSS program version 25.0. The correlation between each analysis was calculated by multiple linear regression analysis.

3. Results and discussion

3.1 Extraction and yield

Table 1 shows the total yield of crude RSF and its fractions produced by two-stage fractionation. Solvent fractionation process led to nearly complete separation of the high melting solid fractions from crude RSF. The total fat content (34.10%) of crude RSF obtained in this study was comparable with previous studies which ranged from 32.60 - 39.13% (Solís-Fuentes *et al.,* 2010; Sirisompong *et al.,* 2011; Lourith *et al.,* 2016; Chai *et al.,* 2018; Chai *et al.,* 2019). The first stage crystallization process of crude RSF yielded first solid fraction (F_1-S) (53.09%) that was significantly (p>0.05) higher than the first liquid fraction (F_1-L) (46.91%). Further fractionation on the first solid fraction (F_1-S) under the same conditions produced more saturated fats. The second stage fractionation from the first solid fraction (F_1-S) produced the second solid (F_2-S) and liquid (F_2-L) fractions yielded 56.96% and 43.04%, respectively. The results corresponded with the previous study conducted by Jin, Wang, Su *et al.* (2017), where the yield of mango seed fat solid fractions decreased after first and second stages of fractionation due to the solid fractions became more saturated. The presence of high melting saturated fatty acids (SFAs) which were mainly arachidic and stearic acids in F_1-S resulted in gradual precipitation of SFAs from the liquids because of

the effect of low-temperature crystallization, hence more of solid fractions yielded (Latip *et al.,* 2013). Meanwhile, the liquid fractions were found to be easily melted at <28°C due to the presence of low melting unsaturated fatty acids (USFAs) which were mainly oleic and linoleic acids causing less production of solid fractions at low temperature of crystallization process in the second stage of fractionation from F_1-L (Jin, Wang, Su *et al.,* 2017).

Table 1. Total yields of crude RSF and its fractions

Sample	Yield (%)
RSF	34.10±0.07[c]
First Stage of fractionation (From RSF)	
F_1-S (First solid fraction)	53.09±0.04[b]
F_1-L (First liquid fraction)	46.91±0.03[c]
Second Stage of Fractionation (From F_1-S)	
F_2-S (Second solid fraction)	56.96±0.04[a]
F_2-L (Second liquid fraction)	43.04±0.05[c]

Mean values ± SD of triplicates; means with different letters are significantly different (p<0.05) as measured by Tukey's test.

3.2 Fatty acids composition

The fatty acids of crude RSF and its fractions are shown in Table 2. Some of the gas chromatograms of RSF and its solid fraction are shown in Figure 2. The major fatty acids found in crude RSF were arachidic ($C_{20:0}$) (40.9%), oleic ($C_{18:1}$) (40.3%) and stearic ($C_{18:0}$) (8.6%) acids. These three fatty acids constituted approximately 89.8% of the total fatty acids. These values were comparable with the values reported by (Solís-Fuentes *et al.,* 2010; Sirisompong *et al.,* 2011; Yanty *et al.,* 2013). RSF also contained significant quantities of palmitic (3.8%), linolenic (3.2%), and behenic (4.5%) acids. These fatty acids affected the physicochemical and thermal characteristics of RSF and its fractions.

Solvent fractionation is mostly used by researchers due to the high separation efficiency and purity for diverse applications (Gibon 2006; Kang *et al.,* 2013; Jin *et al.,* 2016; Jin, Wang, Su *et al.,* 2017). Fractions with narrower and better properties than crude RSF can be obtained through solvent fractionation. It was expected that due to the fractionation process, the USFAs would be concentrated in the liquid fractions and significantly reduced in the solid fractions. A significant increasing and decreasing trends were observed among the fatty acids in the fractions because of the fractionation process. It was found that SFAs such as $C_{20:0}$ (46.9%) and $C_{18:0}$ (21.6%) contents in F_2-S were significantly (p>0.05) higher than F_1-S (42.7 and 15.1%) and crude RSF (40.9 and 8.6%). On the other hand, the USFAs ($C_{18:1}$ and $C_{18:2}$) in both liquid fraction-II and IV (F_1-L

Table 2. Fatty acid composition (%) of rambutan seed fat and its fractions

Fatty acids	RSF (Crude)	First stage of fractionation from crude RSF		Second stage of fractionation from F_1-S		Cocoa butter[1,2,3]
		F_1-S (Solid)	F_1-L (Liquid)	F_2-S (Solid)	F_2-L (Liquid)	
$C_{16:0}$	3.8±0.5[c]	2.5±0.4[d]	5.7±0.1[a]	2.0±0.9[e]	4.2±0.2[b]	25.3-28.1
$C_{18:0}$	8.6±0.3[c]	15.1±0.9[b]	3.9±0.1[e]	21.6±0.1[a]	6.9±0.8[d]	34.5-37.8
$C_{18:1}$	40.3±0.3[c]	35.5±1.3[d]	51.7±0.0[a]	25.0±0.5[e]	42.2±0.0[b]	32.8-33.2
$C_{18:2}$	3.2±0.0[c]	2.2±0.7[d]	3.7±0.3[a]	1.9±1.0[e]	3.6±0.1[b]	2.9
$C_{20:0}$	40.9±0.1[c]	42.7±0.0[b]	33.2±0.3[e]	46.9±0.0[a]	36.8±0.2[d]	1
C_{22}	4.5±0.1[d]	4.93±0.1[b]	2.9±0.1[e]	5.3±0.2[a]	4.6±0.3[c]	0
SFAs	56.8±0.0[c]	64.3±0.5[b]	45.3±0.2[e]	74.7±0.1[a]	49.8±0.0[d]	57-64
UFAs	43.2±0.1[c]	36.4±0.2[d]	54.9±0.0[a]	25.6±0.2[e]	50.3±0.1[b]	36-43

Mean values ± SD of duplicates; means with different letters in the same row are significantly different (p<0.05) as measured by Tukey's test.
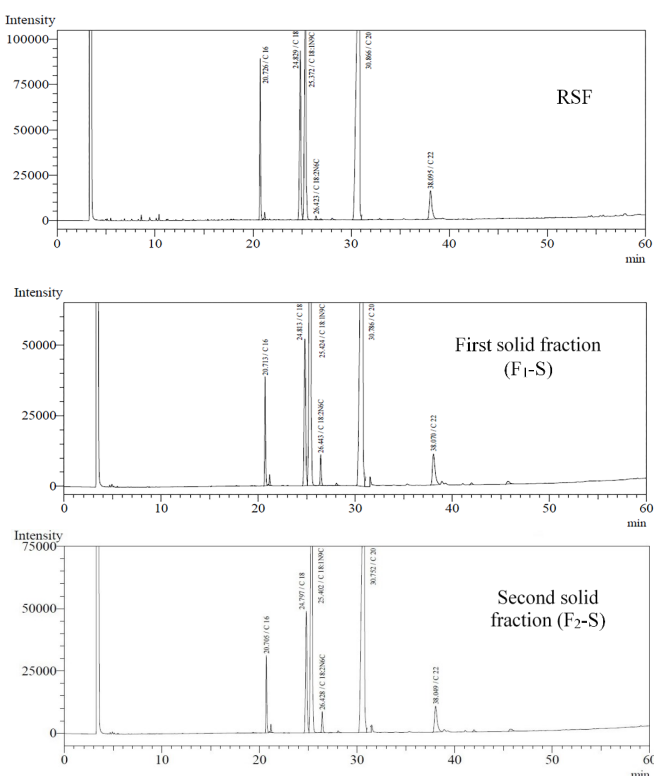[1]Kadivar *et al*. (2016); [2]Yanty *et al*. (2012); [3]Gunstone (2011)

Figure 2. GC chromatogram of fatty acid methyl esters of RSF and its solid fraction-I and III.

and F_2-L) were significantly (p>0.05) increased from crude RSF. However, $C_{18:1}$ and $C_{18:2}$ acids of liquid fraction-II (F_1-L) (51.7 and 3.7%) were found to be higher than liquid fraction-IV (F_2-L) (42.2 and 3.6%). The $C_{16:0}$ content in solid fraction-I and III (2.5 and 2.0%) were found to be significantly (p>0.05) lower than crude RSF. Meanwhile, the $C_{16:0}$ content was increased in both F_1-L and F_2-L (5.7 and 4.2%). The results were comparable with previous study performed by Jin, Wang, Su *et al*. (2017), where the $C_{16:0}$ content managed to be decreased in solid fractions but increased in all liquid fractions. The authors also reported that USFAs ($C_{18:1}$ and $C_{18:2}$) in liquid fractions and SFAs ($C_{18:0}$ and C_{20}) in solid fractions were higher than crude mango seed fat. Similar findings were observed by Kang *et al.*

(2013), who fractionated palm stearin and found to have increasing trends for USFAs ($C_{18:1}$ and $C_{18:2}$) in liquid fractions. Gunstone (2011) stated that the major fatty acids contained in most CBIs are palmitic acid, stearic acid and oleic acid, similar to that of CB, which contributed 57-64% to the total saturated fatty acid present in CB and unsaturated fatty acid about 36-43%. The results in Table 2 demonstrated that the total of saturated and unsaturated fatty acids of crude RSF and its fractions were quite different based on the amount of $C_{16:0}$, $C_{18:0}$ and C_{20}. However, they are still comparable with CB. It might serve as a novel source of CBI due to the presence of high melting fatty acid such as $C_{20:0}$ which can help to increase the hardness of CB to be used as confectionary products ingredients in the country with tropical climates. The unique fatty acid composition of the fractions demonstrated that they could be widely used as a good value-adding alternative in cocoa butter to improve the softness of cocoa butter.

3.3 Physicochemical properties

Table 3 shows the IV, SMP, and FFA of RSF and its fractions. The study of IV is crucial in determining the degree of unsaturation and hardness of oils and fats (Jahurul, Zaidul, Norulaini, Saleha, Kamaruzzaman *et al.,* 2014). High IV indicated higher unsaturation in the fats or oils. In this study, the IV (45.51 g I_2/100 g fat) and SMP (41.57°C) of crude RSF were within the range reported by Solís-Fuentes *et al.* (2010), Sirisompong *et al.* (2011), Sonwai *et al.* (2012), Yanty *et al.* (2013), and Chai *et al.* (2018, 2019).

IVs of all solid fractions (F_1-S and F_2-S) ranged from 27.57 - 35.14 g I_2/100 g fat which was significantly (p>0.05) lower than crude RSF. However, IVs of all liquid fractions (F_1-L and F_2-L) ranged from 47.22 - 48.49 g I_2/100 g fat which was significantly (p>0.05) higher than crude RSF. The SMP of solid fraction-I

Table 3. Physicochemical properties of crude RSF and its fractions

Samples	IV (g I_2/100 g fat)	SMP (°C)	FFA (%) as oleic acid	FFA (%) as palmitic acid
RSF	45.51±0.04[c]	41.57±0.12[c]	2.94±0.12[a]	-
First Stage of fractionation (From RSF)				
F_1-S (First solid fraction)	35.14±0.04[d]	45.57±0.11[b]	2.03±0.11[c]	-
F_1-L (First liquid fraction)	48.49±0.13[a]	25.57±0.11[e]	-	1.87±0.11[a]
Second Stage of Fractionation (From F_1-S)				
F_2-S (Second solid fraction)	27.57±0.03[e]	49.03±0.06[a]	2.25±0.12[b]	-
F_2-L (Second liquid fraction)	47.22±0.17[b]	27.43±0.09[d]	-	1.75±0.07[b]
Cocoa butter[1-5]	34.0-37.9	20.0-37.0	-	1.62-2.08±0.02

RSF, rambutan seed fat; IV, iodine value; SMP, slip melting point; FFA, free fatty acid.

Mean values ± SD of triplicates; means that do not share the same letter in the same column are significantly different ($p<0.05$) as measured by Tukey's test.

[1]Muchiri *et al*. (2012); [2]Yanty *et al*. (2012); [3]Hamid and Damit (2004); [4]Timms (2005); [5]Foubert *et al*. (2004)

(45.57°C) and solid fraction-II (49.03°C) were significantly (p>0.05) higher than crude RSF (41.57°C). On the other hand, the SMP of all liquid fractions were significantly (p>0.05) lower than crude RSF. Low IV usually reflects high SMP due to the low content of unsaturated fatty acids (Jin *et al.*, 2016). The IV of F_2-S was the lowest (27.57 g I_2/100 g fat), reflecting the highest SMP of 49.03°C, while F_1-L, on the contrary, exhibited the highest IV (48.49 g I_2/100 g fat) with the lowest SMP (25.57°C). The results are in line with the results reported by Jin, Wang Su *et al.* (2017), where IVs of solid fractions decreased when the IVs of liquid fractions increased. This was further confirmed by the fatty acids, which showed variations in the content of SFAs and USFAs in all fractions (Table 2). All solid fractions contained significantly high levels of SFAs (long-chain fatty acid with a relatively high melting point) and were found as solid at room temperature, whereas the liquid fractions contained more USFAs (low melting fatty acids) which were melted completely at <28°C. The IV of F_1-S (35.14 g I2/100 g fat) was comparable with those of commercial CB (34.0 - 37.9 g I_2/100 g fat) (Hamid and Damit, 2004; Muchiri *et al.,* 2012; Yanty *et al.,* 2012), but has higher SMP (45.57°C) than CB (20.0 - 37.0°C) (Timms, 2005). This study also found that FFA content of all the fractions ranged from 1.62-2.25% which were significantly (p>0.05) lower than that of crude RSF (2.94% as oleic acid). However, the fractionated fats are still not practically safe to be directly used in food processing purposes, therefore physical and chemical refining is needed to reduce the acidity of the fats to that desired for CB (Jahurul, Zaidul, Norulaini, Saleha, Kamaruzzaman *et al.,* 2014; Jin *et al.,* 2016).

3.4 Solid fat content (SFC)

The SFC is a useful tool to study the suitability of each fraction for a particular purpose in formulated foods (Sonwai *et al.,* 2017). The SFC as a function of temperature for RSF and its fractions are given in Figure 3. SFC of crude RSF was found 66.9% at 10°C and decreased rapidly as the temperature increased before reaching 3.22% at 40°C. It was predicted that the SFC increased in solid fractions and decreased in liquid fractions. This is due to the high content of high-melting TAGs in the solid fractions compared to the pure fat and the liquid fractions (Sonwai *et al.,* 2017). All solid fractions (F_1-S and F_2-S) exhibited SFC higher than crude RSF at all temperatures (Figure 3). The SFC curve of F_2-S was located well above compared to SFC curves of other fractions including crude RSF. Both F_1-S and F_2-S showed slow decreasing of SFC at 10 and 15°C, but sharp decreasing after 20°C. However, it can be seen that 8.5% of SFC remained at 40°C in F_2-S, unlike F_1-S for which only 5.18% of SFC remained at 40°C. The SFC curve of F_1-S was positioned above that of crude RSF but below that of F_2-S, demonstrating that it was a softer solid fraction compared to F_2-S. On the other hand, the SFC curve of liquid fraction (F_1-L) was located below that of crude RSF, suggesting that it contained more liquid oil than crude RSF at any temperature, thereby indicating it as softest fraction compared to crude RSF and other fractions. The SFC curves of all fractions in this study were in line with the findings by Sonwai *et al.* (2017) and Jin, Zheng, Pembe *et al.* (2017), where the texture and specific melting behaviour can be enhanced with the increase of SFC in the solid fractions which make them suitable for food-applications such as cocoa butter alternatives. Jin, Zheng, Pembe *et al.* (2017) reported that at 35°C, CB has melted completely and no solid remaining. Contrary to RSF and its fractions, there are solid remaining even at 40oC. This shows that RSF fractions have higher stability and heat resistance at high temperature that could contribute to enhancing the quality of chocolate and has potential to be blend with CB without significantly altering the melting and crystallization temperature ranges or enthalpies, and SFC of natural cocoa butter (Kang *et al.,* 2013). The results

revealed that the solid fractions are suitable for CBI, where it can be blended with CB to increase the hardness in terms of SFC. These fractions may be beneficial for use in tropical climates.
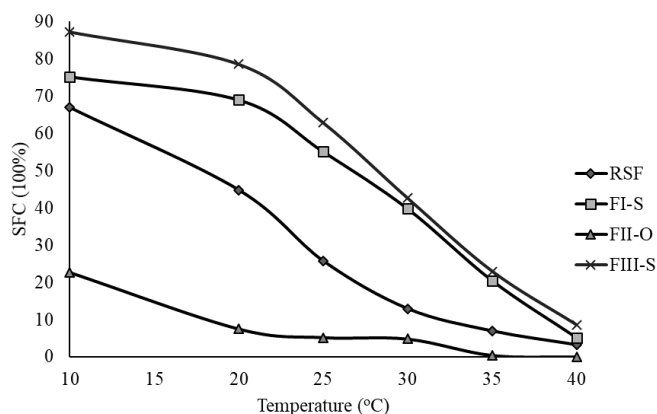

Figure 3. Solid fat content (%), measured at 10–40°C of crude RSF and its fractions represents the average of duplicates and standard deviation.

4. Conclusion

Differences in physicochemical properties (fatty acids, IV, SMP, SFC, and FFA) were observed among crude RSF and its fractions. The solid (F_2-S) fractionated from the first solid fraction (F_1-S) showed better physicochemical properties as CBI in terms of high SMP and SFC as well as a significant improvement of fatty acids. The major fatty acids in F_2-S were stearic, oleic, and arachidic acids. Moreover, the F_2-S exhibited remarkable improvement of SFC as they were not shifted to 0% at body temperature, indicating a better melting resistance at high temperature. The SFC of F_2-S at 20°C and 35°C were also found to be higher than that of F_1-S, indicating a harder solid fraction. Based on the results obtained in this study, F_2-S could be used in formulating confectionery products by blending with CB or other fats such as palm oil mid-fraction or palm stearin.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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