Comparing the Structural Parameters of Rambutan Seed Fat Incorporated Wholly and Partially with Cocoa Butter

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Abstract— The physical characteristics, such as peak maxima during melting and crystallization behavior, polymorphic behavior, microstructure, and solid fat content (SFC), of rambutan seed fat (RSF) and its mixture with cocoa butter (CB) at various ratios were compared to prove that slight changes in the composition of CB, CB:RSF, or RSF can affect its crystal structure, and thereby, its functionality. Physical characteristics were obtained via differential scanning calorimetry (DSC), X-ray diffraction (XRD) analysis, polarized light microscopy (PLM), and SFC analysis. The results showed that mixtures M1 (80% CB:20% RSF) and M2 (60% CB:40% RSF) melted at peak maxima 21.65±0.72°C and 19.51±0.67 °C with a ΔH of 66.77±8.50 J/g and 39.05±1.57 J/g, respectively. These values are nearly similar to that for CB, which is approximately 22.36±0.16 °C with a ΔH of 76.53±3.15 J/g. Meanwhile, M1 exhibited a more similar crystallization pattern than M2, with a single curve that have one peak maxima height at a temperature of 12.17±0.60 °C with a ΔH of 71.29±5.46 J/g relative to that of CB at a temperature of 11.92±0.56 °C with a ΔH of 72.23±2.43 J/g. The XRD pattern showed that M1 had the same short spacing and wide angle reflections as those of CB at a temperature of 20 °C. Furthermore, the microstructure visualized through PLM showed that M1 had a morphology that is similar to that observed in CB, in which small and well-distributed crystal formations provide a hardness with a lower index, whereas large crystal formations exhibit otherwise. The solid fat content of M1 present profiles similar to those of CB, with considerably lower SFC. The findings determined the best mixture with physical characteristics that converge to those of CB.

Keywords— Rambutan fruit, Rambutan seed fat. Cocoa butter. Thermal behavior. Microstructure. Polymorphism. Solid fat content.

1 Introduction

Fats are used as main ingredients in food, cosmetic, and pharmaceutical products [1]. The crystallization of fats has industrial applications when it is controlled; end products such as chocolate, margarine, and whipped cream can be obtained; the crystallization phenomenon of fats can be used to isolate fats from natural resources [2]. Cocoa butter (CB) is one of the natural fats, obtained from cocoa seeds (*Theobroma cacao*); it is typically used as a major component of chocolate and other confectionery products because of its physical and chemical properties [3]. CB is solid at room temperature (below 25 °C) and liquid at body temperature (~37 °C); it consists mostly of palmitic (C_{16}), stearic ($C_{18:0}$), and oleic acids. Virtually all oleic acids are esterified in the central position of all glycerol molecules (Sn-2), whereas saturated fatty acids are typically located in (Sn-1,3) positions. The composition and allocation of fatty acids lead to a symmetrical triglyceride composition of CB that is rich in 1,3-dipalmitoyl1-2-oleoyl-glycerol (pop) and 1-palmitoyl-3-stearoyl-2-oleoyl-glycerol (POS) [4]. This triglyceride composition of CB is generally responsible for its diverse crystalline polymorphic forms, while fatty acid compositions are responsible for fat solidification in its liquid state [5]. CB is known to be more expensive than other vegetable fats because of its specific characteristics and it is cultivated in only a few countries [6]. Therefore, the food industry is keen to find other sources of fats as alternatives to CB in producing chocolates for various reasons, including economic [7]. CB alternatives are defined as non-lauric fats that can replace CB either partially or completely in chocolate or other food products [8].

Rambutan (Nephelium opossum L) is one of the most important tropical fruits that is originally found in Malaysia, Thailand, the Philippines, Vietnam, Borneo and other countries in this region. The industrial processing of this fruit produces seeds and peels as waste materials [9]. Previous studies have reported that rambutan seed possesses a relatively high amount of fat (between 17% and 39%) and some of them have analyzed the fatty acids of rambutan seed fat (RSF) [10], [11]. According to Solis-Fuentes, et *al*. [16], the main fatty acids of RSF are oleic 40.3% and arachidic acid 34.5%. In addition, there are 6.1% palmitic, 1.5% palmitoleic, 7.1% stearic, 6.3% gondoic, and 2.9% behenic. The major triacylglycerol of RSF is an unique due to the high concentration of arachidic acid. Considering the fatty acid composition; the major constituent of RSF was Arachidoyl-dioleoylglycerol (AOO) followed by Arachidoyl-Steareoyl-Oleoylglycerol (ASO) and Arachydoyl-Oleoyl-Palmitoglycerol (AOP) with percentage 49.84%, 15.08% and 12.82% respectively. The other TAGs was occurring in the percentage below than 10% such as Oleic acid (OOO) 1.42%, Arachydoyl-Linolenoyl-Oleoylglycerol (ALnO) 3.03%, Arachydoyl-Linoleoyl-Oleoylglycerol (ALO) 0.97%, Arachydoyl-Linoleoyl- Palmitoylglycerol (ALP) 6.32%, Arachydoyl-Linolenoyl-Steareoylglycerol (ALnS) 1.48% and Arachydoyl-Steareoyl-Palmitoylglycerol (ASP) 9.03%, respectively [12]. CB does not contain many triglycerides and the majority of these are composed of palmitic-oleic-stearic [13]. However, previous studies in [10], [11] have shown that RSF can be used to produce candles and chocolate because it is similar to CB; although RSF possesses several differences in physical properties [14]. Crystalline forms, in which fats may exist, are classified into α, β, and β'. Investigating the distinct crystallization patterns of CB based on behavior observed in industry

standard mixtures may identify other simple alternatives to standardize product quality. The objective of this study is to compare the peak maxima during melting and crystallization behavior, microstructure, and polymorphism of RSF and its mixtures with CB.

2 MATERIALS AND METHODS

2.1 Materials

CB was purchased from an Indonesian coffee company and the Cocoa Research Institute, Jember, East Java, Indonesia. Meanwhile, raw rambutan (*Nepheliumlappaceum L.*) seeds were supplied by a rambutan canning industry in Sungai Petani, Kedah, Malaysia.

2.2 Fermentation and roasting of rambutan seeds

The fermentation process was performed on rambutan seeds, which were still covered by small amounts of rambutan pulp. The rambutan seeds were transferred into plastic baskets (625 mm × 425 mm × 294 mm), which were previously lined with banana leaves. After filling in the baskets with raw rambutan seeds, the baskets were covered with banana leaves for 6 days. The dried rambutan seeds were roasted at 150 °C for 30 minutes by oven-drying (AFOS Mini Kiln, Hull, England). After roasting, the samples were cooled at room temperature and stored until the screw-pressing process for RSF production [15].

2.3 RSF extraction

RSF extraction was performed using a KOMET screw oil expeller DD 85 IG (IBG MonfortsOekotec GmbH & Co. KG, Germany). Dried rambutan seeds were dehusked and heated at 60 °C for 30 minutes by oven-drying (AFOS Mini Kiln, Hull, England). The screw-pressing process produced RSF, which was a viscous mixture of rambutan seed powder and RSF. The separation of RSF from rambutan seed butter was achieved through filtration under a heated condition (60 °C). Afterward, the collected RSF was transferred into inert-screw-cap bottles [15].

2.4 Mixture of CB and RSF

CB was incorporated into RSF in six proportions, namely, 100/0, 80/20, 60/40, 40/60, 20/80, and 0/100 (w/w) CB to RSF, as CB, M1, M2, M3, M4 and RSF respectively. Then, the mixtures were melted in the oven (AFOS Mini Kiln, Hull, England) at 60 °C for 15–20 minutes. The melted mixtures were then homogenized using a vortex and transferred into inert-screw-cap bottles and then stored at −4 °C until they were used for analysis.

2.5 Melting and crystallization via differential scanning calorimetry (DSC)

The characteristic temperature of the polymorphic transition of CB incorporated with RSF was determined based on the method presented in [16]. DSC Q200 equipped with an RC90 refrigeration system (TA Instrument, New Castle, DE, USA), which was previously calibrated with indium (melting point 156.6 °C), was utilized in this analysis. Approximately 9±0.5 mg of the sample was weighted and hermetically sealed. An empty sealed capsule was used as reference. The temperature programs in the calorimeter were as follows:

1. Isothermal heating at 90 °C for 10 min.
2. Cooling at 10 °C/min from 90 °C to −60 °C. Heating at 10 °C/min from −80 °C to 90 °C.

Triplicate measurements were performed for each sample. Thermal analysis, post-processing analysis, and enthalpy calculation were conducted using TA Universal Analysis 2000 (TA Instrument, New Castle, DE, USA).

2.6 Determining polymorphism via X-ray diffraction (XRD)

The polymorphic form of the crystals was determined using the AOCS method Cj 2–95 [17]. The analyses were performed on a Philips diffractometer (PW 1710, Almelo, the Netherlands) using Bragg–Bretano geometry (θ:2θ) with radiation Cu-kά radiation (γ = 1.54056 Å, tension = 40 kV, current = 35 mA). Measurements were obtained with a step width of 0.05° in 2θ and stop time of 4 seconds, operating at 5° to 40°. X-ray data were processed by a computer programmed to calculate absorption intensity-background, intensity/maximum intensity and peak width, in degrees (2 °e), for each crystalline form and the relative content of β and β' crystals. The analyses were performed at 25 °C according to the procedure described in [18]. Prior to measurement of x-ray diffraction, the samples were subjected to temperature cycling, initially at 5°C for one day, followed by two days at 20 °C, one day at 5°C, two days at 20°C. Identifying the polymorphic form was achieved from the short-spacing characteristic of crystals [19].

2.7 Morphological studies via polarized light microscopy (PLM)

The samples were melted at 90 °C for 10 minutes and a drop was placed on a pre-heated glass slide (90 °C). The fat blends were cooled under controlled conditions, and thereafter covered with a cover slip. Then, all these slides were stored at room temperature (25^{o}C) for 24-48 hours until crystal formation occurred. Crystal formation at 25^{o}C of both fats was studied under polarized light microscope (Olympus BX51; Olympus Optical Co., Ltd., Tokyo, Japan) at 40X magnification and analysis LS starter software (Olympus Soft Imaging Solutions) [13], [20]. The pictures were recorded by using a digital

video camera (KP-D50; Hitachi Digital, Tokyo, Japan) mounted to the microscope.

2.8 Solid fat content (SFC) analysis

The quality of solidified fat as a function of temperature was calculated based on DSC data and by using the delineation methodology [21] with the origin software [22]. The partial areas of the DSC curves were calculated and correlated with the percentage of solids.

2.9 Statistical analysis

Statistical analysis was performed using ANOVA. Duncan's new multiple range test was employed to determine the differences among the means at 5% significance level.

3 RESULTS AND DISCUSSION

3.1 Melting and crystallization behavior (DSC)

3.1.1 Melting behavior

The DSC melting curves of CB and those of its mixtures with RSF (e.g., M1 and M2) is shown in Fig. 1. The melting profiles of some mixtures are relatively simple and exhibit considerable resemblance to those of CB. Some mixtures, particularly M1 and M2, reached the peak maxima for melting. The lower melting point fraction represents the first maxima ranges of 14.09±2.45 °C and 12.26±2.36 °C, whereas the higher melting point fraction represents the maxima peaks temperature ranges of 21.65±0.72 °C and 19.51±0.67 °C with a ΔH of 66.77±8.50 J/g and 39.05±1.57 J/g (Table.1). CB presented the first maxima range of 14.94±0.31 °C, whereas the maxima peak range of 22.36±0.16 °C with a ΔH 76.53±3.15 J/g. These results are in good agreement with results reported in [15]. When the mixture of RSF with CB was used, the peak maxima temperatures of 21.31 °C and 21.83 °C was observed, and that of CB was 22.64 °C. In another study [23], two maxima temperatures, namely, 11.60 °C and 22.80 °C, were reported for the mixture of mango seed fat (MSF) with CB. Meanwhile Similar results have been reported for CB by numerous researchers [23], [24, [25], in which M1 and M2 demonstrated melting characteristics similar to those of CB. These results are different from those of [26], wherein the authors found the first maxima range of 17.01–17.61 °C for the mixture of Mango seed fat (MSF) and palm stearin (PS), whereas the peak maxima temperature was found in the range of 21.40^{O} C–23.01°C. On the other hand, the peak maxima of M3, M4, and RSF were observed at 19.30±0.71, 19.18±1.44 and 20.46±1.09 °C with a ΔH 23.06±4.71, 13.55± 2.76 and 9.65± 0.40 J/g, respectively. In addition, RSF exhibited more similar to that of CB in first maxima and second maxima temperature, but with an additional peak as observed in M3 and M4.

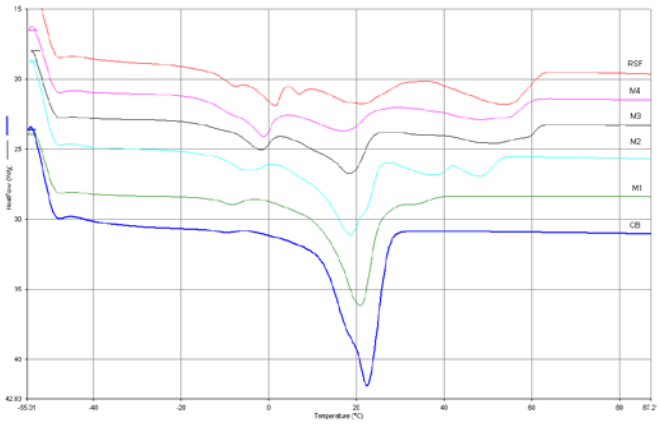

Fig.1 Comparison of DSC melting curves of cocoa butter and its mixture with rambutan seed fat, CB=cocoa butter, M1= 80%CB:20%RSF, M2= 60%CB:40%RSF, M3= 40%CB:60%RSF, M4= 20%CB:80%RSF, RSF= rambutan seed fat

When the amount of CB is less in the mixtures, the size of the endothermic peak increases in the high temperature region and decreases in the low temperature region. As the amount of RSF increases, the enthalpies of all mixtures during melting also gradually increase due to the fermentation and roasting treatment changed the melting profile of RSF, a lower content of long-chain saturated fatty acid can be the differentiating factor. Meanwhile, the roasted RSF showed faster melting than raw RSF because of the fatty acid concentration influences the degree of melting, crystalline formation and distribution of crystal sizes which plays a vital role in the ultimate quality of the RSF [27].

Table 1 Melting behavior of cocoa butter and its mixture with rambutan seed fat

sample	Peak			
	First maxima (^{o}C)	Maxima (^{o}C)	second maxima (^{o}C)	ΔH (J/g)
CB	14.94±0.31^{a}	22.36±0.16^{a}	27.81±0.44^{ab}	76.53±3.15^{a}
M1	14.09±2.45^{a}	21.65±0.72^{ab}	26.27±0.40^{bc}	66.77±8.50^{b}
M2	12.26±2.36^{a}	19.51±0.67^{c}	24.83±0.35^{cd}	39.05±1.57^{c}
M3	11.97±3.07^{ab}	19.30±0.71^{c}	23.98±0.43^{d}	23.06±4.71^{d}
M4	8.24±1.65^{b}	19.18±1.44^{c}	23.98±0.43^{d}	13.55± 2.76^{e}
RSF	11.30±1.47^{ab}	20.46±1.09^{bc}	28.13±2.08^{a}	9.65± 0.40^{e}

CB=cocoa butter, M1=the mixture1, M2= the mixture2, M3= the mixture3, M4= the mixture4, RSF=rambutan seed fat. Different letters indicate significant differences between values ($p<0.05$). Mean ± SD of at least three replicates.

3.1.2 Crystallization behavior

The crystallization thermogram profiles of M1 were different from those of M2, M3, and M4. M1 slowly crystallized, whereas M2, M3, and M4 rapidly crystallized with increasing temperatures. As shown in Table 2 and Fig. 2, the M2, M3 and M4 similar to CB in peak maxima, but they were exhibited more

complex patterns than the crystallization of the M1 and CB.

Table 2 Crystallization behavior of cocoa butter and its mixture with rambutan seed fat

Sample	Peak			
	First maxima (°C)	Maxima (°C)	second maxima (°C)	ΔH (J/g)
CB	17.16±0.05^{c}	11.92±0.56^{b}	5.59±0.46^{a}	72.23±2.43^{c}
M1	18.69±1.20^{bc}	12.17±0.60^{b}	1.88±0.60^{b}	71.29±5.46^{c}
M2	18.97±1.29^{bc}	12.51±0.64^{b}	1.31±0.81^{b}	41.90±5.21^{b}
M3	19.28±0.42^{bc}	12.60±0.90^{b}	0.48±0.10^{b}	39.71±4.59^{b}
M4	9.81±0.53^{ab}	13.40±0.59^{ab}	2.52±0.59^{b}	32.61± 2.72^{a}
RSF	21.56±2.31^{a}	15.47±2.59^{a}	5.35±2.59^{a}	27.31± 1.29^{a}

CB=cocoa butter, M1=the mixture1, M2= the mixture2, M3= the mixture3, M4= the mixture4, RSF=rambutan seed fat. Different letters indicate significant differences between values ($p<0.05$). Mean ± SD of at least three replicates.

The first maxima of M2, M3 and RSF were observed at 18.97±1.29, 19.28±0.42 and 21.56±2.31°C, respectively, which were slightly higher than those of M1 at 18.69±1.20 °C and CB at 17.16±0.05 °C. Meanwhile, the peak maxima in M2, M3, M4, RSF were 12.51±0.64, 12.60±0.90, 13.40±0.59 and 15.47±2.59 °C, respectively.

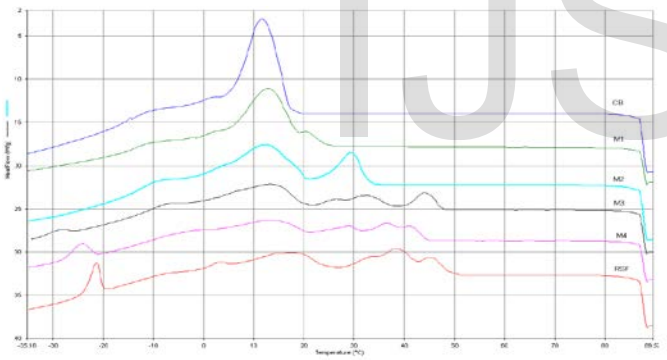

Fig.2. Comparison of DSC crystallization curves of CB and its mixture with RSF, CB=cocoa butter, M1= 80%CB:20%RSF, M2= 60%CB:40%RSF, M3= 40%CB:60%RSF, M4= 20%CB:80%RSF, RSF= rambutan seed fat

These results are similar to those presented in [15], when the mixtures of RSF with CB were used, the maximum peak heights of the mixtures at 14.85–18.33 °C were slightly higher than the other peaks with the maximum temperature of 14.09 °C and that of CB at 12.98 °C. The compatibility of natural fats with CB was previously reported in [26], wherein mixtures of MSF and PS have been used. This previous study found that the maximum peak at high temperature ranged from 17.01–21.50 °C, which was less than the other maximum peaks that ranged from 12.20–12.70 °C. The heating and cooling rates (scanning) have a major effect on the shape of the curves obtained by DSC. When fat is heated, it may exhibit multiple melting phases; moreover, during each crystallization, a stable polymorphic transforms into a more stable one. The peak transition temperature indicates the presence of a crystal because the most stable crystalline form has a high melting point [18, 28]. The limited heat load allows the achievement of molecular rearrangement. Some thermal events, such as polymorph formation and the melting of newly formed polymorphs, can be observed when the sample is melting slowly [29]. The main factors of these changes in polymorphs may be attributed to changes in fatty acid and triacylglycerol (TAG) compositions.
The formation of TAG hydrolase enzymes such as lipase during fermentation and roasting could be the responsible factors, since it is reported that lipase could induce fatty acid exchange through enzymatic inter-esterification in lipid body and these changes also affect the enthalpy of the crystallization of RSF, in addition to that, the roasted RSF showed a faster crystallization than the raw RSF [27]. As mentioned in [20], varying fat proportions directly affect the rates in mixtures, such as phase behavior, crystallization, and hardness. M1 and M2, which have higher proportions in CB, may dominate the overall characteristics of the mixtures. Adding RSF only slightly varies the thermal behavior of CB. Meanwhile, other mixtures with a higher proportion of RSF than CB are probably dominated by the occurrence of high-melting fatty acid and triacylglycerol. The combination of fatty acids in the triacylglycerol (TAG) has a correlation with the crystallization profile of RSF.

3.2 XRD (polymorphism)

In lipids, three specific types of sub-cells are dominant and related to the polymorphs α, β', and β. The α form is stable and has a hexagonal packing. The β' form exhibits intermediate stability and orthorhombic vertical packing. The β form demonstrates high stability and triclinic parallel packing [30]. For CB, six polymorphic forms, namely, γ, α, β'_2, β'_1, β_2, and β_1, have been verified as a result of a triacylglycerol configuration that is homogeneous to a certain extent; these forms are in accord with the current nomenclature [31]. The nomenclature for CB polymorphs can also be given in the form of Roman numerals, with I, II, III, IV, V, and VI corresponding to γ, α, β'_2, β'_1, β_2, and β_1 polymorphism, respectively. XRD is used to identify crystal polymorphism by determining the dimensions of the crystal unit and the sub-cell. Given the variation in geometric configuration, polymorphs diffract X-ray at different angles. In fats, high-diffraction analysis agrees with the short space of the sub-cells and allow checking of different polymorphs. A short spacing can be defined as the distance between parallel acyl groups in the triacylglycerol molecule; it indicates cross packing of the triacylglycerol chains [32], [33]. Thus, the structure, composition, and polymorphic forms of fat crystals are the most important criteria to identify the functional properties of fats [34]. The XRD patterns obtained for the CB sample and its mixture with RSF are shown in Fig. 3.

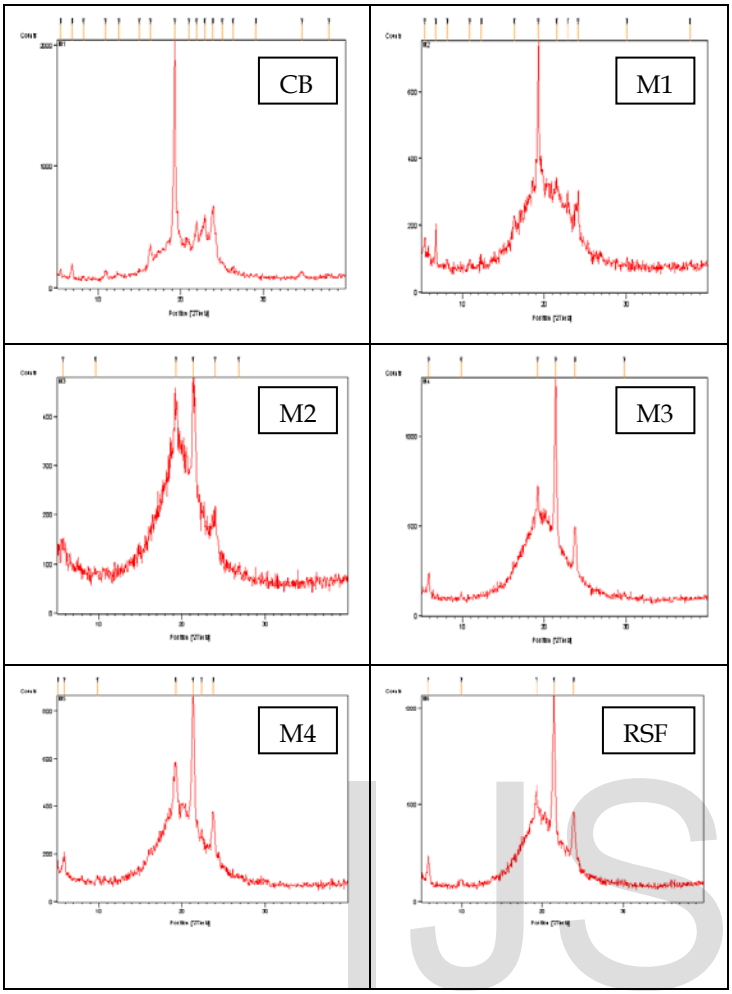

Fig. 3 X-ray diffraction patterns of cocoa butter (CB) sample and its mixture with rambutan seed fat(RSF), CB=cocoa butter, M1= 80%CB:20%RSF, M2= 60%CB:40%RSF, M3= 40%CB:60%RSF, M4= 20%CB:80%RSF, RSF= rambutan seed fat

Meanwhile, Table 3 presents the calculated short spacing. Based on the results, M1 has a more similar crystallization to CB in β form, as shown in Table 3. For CB, the most functional polymorph (form V) is a β type, the approach to form V from the liquid state under static crystallization conditions (17.16±0.05–5.59±0.46°C) can proceed by two different pathways: through the transition sequence I → IV directly from form IV at crystallization temperatures between 20 and 26 °C. The results also show that M1 has the same short spacing and wide angle reflections as those of CB at 20 °C (Fig. 3). These results correspond to the finding for commercial CB in [35]. Furthermore, M2, M3, M4, and RSF, tend to crystallize in β and β′ form as mentioned in (Table 3). Despite some differences in short spacing and wide angle reflections (Fig. 3), the current findings agree with those of [36], which found that similar to CB, rambutan kernel fat tends to crystallize in β form. These results also agree with those of [37], the authors used cupuassu fat, which were identified to be in α, β', and β forms. The β′ crystal in M2, M3, M4 and RSF samples is characterized as a low melting point crystal which can melt easily at 20-28˚C.

Table 3 Short spacing (Å) of CB, M1, M2, M3, M4 and RSF

	Short spacing * (Å)											
Sample	**2.3**	**3.6**	**3.7**	**3.8**	**4.0**	**4.1**	**4.6**	**5.4**	**7.0**	**8.1**	**8.9**	**15.0**
CB	2.37vw	3.60s	–	3.88s	4.05s	–	4.60vs	5.44m	7.08w	8.11w	–	–
M1	2.37vw	3.68s	–	3.88s	4.00s	–	4.60vs	5.42m	7.00w	8.12w	–	–
M2	–	–	3.72m	–	–	4.16s	4.60s	–	–	–	8.99vw	15.09w
M3	–	–	3.74m	–	–	4.15s	4.61s	–	–	–	8.97vw	15.02w
M4	–	–	3.74m	–	–	4.10m	4.61s	–	–	–	8.95vw	15.02w
RSF	–	–	3.72m	–	–	4.15m	4.60s	–	–	–	8.90vw	15.00w

* The relative intensity is noted as very strong (vs), strong (s), medium (m), weak (w), or very weak (vw), CB= cocoa butter, M1= the mixture1 (80%CB:20%RSF), M2= the mixture2 (60%CB:40%RSF), M3=the mixture3(40%CB:60%RSF), M4= the mixture4 (20%CB:80%RSF), RSF= rambutan seed fat.

The tempering process carried out by storing the mixtures and CB at 5˚C for certain time could lead to the formation of desirable β crystal formation. The β crystal formation possesses hard, but brittle texture of cocoa butter, which is usually used for storage and product development. This formation of crystal is also desirable due to its unique snap characteristic and fast melting in the mouth feature.

3.3 Morphological studies

The microstructure level of fat crystal networks can be determined as structures with sizes ranging from 0.5 µm and 200 µm [38]. PLM is the most commonly used technique for visualizing the microstructure network of fats; this method has been applied to clarify differences in the texture of fat mixtures and detect crystalline types or morphological changes during crystal growth. Crystallization at 25°C of cocoa butter and rambutan seed fat was viewed under the microscope at 40X magnification. The polarized light microphotographs were obtained at the end of the crystallization process for CB and its mixture with RSF. The fat crystal network microstructures of the mixtures were a different crystal morphologies. Crystals with different morphologies were observed among the studied mixtures because the crystalline of CB: RSF mixtures were affected by their respective proportions in the mixtures. Fig. 4 showed that M2, M3, and (M4) formed crystal characteristics that are more similar to those of RSF. By contrast, the crystal formation of M1 was more similar to that of CB, which was characterized by its nuclei-centered granular form that resulted from the slow cooling process [13]. These results correspond to the findings of [15], wherein mixtures of CB and RSF were used. The current results also corresponded to those of another study [26], wherein a series of blends (1–10) of MSF and PS could be used as alternatives to CB. A high proportion of CB in M1 (80/20, CB/RSF) corresponds to an increase in the rates of triglycerides because CB contains three main triglycerides, which account for 92%–96% of its total lipid composition[39]. The major fatty acids of CB are palmitic acid (C_{16}),

stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$), which contribute approximately 98% of its total fatty acid [35, 36]. Therefore, the formation of crystal in M1 was mostly induced by a high concentration of medium-chain fatty acids and triacylglycerol. The occurrence of medium-chain fatty acids and triacylglycerol resulted in slower crystal formation, in which lauric-, palmitic-, and oleic-based triacylglycerols behaved as crystal nuclei for other triacylglycerols in the lipid body. However, the crystal formations in M1 were slightly different from those in the CB. Meanwhile, in M2, M3, M4, and RSF, the concentrations of high-melting fatty acids and triacylglycerols were sufficiently high to induce the rapid formation of crystals. On the other hand, the fermentation and roasting process affected the fat crystal size. The raw CB sample generated larger crystals than the roasted sample [27].

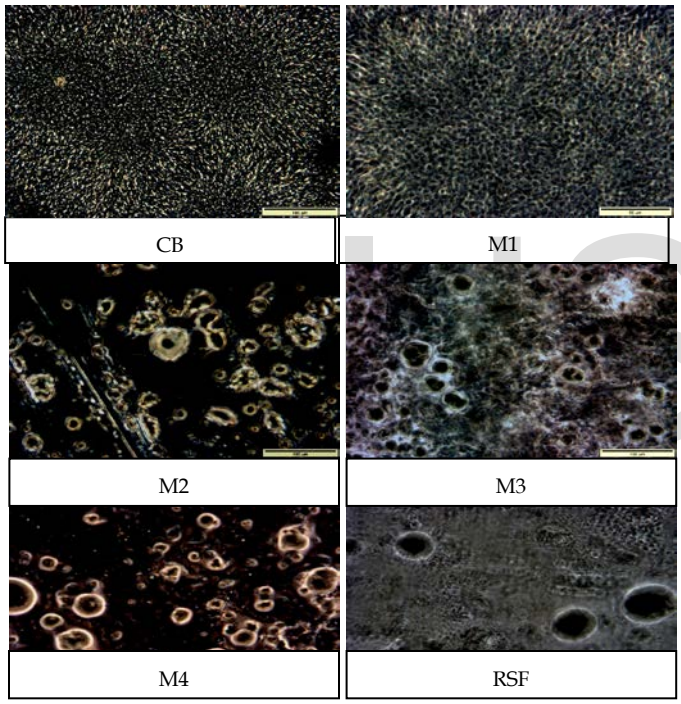

Fig.4. Images of crystal formation in CB and its mixture with RSF at 100x magnification, CB=cocoa butter, M1= 80%CB:20%RSF, M2= 60%CB:40%RSF, M3= 40%CB:60%RSF, M4= 20%CB:80%RSF, RSF= rambutan seed fat

3.4 SFC

Generally, the percentage of SFC is related to temperature and it indicates both the hardness, softness and the melting behavior, the SFC profiles for CB and its mixtures with RSF are shown in Fig. 5. A previous study [40] showed that CB had a unique characteristic profile that made it such a valuable fat. SFC is an excellent indicator of hardness, such that temperatures slightly lower than body temperature (37°C) illustrate a sharp decrease in SFC [37]. A comparison of the SFC curves indicated that M1 exhibited a sharp decrease in SFC at 10–40 °C, with the melting peak temperature 21.65±0.72°C similar to melting peak temperature of CB 22.36±0.16°C, the SFC curve that was nearly indistinguishable from the results reported for CB showed a sharp decrease in SFC between 10 °C and 40 °C and reached SFC of almost zero at temperatures higher than 40˚C, the mixture M1 could be used to increase the hardness of CB. Meanwhile, the mixtures of M2, M3, and M4 were completely melted at 45°C, the SFC of M2, M3, and M4 ranged between the SFC of RSF and CB, which are characterized by high SFC at temperatures of 20–40 °C followed by low SFC after 40°C. The SFC of all mixtures decreased significantly as the temperature increased. These results are in agreement with those reported in [15], which used RSF mixed with CB. This previous study found that a proportion of RSF of less than 30% resulted in greater similarity in SFC between RSF and CB. These results are also similar to those of another study [37], in which 5% cupuassu fat was mixed with 10% CB. The results of fermented and roasted RSF showed higher consistency due to its higher SFC. This higher consistency of RSF samples may be contributed by the crystal formation as affected by its different TAG combination.

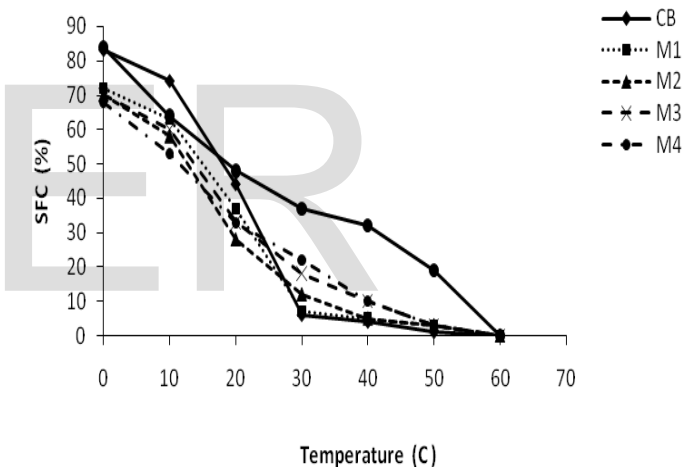

Fig.5. Comparison of solid fat content between CB and its mixtures with RSF, CB=cocoa butter, M1= 80%CB:20%RSF, M2= 60%CB:40%RSF, M3= 40%CB:60%RSF, M4= 20%CB:80%RSF, RSF= rambutan seed fat

CONCLUSION

In this work, the peak maxima during thermal behavior, polymorphism, morphology, and SFC of mixtures of CB and RSF (CB:RSF) were characterized by DSC, XRD analysis, and PLM. The mixtures were analyzed to compare their thermal behavior, polymorphism, and SFC with those of CB. The results suggested that certain mixtures of CB and RSF, such as M1 and M2, exhibited melting characteristics similar to that of CB, with M1 showing a more similar crystallization style with a single curve with one peak to that of CB. The melting temperatures of M1, M2, and CB were observed at peaks maxima 21.65±0.72, 19.51±0.67 and 22.36±0.16 °C, respectively; whereas the crystallization temperature of the M1 and CB were observed at maxima of peaks 12.17±0.60 °C and 11.92±0.56 °C, respectively. The results of the study also show that M1 is

more similar in polymorphism, morphology, and SFC with CB; whereas the other mixtures exhibit some differences with the original CB properties. M1 has similar polymorphism of structure, composition, and polymorphic forms of fat crystals to those of CB. PLM images showed significant changes in the crystal morphology of the mixtures. The results showed that the crystal formation of M1 was more similar to that of CB. The formulation of M1 presented a sharp decrease in SFC at 10–40°C; such formulation is similar to that of CB, which also showed a sharp decrease in SFC at 10–40°C. Based on these results, RSF can be partially incorporated into CB. Other possible applications of RSF in different industries include confectionery and cosmetics products.

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