# Melting behaviour of cocoa butter substitutes synthesised by enzymatic glycerolysis of coconut oil and palm stearin blends

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### Abstract

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# limited and has adverse health effects. Therefore, an alternative to CB is needed. This study aimed to synthesise cocoa butter substitutes (CBS) rich in monolaurin by enzymatic glycerolysis of coconut oil (CNO) and palm stearin (PS) blends. The ratios of CNO to PS used were 7:3, 6:4, and 5:5 (w/w). Several characteristics related to the melting behaviour of CBS were analysed. The results showed that CBS contained monoacylglycerol (MAG) and diacylglycerol (DAG) about 55.89-57.28% and 34.27-39.57%, respectively, while MAG fraction contained monolaurin of 25.31-37.07%. CBS rich in monolaurin produced from the CNO-PS ratio of 6:4 had the slip melting point and the melting point similar to CB, namely, $34.87\pm0.08^{\circ}$ C and $36.75\pm0.14^{\circ}$ C, respectively. In addition, the melting profile and solid fat content (SFC) were also comparable to CB but slightly lower than CB. The obtained CBS had 5% compatibility with CB to maintain the same melting profile as CB. CBS synthesised by enzymatic glycerolysis of coconut oil and palm stearin blends has the potential to partially replace CB.

Cocoa Butter (CB) is a speciality fat required in various products, but its availability is

### 1. Introduction

CB has the unique characteristic that it is a solid state at below 25°C and melts completely at around 37°C (Biswas *et al.*, 2017). CB has several disadvantages because it is expensive, has a limited supply, and high content of saturated fatty acids, so it is not good for health. Therefore, it is necessary to find an alternative for CB in the form of cocoa butter substitutes (CBS) that contain better fat types. One source of oil/fat that is good for health is coconut oil which contains medium-chain fatty acids, namely lauric acid (Lieberman *et al.*, 2006).

Various methods of producing CBS have been carried out either by physical blending or interesterification of fat/oil mixtures. Several types of fat/oil that can be used to synthesise CBS are coconut oil (CNO) and palm stearin (PS). Sonwai et al. (2015) produced CBS in the form of Lauric fat cocoa butter replacer (LCBR) from a mixture of coconut oil and Krabok seed fat; it was found that LCBR has a crystal form of  $\beta$ ' and has 5% compatibility with CB. Biswas *et* al. (2017) produced CBS by blending a mixture of Palm Stearin, Palm Kernel Oil, and palm mid-fraction showing that the crystal morphology and SFC of CBS were comparable to CB, but the melting profile was different with CB. Although the fatty acid content is different from CB, the lauric content in CNO can replace stearate in CB related to the melting profile and texture. Laurate also has a beneficial effect, especially in the form of glycerol monolaurate or monolaurin. Monolaurin can act as an antimicrobial and improve the immune system (Subroto and Indiarto, 2020).

Monolaurin can be produced through glycerolysis between glycerol and lauric-rich fats/oils such as CNO. Enzymatic glycerolysis is an effective method for producing monolaurin and other acylglycerols due to their high catalytic activity on lipases and environmental friendliness (Feltes *et al.*, 2013). CNO contains high lauric acid (48-52%), it has the potential to be modified into monolaurin and other acylglycerols (MAGs and DAGs). MAGs and DAGs can act as emulsifiers and can improve the physicochemical properties of the product (Feltes *et al.*, 2013; Subroto, 2020).

Good quality CBS should have a melting profile that is comparable to CB. CB has a relatively short melting range, with 80% being able to meet at a temperature of 27-35°C and has a high solid fat content (SFC) at temperatures below room temperature, but SFC can reach zero at body temperature (Jahurul *et al.*, 2013). 195

Besides, the melting profile can also affect the lustre, brittleness, and hardness of a product (Basso et al., 2010; Saberi et al., 2011). An appropriate ratio between CNO and PS must be determined to produce CBS with a good melting profile comparable to CB. The synthesis of CBS rich in monolaurin from a mixture of CNO and PS has never been carried out. Therefore, research is needed on the synthesis of CBS rich in monolaurin and melting behaviour in the obtained CBS. In this study, monolaurin -rich CBS was synthesised through enzymatic glycerolysis of CNO-PS blends; and the purpose of this study was to determine the ratio of CNO to PS that produces CBS rich in monolaurin, which has a melting behaviour comparable to CB. Glycerolysis of CNO-PS blend is expected to be an alternative for the production of CBS, which is healthier and has good partial compatibility with CB.

### 2. Materials and methods

### 2.1 Materials

Coconut oil (CNO) and palm stearin (PS) were provided by PT. Barco and PT Smart Tbk, Indonesia, and commercial CB were purchased from PT. Barry Callebaut, Indonesia. Other materials used were immobilised lipase from *Candida antarctica* (Novozyme 435 with the specific activity of 5000 U/g), hexane, glycerol, tert-butanol obtained from Merck, and several analytical grade reagents used for analysis purposes.

# 2.2 Enzymatic glycerolysis of coconut oil-palm stearin blend for cocoa butter substitutes production

The enzymatic glycerolysis was conducted according to Subroto *et al.* (2019). A mixture of CNO and PS was provided in a ratio of 7:3, 6:4, and 5:5 (w/w). Glycerol was entered into the fat mixture at a molar ratio of fat to glycerol 1:3. t-butanol was mixed at a ratio of solvent to the substrate was 2:1 (v/w). Glycerolysis was catalysed by 5% immobilised *Candida antarctica* lipase, and the reaction took place in a water bath shaker at 50°C for 24 hrs. The immobilised lipase was separated after the reaction was completed by filtration using filter paper, and then the filtrate (reaction mixture) was taken. The remaining glycerol was precipitated, and t-butanol was evaporated to obtain CBS for storage and analysis.

### 2.3 Analysis of fatty acid composition

The fatty acid composition was analysed by preparing of 200-300  $\mu$ L sample, then transmethylated by adding 400  $\mu$ L of BF3-methanol complex at 90±5°C for 2 hrs. The residue from the sample was extracted with 500  $\mu$ L of hexane. Samples were analysed using Shimadzu Gas Chromatography-2010 equipped with a CP-Sil 8CB column and a flame ionisation detector. The

analysis was carried out by comparing it with standard fatty acid compounds (AOCS, 2004).

### 2.4 Analysis of acylglycerol composition

Acylglycerol composition was analysed by thin-layer chromatography (TLC) according to Fuchs *et al.* (2011) and Subroto *et al.* (2020) with some modifications. TLC silica gel was activated by heating at 100-105°C for 1 hr. The samples were spotted to the TLC plate and then developed in a chamber containing a solvent consisting of hexane-diethyl ether-acetic acid with a ratio of 80:20:2. The TLC plate was removed from the chamber to be dried, then stained using sublimation from iodine crystals. The spots on the TLC were then analysed quantitatively using the TLC Scanner III Camag.

### 2.5 Analysis of monolaurin content

The analysis of monolaurin content was principally carried out by isolating or separating the MAG fraction from the fat sample using a silica gel TLC plate as in the method described previously. The MAG spots obtained were scraped and extracted using hexane. The isolated MAG was then methylated using a boron trifluoride (BF3) methanol complex and then analysed for its fatty acid content using a Shimadzu Gas Chromatography-2010 fitted out by a CP Sil 8 CB and a flame ionisation detector (AOCS, 2004). The detected methyl laurate represented the monolaurin content in the sample.

### 2.6 Analysis of slip melting point and melting point

Analysis of SMP was determined using the AOCS Cc. 3.25 (AOCS, 1997), and analysis of MP was determined using the AOCS Cc 1-25 (AOCS, 1997).

### 2.7 Analysis of melting profile and solid fat content

The melting profile and SFC were analysed using a Differential Scanning Calorimeter (DSC) (Shimadzu DSC-60 Plus, Japan). The sample was weighed as much as  $10\pm0.5$  mg, and placed in a closed aluminium pan; then, the empty pan was also closed and used as a reference. The DSC instrument was programmed by being heated gradually to 80°C and held for 5 mins, then the sample was cooled to -30°C at a speed of 10°C/min and held for 5 mins. The DSC was then programmed from -30°C to 80°C at 5°C/min. The thermogram obtained from the DSC showed the melting profile and was then used to calculate SFC by transforming the melting energy into melting mass (Márquez *et al.*, 2013).

## 2.8 Compatibility evaluation of cocoa butter substitutes to cocoa butter

CBS rich in monolaurin with the best ratio was added to commercial CB at concentrations of 5, 10, and

15%. Compatibility evaluation was carried out by comparing the melting profile and SFC, which were still almost the same as commercial CB (Çiftçi *et al.*, 2010).

### 2.9. Statistical analysis

Data were analysed by one-way ANOVA followed by Duncan's multiple range test at the significance level (p<0.05) using PASW Statistics 18.

### 3. Results and discussion

### 3.1 Fatty acid composition

The fatty acid composition of CBS produced by glycerolysis at various ratios of CNO to PS can be seen in Table 1.

Based on Table 1, each ratio of CBS contains saturated fatty acids such as caprylic acid, capric acid, lauric acid, myristic acid, stearic acid, and palmitic acid, namely 3.32-4.63%, 2.92-4.02%, 22, 78-32.18%, 9.04-12.77%, 4.25-6.17%, and 20.75-26.03%, respectively. CBS also contain unsaturated fatty acids such as oleic acid, about 21.4-29.73%. Lauric acid, capric acid, myristic acid, and caprylic acid tend to increase along with the higher ratio of addition of coconut oil; while stearic acid, palmitic acid, and oleic acid tend to decrease. This was due to the effect of the fatty acid composition of coconut oil, which is dominated by medium-chain fatty acids (C8-C12), while palm stearin is dominated by longer chain fatty acids (C16-C18).

The CBS had the highest lauric acid content, which indicated that it was different from the fatty acid composition of CB, which had a high stearic acid content. However, the lauric acid content of CBS can also affect the melting point of CBS to remain compatible with commercial CB. This is in accordance with Podchong *et al.* (2020), who reported that CBS produced from lauric-rich oil has a lauric acid content of 53.3% with a melting point of 37°C, indicating this physical property can be potentially compatible with CB. The high content of lauric acid can replace the role of stearic acid on the physical properties of CB; also be beneficial for health.

# 3.2 Acylglycerol profile of cocoa butter substitutes and coconut oil-palm stearin blend

The acylglycerol profile described the effectiveness of the enzymatic glycerolysis reaction converting TAG into MAG and DAG. Acylglycerol profiles of CBS from glycerolysis and CNO-PS blend from physical blending, which includes MAG, DAG, and TAG content, were presented in Table 2.

Based on Table 2, there are differences in the MAG, DAG, and TAG content between CBS and CNO-PS blends. The MAG content that was not detected in the CNO-PS blend increased significantly to around 55.89-57.28% in the CBS. The CNO and PS used have been refined not to contain MAG, then increase significantly after glycerolysis because TAG in CNO and PS reacted with glycerol to form MAG and DAG. The content of DAG in CBS was about 34.27-39.57%. CBS with a ratio of 7:3 and 6:4 increased in the amount of DAG compared to the CNO-PS blend, while CBS with a ratio of 5:5 decreased in DAG. These differences were due to the DAG contained in the CNO-PS blend was also converted into MAG after glycerolysis. The content of MAG and DAG contained in the reaction system during glycerolysis also acted as an emulsifier in the glycerolysis step, increasing the conversion rate of

Table 1. Fatty acid composition of CBS resulting from enzymatic glycerolysis at various ratios of CNO to PS.

Ratio of	Fatty acid content (%)						
CNO:PS	C8:0	C10:0	C12:0	C14:0	C16:0	C18:1	C18:0
7:3	4.63	4.02	32.18	12.77	20.75	21.40	4.25
6:4	4.30	3.73	28.74	11.03	21.94	25.01	5.26
5:5	3.32	2.92	22.78	9.04	26.03	29.73	6.17
CNO	7.60	7.30	48.20	16.60	8.00	5.00	3.80
PS	nd	nd	0.14	1.10	56.34	33.16	7.72
CB	nd	nd	nd	0.20	28.35	31.18	37.92

Table 2. Acylglycerol profile of CBS and CNO-PS blend.

Ratio of		CBS			CNO-PS Blend	
CNO:PS	MAG (%)	DAG (%)	TAG (%)	MAG (%)	DAG (%)	TAG (%)
7:3	$56.94{\pm}3.06^{a}$	$38.77 \pm 3.39^{a}$	4.54±3.13 <sup>ab</sup>	Not detected	$36.62{\pm}2.73^{a}$	63.38±2.73 <sup>a</sup>
6:4	$57.28{\pm}2.90^{a}$	$39.57{\pm}1.73^{a}$	$3.15{\pm}0.08^{\mathrm{a}}$	Not detected	$37.70{\pm}0.95^{a}$	$62.30{\pm}0.95^{a}$
5:5	$55.89{\pm}5.60^{a}$	$34.27{\pm}10.69^{a}$	$9.31{\pm}5.12^{b}$	Not detected	$36.73{\pm}0.59^a$	$63.27{\pm}0.59^{a}$

Values are presented as mean $\pm$ SD. Values with different superscripts within the same row are statistically significantly different at the 5% level (p<0.05).

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### further MAG formation (Pawongrat et al., 2007).

The conversion of TAG to MAG was greater than the conversion of TAG to DAG. The amount of glycerol used in the oil mixture in the enzymatic glycerolysis affected the conversion of acylglycerol composition. The more glycerol used the tendency towards the formation of MAG rather than DAG. This matter was in line with Wang *et al.* (2011) and Naik *et al.* (2014), who reported that differences in the amount of glycerol used caused differences in the conversion of MAG or DAG. This showed that the use of glycerol in oil mixtures with a molar ratio of fat to glycerol 1:3 was the right ratio to further increase the amount of MAG conversion compared to DAG, so it was expected that the monolaurin formed was also higher.

Based on Table 2, glycerolysis produced different acylglycerol profiles (MAG, DAG, and TAG) from each ratio of CNO-PS, but the amount of MAG from each ratio was not significantly different. This was due to differences in the fatty acid composition and the position of the fatty acids on the TAG in each oil used. This was also supported by Adamczak (2003), which reported that the lipase activity of *C. antarctica B* (CALB) was higher in short and medium-chain compared to long-chain fatty acids. CBS with more coconut oil blends, especially at ratios of 7:3 and 6:4, had higher MAG and DAG content than the ratio of 5:5.

CBS produced by the enzymatic glycerolysis had relatively high MAG and DAG content at all ratios of CNO-PS (Table 2). The high content of MAG and DAG can affect the physical properties possessed, such as the profiles of melting, crystal, texture, and other physical properties (Subroto, 2020). The high content of MAG and DAG made the CBS have the ability as surfactants, making it easier to apply to various products that require emulsifiers. MAG in CBS that contains a lot of lauric acids will certainly produce monolaurin, which is good for health to be a functional food (Subroto and Indiarto, 2020).

# 3.3 Monolaurin content in the monoacylglycerol fraction of cocoa butter substitutes

Monolaurin is a MAG in the form of an ester of glycerol with one molecule of lauric acid obtained from the glycerolysis reaction of lauric acid-rich fat with glycerol as contained in CBS. The content of monolaurin could be determined by isolating the MAG contained in CBS, which was then analysed for lauric acid content. The monolaurin content in CBS from the MAG fraction can be seen in Figure 1.

Based on Figure 1, the monolaurin content showed different results for each CNO-PS ratio in CBS, namely

25.31-37.07%. The monolaurin content of CBS increased with the increasing proportion of coconut oil. This was due to the higher the proportion of CNO, the higher the lauric acid content so the MAG fraction of CBS also contained higher lauric acid. Abdul Halim et al. (2019) reported that the more CBS from coconut oil was added to the chocolate, the lauric acid also increased. Candida antarctica lipase used as a catalyst had high specificity in reactions involving (MCFAs), including lauric (C12:0). Therefore, in this glycerolysis, lauric acid from coconut oil TAG was easily released and esterified on a glycerol substrate available to become MAG, called monolaurin. The high monolaurin content in CBS was expected to improve the melting profile and other functional properties of CBS. Monolaurin is multifunctional with characteristics that can improve physicochemical properties, act as a surfactant, and act as an antimicrobial agent. Therefore, the CBS rich in monolaurin can improve the shelf life of a product. This was consistent with Yu et al. (2017), who stated that monolaurin could improve the quality and sustain the shelf life of food products. The quality parameters that can be improved by the presence of monolaurin include emulsification ability, melting properties, crystallisation, and antimicrobial activity.

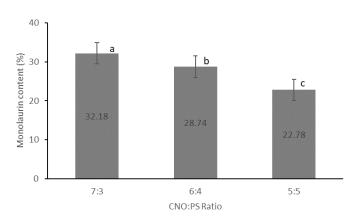


Figure 1. Monolaurin content of MAG fraction from CBS at various ratios of CNO-PS. Bars with different notations are statistically significantly different at the 5% level (p<0.05).

### 3.4 Slip melting point and melting point

Slip melting point (SMP) and melting point (MP) relate to the shape and appearance of the product. CBS with good quality must have SMP and MP, which are almost similar to commercial CB with the characteristics of having a solid-state at room temperature and liquid at body temperature (Biswas *et al.*, 2017). SMP and MP in CBS and CNO-PS blend at various ratios can be seen in Table 3.

Table 3 shows that SMP and MP in CBS were higher than in CNO-PS blends. This was due to higher amounts of MAG and DAG in CBS than in CNO-PS blends (Table 2). In general, MAGs and DAGs have higher

Ratio of	Slip Melting	Point (°C)	Melting Point (°C)		
CNO:PS	CNO-PS Blends	CBS	CNO-PS Blends	CBS	
7:3	$26.70{\pm}0.10^{a}$	$32.38{\pm}0.35^{\text{b}}$	28.73±0.12 <sup>a</sup>	$34.70 \pm 0.10^{b}$	
6:4	$30.50{\pm}0.17^{a}$	$34.87{\pm}~0.08^{\text{b}}$	$32.87{\pm}0.15^{a}$	$36.75 {\pm} 0.14^{b}$	
5:5	$33.70{\pm}0.10^{a}$	$36.03{\pm}~0.42^{\text{b}}$	35.57±0.12 <sup>a</sup>	$38.20{\pm}0.22^{b}$	
CB	34.13±0	0.06	36.20±0.17		

Values are presented as mean $\pm$ SD. Values with different superscripts within the same row are statistically significantly different at the 5% level (p<0.05).

melting points than TAGs. This was also supported by research conducted by Saberi *et al.* (2011), which showed that palm oil added PO-DAG resulted in higher melting endothermic peaks and temperatures.

SMP and MP in CBS with various CNO-PS ratios also increased. SMP in CBS increased from 32.30 to 36.03°C, while MP in CBS raised from 34.70 to 38.20° C. This occurred in line with the large proportion of palm stearin. The more palm stearin added, the higher the SMP and MP of CBS. This was due to the high amount of fatty acids with high melting points, such as palmitic acid in palm stearin (Norizzah *et al.*, 2004; Subroto and Nurannisa, 2020).

Commercial CB has SMP and MP of 34.13 and 36.20°C. Based on the approach to SMP and MP, the CBS from the CNO-PS ratio of 6:4 was the ratio that had the closest SMP and MP to commercial CB, which was 34.87 and 36.75°C. More than 80% of CB melts at temperatures between 27-35°C which reflects that commercial CB has a relatively short melting temperature range. This property was highly desirable for chocolate products (Jahurul *et al.*, 2014). Therefore, CBS with a ratio of 6:4 potentially could be used as a substitute for CB but with a limited percentage.

### 3.5 Melting profiles

The melting profile is important to determine the interaction of components, complex structures, and the basics that explain the physicochemical properties of fat. The melting profile can be analysed using DSC, which describes the thermodynamic properties of fat in a thermogram that displays the temperature range on the melting profile. The melting profile thermogram of CBS at various CNO-PS ratios can be seen in Figure 2.

Figure 2 shows that the melting peak temperature of CBS at various ratios gradually increased and shifted towards higher temperatures as the PS proportion increased. This was due to an increase in the proportion of long-chain saturated fatty acids, namely palmitic acid and stearic acid, which triggers an increase in melting point and higher melting enthalpy along with the use of more palm stearin (Liu *et al.*, 2018).

The melting profile of CBS with a CNO-PS ratio of 7:3, 6:4, and 5:5 showed one endothermic peak, and no new peak was seen. The peaks formed were relatively smooth and did not form new peaks because lauric oil is a medium-chain oil that melts and dissolves. So, if there is a change in TAG to DAG and MAG, there is no significant change because the melting that occurs is still in the melting range with other fatty acids. According to Chaleepa *et al.* (2010), lauric oil has a relatively simple melting curve with one major endothermic peak and a small shoulder at lower temperatures. This was also supported by Sonwai *et al.* (2015), who observed that coconut oil has one main endothermic peak at 23.64°C and another endothermic peak at a lower temperature (14.75°C).

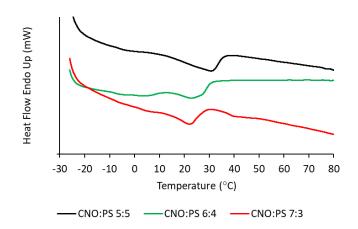


Figure 2. The melting profile thermogram of CBS rich in monolaurin at various CNO-PS ratios.

### 3.6 Solid fat content

SFC was used to determine the solid/liquid ratio of fats at various temperatures. The three temperature measurements used were cold temperature, room temperature, and body temperature, which were related to the ability to spread, product stability, texture, and taste in the mouth of each sample. The ideal SFC for CBS was to have a high SFC at low temperature to maintain solid form and a low SFC at high temperature (35-37°C) (Biswas *et al.*, 2017). The solid fat content of CBS rich in monolaurin at various ratios of CNO to PS can be seen in Figure 3.

Based on Figure 3, it can be seen that all ratios of

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CBS had high SFC at a temperature of 0-10°C, then decreased drastically at 15-35°C. SFC became 0% at 40° C for CBS from the ratio of 7:3 and 6:4, while CBS from the ratio of 5:5 had an SFC of about 7.17%. A drastic decrease at a temperature of 15-35°C occurred because most of the TAG dissolved at that temperature. However, CBS with a CNO-PS ratio of 7:3 has an SFC of 0% at a lower temperature of 35°C, while CBS at a CNO-PS ratio of 6:4 still has an SFC at a temperature of 35°C, which was 5.95%, but an SFC of 0% at 37°C. When compared with all the ratios, it can be seen that the increasing proportion of palm stearin leads to a higher SFC. This was supported by the research of Jahurul et al. (2014), which used palm stearin as a substitute for the middle fraction of palm oil, and it was seen that the increase in SFC was in line with the proportion of the addition of palm stearin because palm stearin had a high melting fraction.

Figure 3 also shows that CB has a high SFC (> 70%) at 0-10°C, followed by a sharp decrease at 15-35°C, and at the temperature of <40°C, the SFC was 0%. When compared to all ratios, CBS with a ratio of 7:3 and 6:4 has an SFC that was almost close to CB even though the SFC at that ratio was lower, especially at temperatures <30°C, but at a temperature of 40°C, the SFC in both ratios was the same with a CB namely 0%. Therefore, there was no unwanted waxy texture or a greasy feeling on the palate (Biswas et al., 2017). The lower SFC in CBS compared to CB was due to differences in fatty acids, namely, CB was rich in stearic acid while CBS were rich in lauric acid. In addition, there were also differences in the solubility of saturated fatty acids with unsaturated fatty acids in mixed TAG. CBS with a CNO-PS ratio of 6:4 was a more appropriate ratio because it had an SFC almost close to CB and higher monolaurin. CBS with this ratio had the potential to be used as a substitute for CB but with a limited percentage.

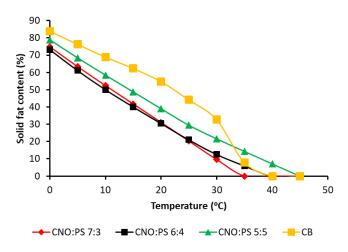


Figure 3. The solid fat content of CBS rich in monolaurin for various ratios of CNO to PS at various temperatures.

3.7 Compatibility evaluation of cocoa butter substitutes rich in monolaurin to cocoa butter

CBS rich in monolaurin from the ratio of CNO-PS of 6:4, which has the most comparable melting profile to CB, was then evaluated for compatibility with CB. Compatibility was evaluated to find out how high the level of compatibility of CBS is when mixed with CB to be applied to certain food products. Evaluation of the compatibility of CBS to CB can be conducted by comparing the melting profile and SFC at various percentages of the addition of CBS to CB.

The compatibility of fat in a food product was highly dependent on its melting profile. Good quality CBS should have a melting profile similar to CB when mixed with CB. The thermogram of the melting profile of CB and a mixture of CBS in CB at various concentrations can be seen in Figure 4.

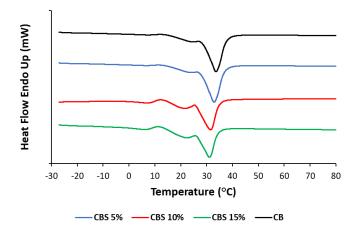


Figure 4. The thermogram of the melting profile of CB and the mixture of CBS in CB at various concentrations.

CB has a melting peak at  $32.87^{\circ}$ C, with T<sub>onset</sub> at  $6.21^{\circ}$ C and T<sub>endset</sub> at  $40.09^{\circ}$ C. The increasing proportion of CBS in CB caused the melting peak to shift to the left at lower temperatures, and some peaks were seen at lower temperatures (10-20°C). This was because the material used in CBS was coconut oil which contained lauric acid, which was categorised as a medium-chain fatty acid that melted at lower temperatures than stearic acid in CB.

Based on the melting profile in Figure 4, it can be seen that the CBS compatibility was only 5% because a higher proportion of CBS has changed the melting profile even though the melting point was still around 37°C. This may be due to CBS having a different fatty acid composition and triacylglycerol type from CB. This change can be seen in the melting profile at the initial temperature (10-20°C), where there was a new peak. The variation in the melting profile was due to the happening of polymorphic transitions resulting from TAG interactions (Biswas *et al.*, 2017; Liu *et al.*, 2018).

Hence, the use of CBS rich in monolaurin needs to be limited to only 5% to maintain the same melting profile as CB.

The compatibility of CBS was also highly dependent on its SFC. SFC that was too low can reduce the plasticity of fat substitutes for chocolate; on the other hand, it can cause a waxy sensation in the mouth when the SFC is too high. The ideal SFC for CBS was to have a high SFC at low temperature for the ability to maintain its shape and a low SFC at body temperature (Zhang et al., 2020). The SFC of CB and a mixture of CBS in CB at various concentrations can be seen in Figure 5.

Based on Figure 5, it can be seen that all concentrations of addition of CBS into CB have high SFC at a temperature of 0-10°C, then experienced a drastic decrease at a temperature of 15-35°C, and had an SFC of 0% at 40°C. The drastic decrease at 15-35°C occurred because most of the TAG melted rapidly in this temperature range (Biswas et al., 2017).

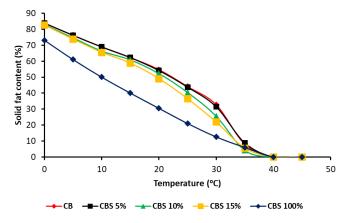


Figure 5. The SFC of CB and the mixture of CBS in CB at various concentrations.

Based on the SFC profile, it can be seen that the compatibility of CBS to CB was only 5%. The use of CBS by more than 5% resulted in a decrease in SFC, which began to be seen at temperatures above 20°C. This change in SFC was in line with the melting profile shown in Figure 4, which shows the melting peak shifted to the left. Therefore, the use of CBS rich in monolaurin needs to be limited to only 5% to maintain the solid fat content and melting profile that remains the same as CB.

### Conclusion 4.

The differences ratio of CNO-PS in glycerolysis of a mixture of CNO and PS affected the fatty acid composition, monolaurin content, melting profiles, and SFC of CBS. CBS with a CNO-PS ratio of 6:4 had a high monolaurin content (28.74%) from the obtained MAG fraction and melting profile which was comparable to CB. However, it had SFC that was lower than CB. Based on the melting profile and solid fat

content, the proportion of CBS rich in monolaurin had compatibility with CB up to 5%, so the use of CBS should be limited to maintain the same melting profile as CB.

### **Conflict of interest**

The authors declared no conflict of interest.

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