



Physical, rheological and sensorial properties, and bloom formation of dark chocolate made with cocoa butter substitute (CBS)



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ABSTRACT

This study examined the physical properties of enzymatically produced palm oil-based cocoa butter substitute (CBS) in dark chocolate. Melting profile, particle size distribution (PSD), rheological, textural behaviors, bloom formation and polymorphism were analysed using differential scanning calorimetry (DSC), master-sizer/polarized light microscopy (PLM), rheometer, stereomicroscope and x-ray diffraction (XRD), respectively. Dark chocolates were produced with cocoa butter (CB, without CBS), 5 g CBS (formulation-1) and 20 g CBS/100 g blend (formulation-2). Both chocolates with addition of CBS showed maximum melting temperature similar to CB-chocolate. However, the peak area and melting enthalpy for formulation-2 were significantly ($P < 0.05$) different from CB-chocolate. Significant differences ($P < 0.05$) in PSD, flow behavior, hardness and sensory characteristics were observed for formulation-2 whilst no significant difference ($P \geq 0.05$) was observed for formulation-1. Stereomicroscope images of all the chocolate samples did not show bloom at 24 °C for up to 8 weeks. Conversely, at 29 ± 1 °C, bloom formation was only observed for CB-chocolate and formulation-1 after two weeks of storage. Noticeable changes in XRD peaks were observed for bloomed chocolate. Overall, chocolate with formulation-1 was similar to CB-chocolate in terms of physical and sensory properties. However, chocolate with formulation-2 exhibited significantly lower sensory profiles particularly taste acceptance and hardness compared to CB-chocolate.

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1. Introduction

Enzymatic interesterification of fats and oils for the formulation of cocoa butter substitute (CBS) has been receiving a lot of attention. Triacylglycerol (TAG) composition of fats and oils is modified to change the physical properties particularly melting profile similar to cocoa butter (CB) for using in confectionery applications (Asgar, Pasha, Murtaza, & Ali, 2017; Sridhar, Lakshminarayana, & Kaimal, 1991). Chemical modification in the production of these types of TAGs is generally not applicable because of deficiency in positional specificity (Kadivar, De Clercq, Mokbul, & Dewettinck, 2016; Xu, 2000).

CB, the main ingredient of chocolate, is expensive among all the vegetable fats/oils due to its limited supply and high market demand. Hence, researchers are looking for alternatives to CB. CBS is an alternative to CB and have a similar melting profile to that of

cocoa butter, but with different chemical composition (Biswas, Cheow, Tan, & Siow, 2016; Calliauw et al., 2005; Garti & Widlak, 2015; Talbot, 2006). In the literature, modification of kokum fat, mango fat, sal fat with methyl palmitate-stearate (Sridhar et al., 1991); and palm oil with soybean oil (Abigor et al., 2003) using enzymatic interesterification has been studied in producing CBS. Reports on production of CBS from cheap and available palm oils fraction are not available. In our preliminary study (Biswas, Cheow, Tan, Kanagaratnam, & Siow, 2017), palm mid-fraction was mixed with refined, bleached and deodorized palm kernel oil and palm stearin to produce CBS and the results showed several melting temperatures instead of a single melting peak between 30 and 35 °C as shown by CB. Melting is the main characteristic that is used for the evaluation of an enzymatically modified CB-like fats (Çiftçi, Fadiloğlu, & Gögüş, 2009). Therefore, enzymatic interesterification was used to modify CBS to show similar melting characteristic and triacylglycerol composition as per CB in our earlier phase of this study (unpublished data). Subsequently, 5–50 g of CBS/100 g blend were mixed with CB in order to investigate the compatibility of CBS/CB mixture; in which 5–20 g CBS/100 g blend was found to be

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compatible with CB in terms of solid fat content as a function of temperature and polymorphism.

European Union Directive 2000/36/EC and Food Standards Agency 2003 limit the use of vegetable fats in chocolates to 5 g/100 g blend. The non-EU countries have their own regulations. For example, the United States does not allow the use of CB alternatives in chocolates, but permits its use as coatings on chocolate products. Generally, most countries permit more than 5 g CB alternatives/100 g blend in chocolates, but this is labelled as “compound chocolate” (Beckett, 2011; De Clercq et al., 2016; Kadivar, De Clercq, Van de Walle, & Dewettinck, 2014).

In the present study, dark chocolate is used as model chocolate, which is made up of CB along with sugar, ground cocoa solids and soy lecithin (Asghar et al., 2017; Zarringhalami, Sahari, Barzegar, & Hamidi-Esfehani, 2010). However, milk chocolate has not been considered in this study to avoid any overlapping melting or other interaction between CBS and milk fat (i.e., milk fat may interfere on the physical properties of CBS). The important ingredient of CB and its crystal structure is responsible for the rheological properties, appropriate texture and sensory perception of chocolate (Aidoo, Afoakwa, & Dewettinck, 2015; Loisel, Lecq, Keller, & Ollivon, 1998; Timms, 2003). The rheological properties of the chocolate depend on many factors such as temperature, composition and processing conditions. During dark chocolate processing, composition and crystallization of CB play an important role in obtaining good quality product. The chocolate composition determines different interactions that occur between ingredients whilst crystallization is the most important step in chocolate processing i.e. refining, conching and tempering (Glicerina, Balestra, Dalla Rosa, & Romani, 2013; Servais, Ranc, & Roberts, 2003) to ensure the desired sensory characteristics.

The melting characteristic of dark chocolate is very important in order to evaluate the effects of CB polymorphism. CB has complex polymorphs namely γ , α , β' and β (or Roman numbering, I–VI) in ascending stability (Marangoni & McGauley, 2003; Rousseau & Smith, 2008). Polymorphic transitions of CB take place via either a solid-state transition or by melt-mediation. During the manufacturing process, tempering is usually conducted at set temperature and time regime to get the desirable $\beta(V)$ crystal form with melting temperature of 32–34 °C which is preferred in chocolate to impart the desired good snap, glossy appearance and sensory mouth-feel (Talbot, 2009). However, poorly tempered chocolate can develop a sticky greyish-white surface namely fat bloom upon storage. Bloom formation may also arise due to slight (i.e., ± 2 – 3 °C) or larger temperature variations which cause the melting and re-crystallisation of TAG in CB, where the liquid fat from the chocolate matrix migrates through pores and micro-fractures to the surface forming bloom (Rousseau & Smith, 2008).

Since the melting, rheological, textural and polymorphism are the main properties used for the quality assurance of chocolate, it is important to understand how the enzymatically produced palm oil-based CBS influences the physical properties of CB. Therefore, the objective of the present work was to investigate the melting, rheological, textural properties, bloom formation and sensory profile of dark chocolate made with enzymatically produced CBS.

2. Materials and methods

2.1. Materials

Cocoa powder (10–12 g fat/100 g, pH 6.8–7.2, moisture 5 g/100 g, Guan Chong Cocoa Sdn. Bhd), icing sugar (MSM Prai Sdn. Bhd.), Cocoa butter (Le Bourne Sdn. Bhd.), Soy lecithin (moisture 0.19 g/100 g, Cargill, Shanghai, China) were employed. Palm mid-fraction, refined bleached deodorized palm kernel oil and palm

stearin were obtained from Sime-Darby Research Sdn. Bhd. In our preliminary study (Biswas et al., 2017), the major palmitic, oleic acids and POP composition of the mixture of palm mid-fraction/refined bleached deodorized palm kernel oil/palm stearin closely approximated those of CB, although its melting profile was different from CB. Subsequently, CBS with desirable melting profile and comparable fatty acids/TAGs composition as per CB was produced from palm mid-fraction/refined bleached deodorized palm kernel oil/palm stearin mixture added with commercial stearic-oleic acid through enzymatic interesterification (unpublished data) and was used in the current study. TAG standards were obtained from Sigma Aldrich (St. Louis, MO). All other reagents and solvents were of analytical or HPLC grade.

2.2. Chocolate production

Production of standard dark chocolates was performed using the method described by Kadivar et al (Kadivar et al., 2016), with minor modification at Cocoa processing lab, Malaysian Cocoa Board, Nilai. Formulation of standard dark chocolate was according to the following composition (w/w): 48 g icing sugar/100 g blend, 30.90 g CB/100 g blend, 20.5 g cocoa powder/100 g blend and 0.6 g soy lecithin/100 g blend. For two different chocolate formulations, CBS was added at the levels of 5 g and 20 g in 100 g CB (1.5 g and 6.2 g of CBS were replaced from 30.90 g of CB). Experimental samples (1 kg batch for each formulation) were prepared by mixing sugar, cocoa powder and 2/3 of melted CBS + CB fat in a mortar and pestle mill (Pascal, UK) at low speed for 10 min at 45 °C. Then the mixture was refined using a 3-roll refiner (Pascal, UK) at ambient temperature to get a particle size <35 μ m. After refining, the mixture was transferred to the conche (Mortar and Pestle mill, Pascal, UK) and then mixed with the rest of the melted CBS + CB fat at 45 °C for 4 h. In the following phase, the mixture became a paste as the viscosity reduced. To obtain the desired flow characteristics, soy lecithin was added and mixed for another 2 h.

In the next step, the liquid chocolate was tempered manually according to the method described by Talbot (Talbot, 1994). Tempering was performed to produce the desired β crystals in the chocolate products. Tempering was carried out as follows: i) chocolate was melted at 45 °C to remove crystal history, ii) approximately 2/3 of the chocolate mixture was poured onto a marble slab and mixed with a flexible spatula until the product reached 27 °C to produce seed crystals and iii) the thickened chocolate was then mixed with the remaining 1/3 warm chocolate (40 °C) to get the overall temperature of 31–32 °C, in order to melt the unstable crystal polymorphs (Bricknell & Hartel, 1998; Briggs & Wang, 2004; Talbot, 1994). Temperature was measured using an Aasted-Mikroverk Chocometer (Aasted-Mikroverk ApS, Farum, Denmark) to ensure accurate tempering temperature for chocolate. The tempered chocolate was poured into plastic chocolate molds (dimensions bar, 98 mm \times 30 mm \times 11 mm) and cooled at 13 ± 1 °C for 60 min to solidify the chocolate (De Clercq et al., 2012). The chocolate bars were de-moulded and subsequently stored at room temperature.

2.3. Triacylglycerol (TAG) analysis

The TAG profiles of individual CB and enzymatically produced CBS were analysed using high performance liquid chromatography (Agilent HPLC series 1260, Santa Clara, USA) equipped with Column ZORBAX C-18 (4.6 \times 250 mm, 5 μ m, Agilent Technologies, Santa Clara, USA) according to AOCS Official Method Ce 5b-89 as discussed in our previous study (Biswas et al., 2016).

2.4. Melting profile

Melting profile of the dark chocolates was determined using differential scanning calorimetry (DSC Pyris 4000 DSC, Perkin-Elmer Ltd., Norwalk, USA) equipped with nitrogen gas flow rate of 20 ml/min, following the method of Kadivar et al. (2016). Surface of the chocolate was scraped off with a scalpel and 2–4 mg of the chocolate slivers was hermetically sealed in an aluminum pan. An empty, covered aluminum pan was used as the reference. When the system reached the equilibrium conditions at 20 °C, the pan was put in the DSC cell and the melting thermograms were recorded by heating from 20 to 65 °C at 5 °C/min. Onset temperature (T_{onset}), endset temperature (T_{end}), maximum peak temperature (T_{max}), melting enthalpy (ΔH) and peak area were calculated from the melting thermogram using DSC software. T_{onset} is the temperature at which the corresponding crystal form starts to melt; T_{end} represents the temperature at which liquefaction of the sample is completed; ΔH_{melt} is the amount of energy required for complete melting of the sample; T_{max} is the temperature at which maximum melting occurs; and peak area is equivalent to the heat taken up by the sample during melting (Afoakwa, Paterson, Fowler, & Vieira, 2008a; De Clercq et al., 2014).

2.5. Polarised light microscopy (PLM)

To measure the micrographs in terms of fat particle size of the chocolates, a polarized light microscope (PLM, Olympus BX51, Tokyo, Japan) fitted with a digital camera (Nikon, DS-Filc, Tokyo, Japan) was used at room temperature. Approximately 10 μg of the melted chocolate was then placed on a glass microscope slide. A coverslip was placed on top of the sample and centred on the melted sample to ensure uniform thickness. PLM images were captured using 20 \times objective lens after the slides were kept at room temperature for 4 h to solidify the particles.

2.6. Particle size distribution (PSD)

To measure the PSD of the chocolates, a MasterSizer 3000 (Laser Diffraction Particle Size Analyser, Malvern Instruments Ltd., Malvern, Worcestershire, UK) equipped with a Hydro EV was used. Approximately 0.5 g of chocolate slivers was mixed with 10 ml of isopropanol. The sample (~0.2 ml) was dispersed in isopropanol until an obscuration of 10.5% as recommended by the instrument software. The sample was sonicated for 2 min to ensure particles were independently dispersed and thereafter maintained by stirring during the measurement. PSD was determined based on the Mie-Theory using the refractive index 1.59 for dark chocolate (Afoakwa, Paterson, Fowler, & Vieira, 2008b). Results were provided as a relative volume (%) of particles in size compared with particle size curves (Malvern MasterSizer Micro Software). PSD parameter was obtained at the largest particle size D_{90} (>90% finer) (Beckett, 2008).

2.7. Flow behavior

In order to examine flow behavior of the chocolates, a Rheometer RS600 (HAAKE RheoStress 600, Thermo Electron Corp., Karlsruhe, Germany) fitted with a plate-plate geometry was used. Chocolate samples were melted at 50 °C for 1 h and approximately 2 g of the samples was placed onto a preheated plate in a gap of 1 mm. The measurement procedure was based on the method of ICA. (2000) and Afoakwa, Paterson, and Fowler (2008) with minor modifications. Temperature of the bottom plate was set at 40 °C to prevent solidification of the fat crystals. A stepped flow procedure was applied by increasing the shear rate logarithmically from 2 s^{-1}

to 110 s^{-1} . Yield stress (Pa) and viscosity (Pa s) were measured at 65 s^{-1} (Kadivar et al., 2016).

2.8. Texture analysis

Hardness (N, the maximum force required to penetrate the sample) of the chocolates was measured with a Texture Analyser (TA-XT plus, Stable Microsystems Ltd., Surrey, UK) equipped with 2 kg load cell and probe (P/2N needle stainless) using the following parameters: product height 10 mm, penetration depth 5 mm, pre-speed 1 mm/s, test speed 2 mm/s, post speed 10 mm/s and the duration of the test at ~1–2 min (Kadivar et al., 2016).

2.9. Bloom formation on chocolate surface

Bloom formation on chocolates, stored at 24 ± 1 °C and 29 ± 1 °C, was captured and examined every two weeks for a total of 3 months using a stereomicroscope (Nikon, SMZ1500, Tokyo, Japan) fitted with a digital Nikon camera (Nikon, Digital Sight DS-2Mv, Tokyo, Japan), according to previously described method (Kinta & Hartel, 2010).

2.10. Polymorphism

To identify the polymorphic transformations of chocolate, a D8 Discover X-ray Diffraction (Bruker, Karlsruhe, Germany) fitted with Cu-K α radiation ($k = 1.5418$ Å, voltage 40 kV and current 40 mA) was used at room temperature. Surface of the chocolates was chopped with a scalpel. To eliminate the interference of sugar crystals, approximately 5 g of the chocolate slivers were mixed in 500 ml of cold water, shaken and allowed to stand at room temperature for 4 h to dissolve the sugar, according to the method of Cebula and Ziegleder (1993). The suspension was filtered through a Buchner funnel with whatman filter paper (0.45 μm) under a vacuum pump to dry the samples. The sugar free chocolate samples were mounted onto the XRD sample holder. The samples were analysed at 2θ angles of 10° – 30° with a scan rate of $1.5^\circ/\text{min}$. Short (d) spacing (Å) was determined using the EVA-diffraction software (Bruker, Karlsruhe, Germany). Assignments of polymorphs were based on the following short spacing characteristics of CB: α form ($d = 4.15$ Å); β' forms ($d = 3.8$ – 4.3 Å) and β forms ($d = 4.5/4.6$ Å) (D'Souza, 1990). Unbloomed chocolate was used as a control.

2.11. Sensory evaluation

Sensory evaluation was performed using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) where participants evaluated the sensory attributes i.e. glossiness, hardness, waxiness, greasy to touch (sticky), mouth-feel (smooth, melting), overall acceptability and taste acceptance of the dark chocolates. One hundred participants (students and staffs) from Monash University Malaysia were selected. Participants were asked to pick one sample which is different from the other two in a triangle test. Three formulations of dark chocolate (each weighing ~3 g) were served at 23 ± 1 °C in sealed plastic bags. Samples were coded using three digit random numbers. Participants were asked to take a small bite of biscuit and then drink water to rinse their palate after tasting each chocolate.

2.12. Statistical analysis

Data were statistically analysed by t -test and one-way analysis of variance using the SPSS software, version-20 (IBM Corp., Chicago, USA). Tukey's test was applied to determine the significant differences at $P < 0.05$ level. DSC, PSD, Rheometer, XRD, TA and PLM

analyses were conducted in triplicate.

3. Results and discussion

3.1. TAG composition

The TAG profiles of individual CB and enzymatically produced CBS are presented in Table 1. CB contained three main TAGs: POSt (39.2 g/100 g), StOSt (29.7 g/100 g) and POP (18.1 g/100 g), which is in agreement with previous studies (Biswas et al., 2016; Bootello, Hartel, Garcés, Martínez-Force, & Salas, 2012). As shown in Table 1, enzymatically produced CBS had comparable POP, while significantly ($P < 0.05$) lower composition of POSt and StOSt to CB. In addition, CBS contained additional tri-saturated TAGs (PPP, LaLaLa, PPSt and LaLaM) and di-unsaturated TAG (PLO) which appear only in traceable quantity in CB.

3.2. Melting behavior

Melting properties of different chocolate samples were shown in Fig. 1 and Table 2. Thermal parameters: T_{onset} , T_{end} , ΔH_{melt} , T_{max} and peak area of the melting endotherms of dark chocolate samples were compared.

In the present study, chocolate formulated with CB showed a sharp melting peak near 33 °C with T_{onset} (27.5 °C), T_{end} (35.5 °C), area (64.2 mJ) and ΔH_{melt} (34.1 J/g). These findings were consistent with the literature (De Clercq et al., 2016). The melting peak and T_{end} of the chocolate with 5 g CBS/100 g blend and 20 g CBS/100 g blend were similar to CB-chocolate (Fig. 1, Table 2). However, chocolate with 20 g CBS/100 g blend had significantly ($P < 0.05$) higher ΔH_{melt} and broader peak area with a small shoulder peak compared to the CB-chocolate. This is probably due to the lower melting TAGs specially LaLaLa and PPP (Table 1). Similar results were also reported in a previous research (Kadivar et al., 2016), where chocolate with 25 g CB-like fat/100 g blend showed a broader melting peak and lower T_{onset} . According to De Clercq et al. (2014), the melting profile of dark chocolate should have a narrow

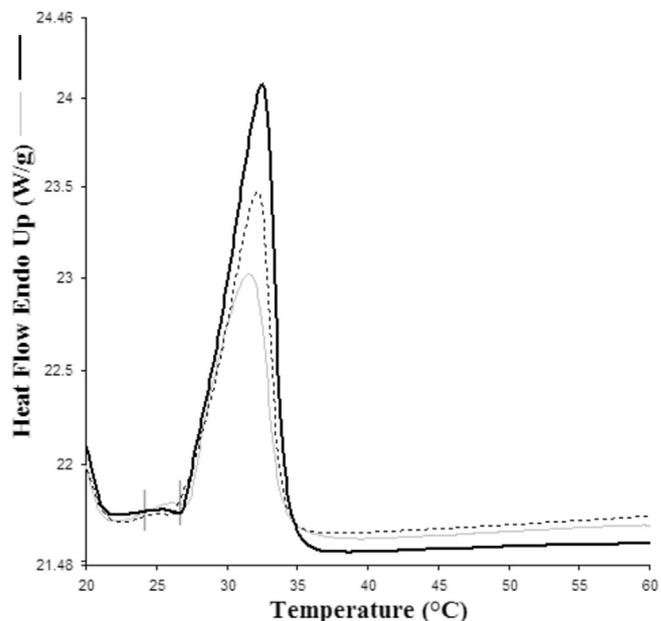


Fig. 1. DSC Melting thermograms of — CB-chocolate, 5 g CBS/100 g chocolate blend and — 20 g CBS/100 g chocolate blend.

melting peak leading to a quick melt down at 37 °C (body temperature), producing a cool sensation and smooth mouth-feel. In the current study, there were no significant ($P \geq 0.05$) differences in ΔH_{melt} and peak area between 5 g CBS/100 g chocolate blend and CB-chocolate (Table 2), indicating 5 g CBS/100 g chocolate blend is comparable to CB-chocolate.

3.3. Particle size distribution (PSD) of the dark chocolates

Polarised light microscopy (PLM) was used to observe the

Table 1
Overview of TAGs composition of individual CB and enzymatically produced CBS.

| TAG (area %) ^a | | CB | CBS |
|---------------------------|--------|-------------|-------------|
| SSS | PPP | 0.1 ± 0.0a | 8.4 ± 0.2b |
| | PPSt | 0.5 ± 0.0a | 2.4 ± 0.4b |
| | StPSt | — | 1.2 ± 0.2 |
| | LaLaLa | — | 4.7 ± 0.3 |
| | LaLaM | — | 2.3 ± 0.1 |
| | MMM | — | 1.1 ± 0.2 |
| | Total | 0.6 | 20.1 |
| | SUS | POP | 18.1 ± 0.8a |
| POSt | | 39.2 ± 1.2a | 28.4 ± 1.2b |
| StOSt | | 29.7 ± 0.7a | 19.5 ± 0.8b |
| LaOLa | | — | 0.7 ± 0.2 |
| AOSt | | 1.9 ± 0.5a | 0.6 ± 0.1b |
| PLP | | 1.1 ± 0.0a | 1.4 ± 0.3a |
| MLP | | 0.6 ± 0.0a | 1.4 ± 0.2b |
| Total | | 90.6 | 69.7 |
| SUU | PLL | 0.5 ± 0.0a | 0.8 ± 0.0a |
| | PLO | 0.6 ± 0.2b | 6.1 ± 0.7a |
| | POO | 2.8 ± 0.4a | 1.3 ± 0.2b |
| | StOO | 2.4 ± 0.1a | 1.3 ± 0.0b |
| | Total | 6.3 | 9.5 |
| UUU | LLO | 0.4 ± 0.0a | 0.1 ± 0.0a |
| | OOL | 0.7 ± 0.1a | 0.2 ± 0.0a |
| | OOO | 1.4 ± 0.2a | 0.4 ± 0.0b |
| | Total | 2.5 | 0.7 |

Values within the same row with different letters are significantly different ($P < 0.05$). Each value in the table represents the mean ± SD of two measurements.
^a Abbreviations: Triacylglycerol (TAG), cocoa butter (CB), cocoa butter substitute (CBS), total content of tri-saturated (SSS), total content of mono-unsaturated (SUS), total content of di-unsaturated (SUU), total content of poly-unsaturated (UUU), P palmitic, St stearic, O oleic, La lauric, L linoleic, M myristic, A arachidic.

Table 2
Overview of Melting profile of CB-chocolate, 5 g CBS/100 g chocolate blend and 20 g CBS/100 g chocolate blend.^a

| Melting profile | CB-chocolate | 5 g CBS/100 g chocolate blend | 20 g CBS/100 g chocolate blend |
|--------------------------|--------------|-------------------------------|--------------------------------|
| T _{onset} (°C) | 27.5 ± 0.2a | 27.4 ± 0.6a | 26.6 ± 0.3a |
| T _{endset} (°C) | 35.4 ± 0.2a | 35.5 ± 0.2a | 35.2 ± 0.2a |
| T _{max} (°C) | 33.2 ± 0.6a | 33.2 ± 0.4a | 33.1 ± 0.5a |
| ΔH _{melt} (J/g) | 34.1 ± 0.4a | 33.8 ± 0.6a | 22.7 ± 0.6b |
| Peak area (mj) | 64.2 ± 0.6b | 65.1 ± 0.6b | 69.4 ± 0.6a |

^a Values within the same row with different letters are significantly different ($P < 0.05$). Each value in the table represents the mean ± SD of three measurements.

variations in fat-particle phase, sugar crystalline network and cocoa particle interaction from the dark chocolates as shown in Fig. 2. All three samples showed similar micrographs under PLM (Fig. 2A–C). Sugar and fat crystals exhibited polarization (bright image), whereas cocoa solids (amorphous) did not show polarization, or appeared as dark. This finding was in accordance with the literature (Afoakwa, Paterson, Fowler, & Vieira, 2009b). In addition, non-fat particulates were visible in the chocolates, such as irregularly large-shaped sugar crystals (~25 μm, bright image) and brownish-red small cocoa particles.

In this study, master-sizer was used to confirm the PSD of the chocolate samples. Fig. 3 shows volume density of the selected samples against size distributions using D₉₀ (>90% finer). Chocolate produced with CB showed particle size approximately 18 μm, which is in agreement with the previous research (Afoakwa et al., 2008). Similar PSD was observed for chocolate made with 5 g CBS/100 g blend. However, chocolate made with 20 g CBS/100 g blend had a slightly higher PSD (~24 μm) than the CB-chocolate (Fig. 3). As stated by Beckett, PSD has a direct influence on rheological, textural and sensory characteristics. The largest particles are responsible for mouth-feel notably grittiness, whereas smaller particles improve flow properties and hardness (Beckett, 2011). For good dark chocolate, PSD should be less than 35 μm (Afoakwa et al., 2008). Overall, chocolate made with 5 g CBS/100 g blend and 20 g CBS/100 g blend was within the range of PSD of less than 35 μm.

3.4. Flow behavior

Dark chocolate is a solid suspension of sugar and cocoa powder in cocoa butter, which shows non-Newtonian flow behavior (De Clercq et al., 2016). The Casson model was fitted to the flow curves to study the Casson yield stress and viscosity. Yield stress is the amount of energy required to initiate chocolate flow. Plastic viscosity is related to the energy needed to maintain the flow of a fluid. High viscous chocolates are not desirable due to sticky mouth-feel (De Clercq et al., 2016).

Fig. 4 shows the Casson yield stress and viscosity as a function of shear rate between 2 and 65 s⁻¹ for the chocolate samples. When the shear rate gradually increased, the Casson plastic viscosity correspondingly decreased while yield stress increased. The viscosity and yield stress of the CB-chocolate were found

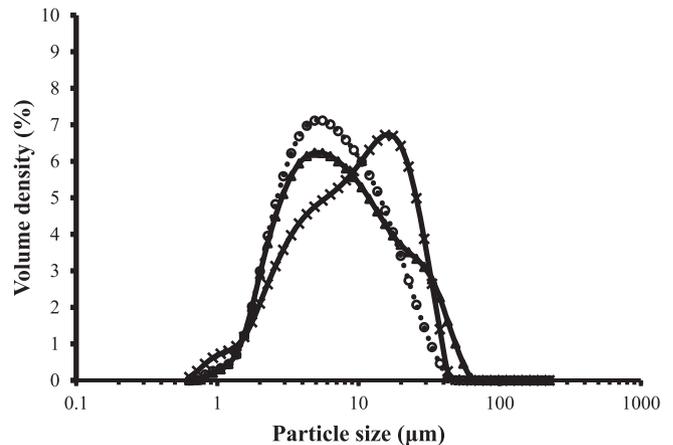


Fig. 3. Particle size distribution of ••••• CB-chocolate, —■— 5 g CBS/100 g chocolate blend and —▲— 20 g CBS/100 g chocolate blend at D₉₀ (>90% finer).

approximately 2 Pa s and 15 Pa, respectively, at shear rate 65 s⁻¹ (Fig. 4A&B). These results were in accordance with a previous study (Kadivar et al., 2016) for dark chocolate. Similar trend in plastic viscosity was also observed for the chocolate with 5 g CBS/100 g blend and 20 g CBS/100 g blend (Fig. 4). Likewise, chocolate with 5 g CBS/100 g blend showed similar yield stress to the CB-chocolate, whereas chocolate with 20 g CBS/100 g blend had slightly lower yield stress (12.2 Pa). This variation was likely due to the function of CB replaced by enzymatically produced CBS and the high PSD (24 μm) (Fig. 3). PSD and rheological parameters are highly linked as the higher PSD denotes lower surface area available to interact, which leads to higher viscosity and/or yield stress (Aidoo, Clercq, Afoakwa, & Dewettinck, 2014).

3.5. Textural behavior

As stated by Afoakwa (2016), a good quality chocolate is a solid product with a good snap at 24 °C (room temperature) and shiny appearance along with easy melting in the mouth, giving a pleasant mouth-feel sensation. Hardness of dark chocolate samples, expressed in maximum force (N), was measured by penetration test

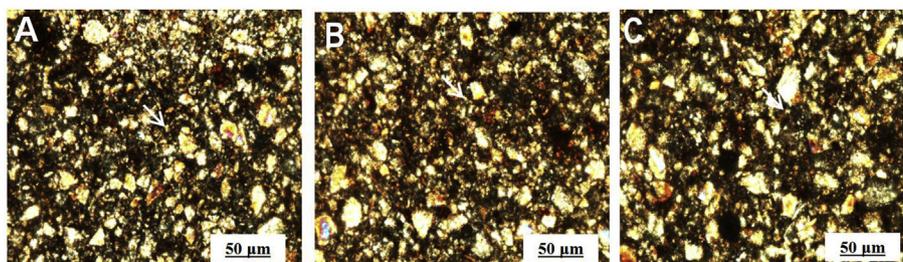


Fig. 2. PLM micrographs (20 × lens) of A) CB-chocolate, B) 5 g CBS/100 g chocolate blend and C) 20 g CBS/100 g chocolate blend at 24 °C.

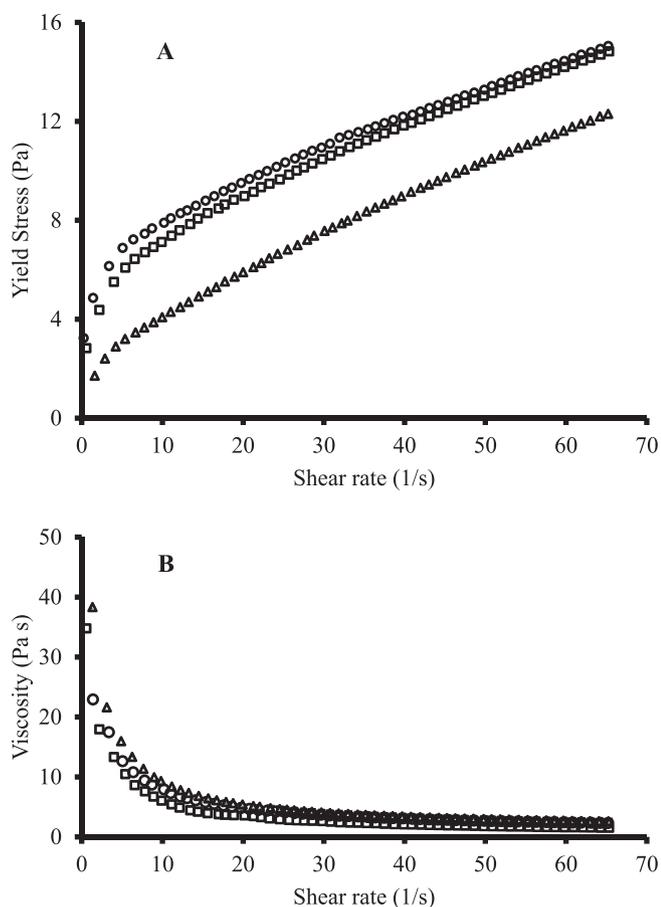


Fig. 4. A) Casson yield stress (Pa) and B) Casson viscosity (Pa s) of □ CB-chocolate, ○ 5 g CBS/100 g chocolate blend and △ 20 g CBS/100 g chocolate blend.

as shown in Fig. 5. Hardness of the CB-chocolate at 24 °C showed approximately 13 N, which is in agreement with previous studies (De Clercq et al., 2016; Kadivar et al., 2016). Similar hardness value was observed for 5 g CBS/100 g chocolate-blend (Fig. 5). However, there was significantly ($P < 0.05$) lower hardness of the chocolate with 20% CBS in comparison to the CB-chocolate and 5 g CBS/100 g chocolate-blend. This may be due to the broader melting

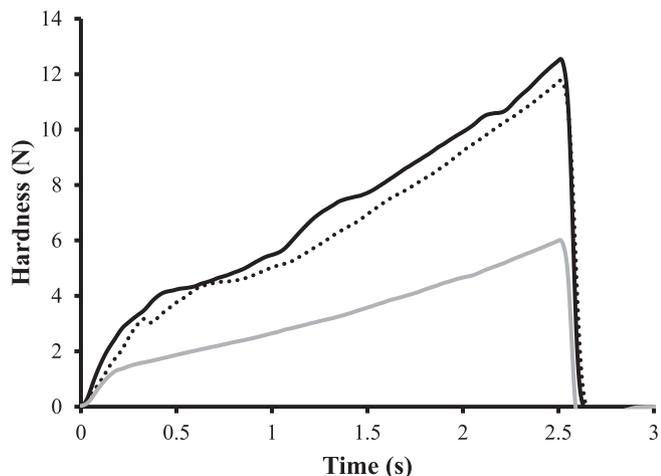


Fig. 5. Hardness of — CB-chocolate, 5 g CBS/100 g chocolate blend and - - - 20 g CBS/100 g chocolate blend at 24 °C.

temperature (Table 2) and high PSD (Fig. 3). The large particle size in the chocolate creates weak interaction between particles, resulting in a lower maximum force required to penetrate the product (Afoakwa, Paterson, Fowler, & Vieira, 2009a). Zarringhalami et al. (2010) also found no significant differences between a reference chocolate and the chocolates with 5 g and 10 g CB-like fat/100 g blend, but the chocolates with 15 g and 20 g CB-like fat/100 g blend had significantly lower hardness. In the current study, 5 g CBS/100 g chocolate-blend had a textural behavior in terms of hardness similar to that of CB-chocolate (Fig. 5).

3.6. Bloom formation and X-ray diffraction during storage

Fat bloom is a physical defect that involves the loss of gloss, smoothness and an undesirable discoloration on the chocolate surface which is the main concern for chocolate manufacturer. Bloom formation is determined by stereomicroscope and confirmed by X-ray diffraction of the chocolate samples during storage at 24 ± 1 °C and 29 ± 1 °C until 12 weeks as shown in Fig. 6.

Chocolate produced with CB, 5 g CBS/100 g blend and 20 g CBS/100 g blend at 24 ± 1 °C and stored up to 8 weeks did not show bloom, however after 8 weeks the CB-chocolate and 5 g CBS/100 g chocolate blend began to form white-greyish haze on the surface. At 29 ± 1 °C, noticeable bloom formation was observed for CB-chocolate and 5 g CBS/100 g chocolate blend after 2 weeks. This phenomenon can be explained by many factors such as fluctuation of temperature during storage time, recrystallization of fats and sugar crystal, and poor tempering (Hartel, 1999; Lonchampt & Hartel, 2006). However, no obvious bloom formation was observed for chocolate formulated with 20 g CBS/100 g blend at 24 ± 1 °C and 29 ± 1 °C until 12 weeks (Fig. 6). This may be likely due to the more complex crystalline structures that inhibit bloom (Lonchampt & Hartel, 2006; Osborn & Akoh, 2002; Zarringhalami et al., 2010). Another possible reason may be the presence of medium and high-melting TAG in CBS (Table 1). Due to fluctuation of temperature, phase behavior of the TAG becomes disrupted, leading to the formation of large surface crystals (Cebula & Ziegleder, 1993; Couzens & Wille, 1997; Hodge & Rousseau, 2002). Beckett stated that the use of high-melting TAG in chocolate allows the melted chocolate to set again with temperature fluctuation. However, the medium-melting TAGs inhibit fat bloom if the storage temperature is higher than the melting temperature of those TAGs (Beckett, 2008). In summary, both chocolates with 5 g and 20 g CBS/100 g blend can be stored at 24 °C to prevent bloom formation.

XRD patterns of bloomed and control chocolates are presented in Fig. 7. Control chocolates showed a major diffraction peak at $d = 4.5$ Å, indicating β polymorphs and multiple peaks at $d = 3.7$ – 4.1 Å, corresponding to the characteristic of β' polymorphs. For bloomed chocolate with CB and 5 g CBS/100 g blend, the peak intensity at $d = 3.7$ and 4.0 Å increased slightly, whereas the 4.5 Å peak remained unchanged. This result was in agreement with the previous research (Sonwai & Rousseau, 2006) for bloomed chocolate, who reported that the peak intensity at $d = 3.6$ and 3.8 Å increased along with a reduced peak at $d = 3.9$ Å. Wang et al. (2010) also found the diffraction peaks at $d = 3.7$, 3.8 , 4.2 and 4.6 Å for bloomed CBS-chocolate while peaks at $d = 3.8$ and 4.2 Å for control CBS-chocolate. Likewise in the present study for the bloomed chocolates, the diffraction peak at $d = 4.1$ Å shifted toward larger d -spacing along with an increased peak height tending to the β polymorphism (Fig. 7A&B). However, the major diffraction peak at $d = 4.5$ Å with two peaks at 3.7 and 3.9 Å were observed for the control chocolate with 20 g CBS/100 g blend, indicating no change in the polymorphism transformation during storage at 24 ± 1 °C and 29 ± 1 °C (Fig. 7C). This finding was also confirmed by stereomicroscope (Fig. 6).

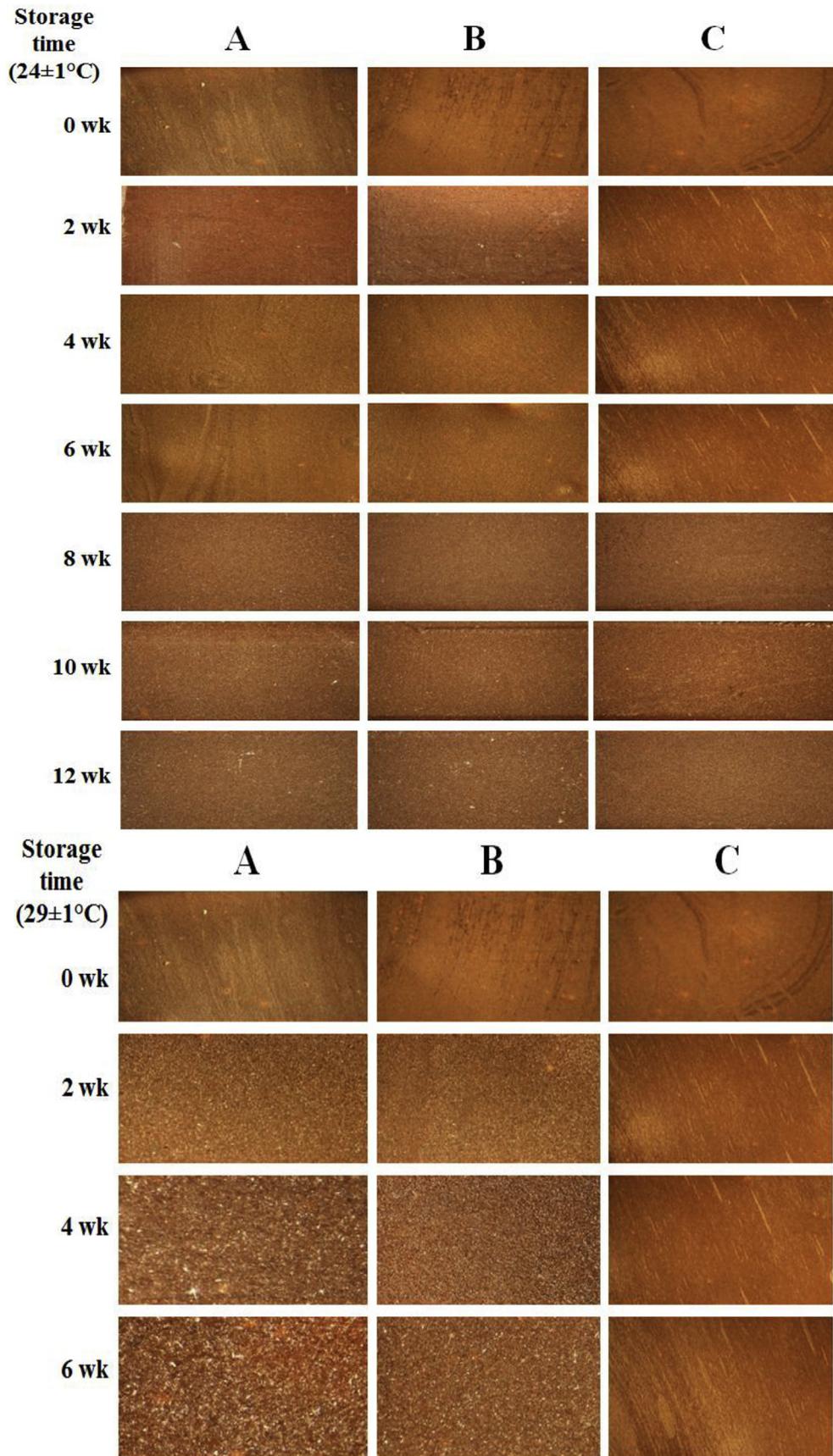


Fig. 6. Bloom formation of A) CB-chocolate, B) 5 g CBS/100 g chocolate blend and C) 20 g CBS/100 g chocolate blend stored at 24 ± 1 °C and 29 ± 1 °C.

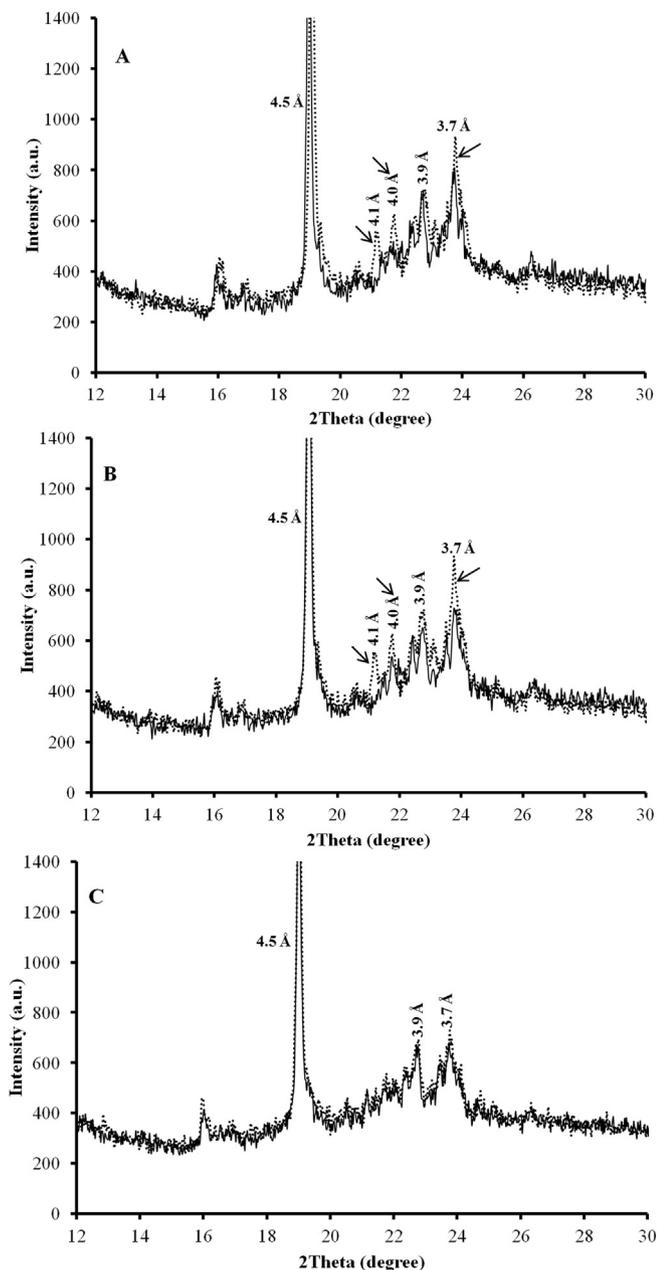


Fig. 7. X-ray diffraction spectrum of **A** — CB-chocolate and bloomed-chocolate, **B** — 5 g CBS/100 g chocolate blend and bloomed-chocolate and **C** — 20 g CBS/100 g chocolate blend and bloomed-chocolate.

3.7. Sensory evaluation

There was no significant ($P \geq 0.05$) difference in sensory characteristics between CB-chocolate and 5 g CBS/100 g chocolate blend (Fig. 8). However, chocolate with 20 g CBS/100 g blend had significantly ($P < 0.05$) different sensory characteristics in terms of taste acceptance, overall acceptability and hardness compared to the CB-chocolate. In triangle test, 52 out of 100 panelists were able to identify chocolate with 20 g CBS/100 g blend as different from the other two samples of CB-chocolate and 5 g CBS/100 g chocolate-blend (data not shown). Therefore, it could be concluded that panelists did not find any difference between the CB-chocolate and 5 g CBS/100 g chocolate-blend. De Clercq et al. found that full CB-like fat replacement of CB influenced the sensory characteristics

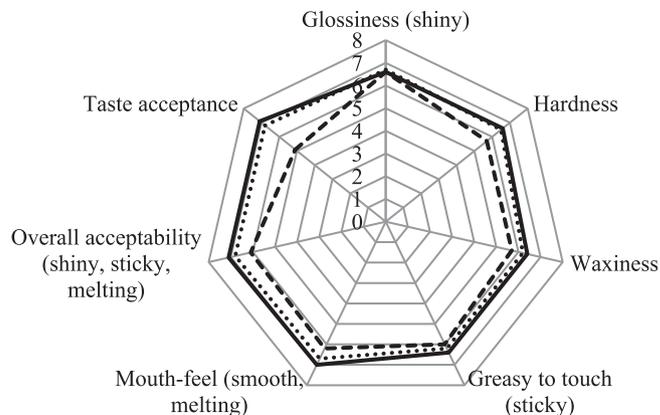


Fig. 8. Sensory evaluation of — CB-chocolate, 5 g CBS/100 g chocolate blend and ----- 20 g CBS/100 g chocolate blend.

of chocolate (De Clercq et al., 2016). In the current study, panelists were not able to distinguish any differences between CB-chocolate and chocolate made with 5 g CBS/100 g blend, thus indicating comparable sensory profile between CB-chocolate and 5 g CBS/100 g chocolate blend.

4. Conclusion

Dark chocolate formulated with CB (without CBS), 5 g CBS and 20 g CBS/100 g blend was characterized using DSC melting, PSD, rheological, bloom formation and sensory properties. There was no significant ($P \geq 0.05$) difference in melting behavior between the CB-chocolate and chocolate with 5 g CBS/100 g blend. Although all the chocolates remained similar melting peak temperature, there were significant ($P < 0.05$) differences in peak area and melting enthalpy between 20 g CBS/100 g chocolate blend and CB-chocolate. Chocolate made with 20 g CBS/100 g blend had significantly higher PSD with lower hardness and yield stress compared to CB-chocolate, but its viscosity was comparable to 5 g CBS/100 g chocolate blend and CB-chocolate. Stereomicroscope images of all the chocolate samples did not show bloom at 24 °C for up to 8 weeks. However, at 29 ± 1 °C, bloom formation was observed for chocolate with 5 g CBS/100 g blend and CB-chocolate after two weeks. Noticeable changes in X-ray diffraction peaks (polymorphism) were observed for the bloomed chocolate. There was no significant difference in sensory characteristics between 5 g CBS/100 g chocolate blend and CB-chocolate. Additionally, chocolate with 20 g CBS/100 g blend was found to be different in terms of taste from the other two samples in a triangle test. The overall characterization suggested that the chocolate with 5 g CBS/100 g blend was comparable to CB-chocolate in terms of physical and sensory characteristics. However, chocolate with 20 g CBS/100 g blend showed significantly lower sensory properties particularly taste acceptance and hardness compared to CB-chocolate. All in all, both chocolates made with 5 g and 20 g CBS/100 g blend need to be stored at 24 °C to prevent bloom formation.

Declaration

The authors have declared no conflicts of interest.

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