Pertanika J. Sci. & Technol. 27 (1): 331 - 342 (2019)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

The Physicochemical Properties of Cocoa Butter Equivalent Produced From Lipase-Catalyzed Palm Oil and Hydrogenated Palm Oil via Physical Fractionation

Siti Maslina Mohamad Alwi[1,2]* and Lai Oi Ming[3]

[1]Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2]FGV Innovation Centre, Bandar Enstek, 71760 Nilai, Negeri Sembilan, Malaysia
[3]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

ABSTRACT

Palm oil (PO) and fully hydrogenated palm oil (FHPO) were subjected to enzymatic interesterification using 9.5% of TLIM Lipozyme. The optimum condition for this process occurred at 62.75°C, with reaction time 172.50 minutes with the ratio of 1:1 for palm oil to hydrogenated palm oil respectively. The Palmitoyl-Oleoyl-Stereoyl (POS) yield obtained was approximately 15%. Product was subsequently subjected to a fractionation process at various cooling temperatures and reaction time. At 34°C, POS achieved was at the highest level which was approximately 31% after 12 hours cooling process. The study of physiochemical properties of the Cocoa butter Equivalent (CBE) fat was determined for the purpose of characterization identification. The properties identified were solid fat content, slip melting point (SMP) and iodine value (IV). The IV and SMP values obtained were 44.30 and 29°C respectively. However, CBE produced almost 0% of Solid Fat Content (SFC) at 30°C. Apart from the high yield of POS, the physicochemical characteristics showed significant compatibility with that of CB. In addition, the crystal polymorph of CBE 34 physicochemical characteristics of CBE34 (β′+β) was similar to CBE. Hence, from this study, CBE 34 is recommended for utilization in the confectionery industry as CBE.

Keyword: Lipase-catalyzed cocoa butter equivalent, fractionation, physicochemical properties

ARTICLE INFO

Article history:
Received: 03 April 2018
Accepted: 27 September 2018
Published: 24 January 2019

E-mail addresses:
nina.alwi@gmail.com (Siti Maslina Mohamad Alwi)
omlai.biotech@gmail.com (Lai Oi Ming)
* Corresponding author

INTRODUCTION

Modification of fats and oil is extensively applied to attain products with appropriate properties for their specific use. Since most natural oils and fats have limited application due to their inherent chemical composition,

ISSN: 0128-7680
e-ISSN: 2231-8526

modification through hydrogenation, interesterification and fractionation processes is required. Modifications were implemented generally to increase oxidative stability, enhance textural characteristics and to alter fatty acid composition (Rasor & Duncan, 2014). These modification processes can change the physiochemical properties of oils and fats by reducing the degree of unsaturation of the acyl groups, either by hydrogenation or interesterification; which the fatty acids chains are redistributed. Another process that can also affect the physicochemical properties of oils and fats is physical separation of the triacylglycerols through selective crystallization and filtration (Hassim & Dian, 2017).

Fractionation process enables industry to obtain several fractions of different melting points, hardness and solid fat content from solid or semisolid oils and fats, making it possible to extend the application of edible oils and fats in the related food products. Palm oil is rich in Palmitoyl-Oleoyl-Palmitoyl (POP), which is a very suitable source of CBE especially after concentrating it by modifying the fat. Since palm oil is undoubtedly the most versatile oil to be used in food products, fractions of distinct different physical characteristics can be produced, by applying multiple stage dry fractionations (Desmet Ballestra, 2015).

Palm oil can be applied in many diverse areas, both edible and non-edible usages by various modification processes such as blending, hydrogenation, fractionation crystallisation and interesterification. Fractionation process plays a big part in cocoa butter industry. Fractionation is used to isolate desirable triacylglycerols. Palmitoyl-Oleoyl-Stereoyl (POS), Palmitoyl-Oleoyl-Palmitoyl (POP) and Stereoyl-Oleoyl-Stereoyl (SOS) are important triacylglycerols in cocoa butter that are responsible to give required attributes of a good chocolate (Oracz et al., 2015). However, this research study only focused on POS yield.

Fractionation refers to the mechanical separation of the liquid from the solid of the oils and fats components. Fractionation has two stages. The first stage involves the formation of stable, larger and uniform crystals partial produced under the controlled gradual cooling to the desired temperatures in the crystallizer. Subsequently, filtration method is used to separate the solid and liquid fractions (Normah et al., 2012).

Dry fractionation is qualified as a natural and green technology because it produces less loss, and effluent and use less chemicals (Kellen et al., 2007). This method can be used especially when fats such palm oil is used in the formulation. Palm oil and palm kernel oil comprises a mixture of high and low melting triacylglycerols making it easier to separate by a simple dry fractionation process (Norizzah et al., 2014). This process consists of crystallization stage where solid crystals are produced in a liquid matrix where liquid is separated from the crystals at the separation stage. This process separated palm oil into two parts, namely liquid (olein) and numerous grades of palm stearin (Zaliha et al., 2004). It is commonly used because it is cost effective and produces considerably good yield. It is also known to improve product and nutrition functionality, improve oxidative stability and increase cold stability via winterization process. Organoleptic properties in terms of taste and flavor can be retained as well (Deffense, 2008).

Physicochemical properties of oils and fats in this case include melting point, solid fat content and iodine value. Melting point increases when fatty acids length increases. It is also influenced by the complexity of TAG components, saturation degree and *trans* fatty acids content. Solid fat content is an important indicator of melting and crystallization behaviors. It therefore determines the choice of fats to be used to achieve specific product functionality. Iodine value (IV) measures the degree of unsaturation of fat and a predictor of its oxidative stability. Physiochemical properties are vital in defining the fats produced according to specification of desired functionality (Rasor & Ducan, 2014). Fats possess the ability to crystallize in multiple forms and this influenced the consistency of the fats. The α form is the least stable and tends to impart a waxy texture. β'crystals are fine needle-like form which is less stable, but gives a smooth structure. While, β crystals are large, have grainy texture. It is the most stable crystal form. So, crystallization in β form is preferred for chocolate products due to desired gloss and snapping attributes (Rasor & Ducan, 2014). CBE and CB have the same crystals form, therefore, both need to be tempered (Lawler & Dimick, 2008). The physicochemical characteristics of CBE are close to CB in terms of crystallization, texture and melting properties due to the similar Triacylglycerol (TAG) composition which makes them compatible when mixed partially or replaced completely (Gunstone, 2011).

POS is the highest TAG percentage in CB. Many studies have concentrated on producing CBE with high SOS and POP. Thus, this study focused on producing high POS CBE instead. In this study, the enzymatically interesterified fat was subjected to dry fractionation process at various cooling temperatures and reaction time to see the effects of these parameters on the properties and yield of the solid portions of CBE fat. Based on the results obtained, cooling temperature can be defined so that optimum POS yield can be achieved.

MATERIALS AND METHOD

Materials

CBE fat was produced from refined palm oil (RBDPO) provided by Sime Darby Jomalina Food Industries Sdn. Bhd. (Telok Panglima Garang, Selangor, Malaysia) and fully hydrogenated palm oil (FHPO) from Unimills, Netherlands, via enzymatic intesterification using TLIM (Novozyme, Denmark). All chemicals used were of analytical grade except for GC and HPLC purposes, the solvents used were HPLC grade.

Methods

Fractional Crystallization. The interesterified fats (500 g) were subjected to dry fractionation using a jacketed vessel reactor. The oil was first heated in the reactor for 20 min at 70°C with stirring at 100 rpm to eliminate all crystals. The oil was then agitated and cooled at

controlled condition to the desired end-temperature. The oil was held in the crystallizer for stabilization followed by separation of the semi-slurry into olein and stearin using hydraulic filter press. The slurry was first fed into the filter press with a minimum pressure 2.0 bar/min. The filling period was 10 min with a maximum pressure 6.0 bar/min. The olein and stearin fractions were weighed and analyzed, respectively.

Analysis of Triacylglycerol (TAG) Species. This method is determined by AOCS Official Method Ce 5b-89 for the separation and quantitative determination of the triacylglycerols in oil samples in terms of their molecular weight and degree of unsaturation as a function of their equivalent carbon number (ECN) which is sometimes referred to as partition number, using high-performance liquid chromatography. The triacylglycerol profile was analyzed using Waters High Performance Liquid Chromatography (HPLC) e2695 (Milford Massachusetts, USA) separation module with Evaporative Light Scattering Detector (ELSD). The mobile phases used are Acetonitrile and Acetone (40:60). Column of stainless steel tube 250 mm in length and 4.6mm i.d. packed with 5-µm diameter particles for silica Supercosil LC-18 (Sigma-Aldrich, USA) is used and the temperature is at 40.0°C. Gas pressure (O_2FN) fixed at 25.0 (psi). Pump mode was Isocratic and flow rate set at 1.0ml. Injections of samples are 5 µl. Standards used for determination of triacylglycerol species were bought from Sigma Aldrich Inc (Sigma Chemical Co., USA). Triglyceride peaks were identified and recorded.

Solid Fat Content (SFC). SFC was measured according to Malaysian Palm Oil Board (MPOB) Test Method p4.8 (2004) using pulsed nuclear magnetic resonance (NMR) spectrometry (Bruker NMS 120 minispec). The SFC of PDAG fat, olein and stearin fractions was measured at each separation temperature. The sample in the NMR tube was first melted at 70°C for 30 minutes, followed by chilling at 0°C for 90 minutes prior to measurement. Melting, chilling and holding of sample were carried out in pre-equilibrated thermostat water bath. The SFC temperature was set to 10, 20, 25, 30, 35, and 40°C. The percentage of SFC was based on three measurements.

Iodine Value (IV). Approximately, 0.130 g of all the blended oil was weighed into 500 ml conical flask with glass stopper. A blank flask which contains no oil was prepared. About 15 ml of cyclohexane and acetic acid solution were mixed in a 1:1 ratio, and then added into the sample flask and blank flask. Then, 25 ml of Wij's solution was added to both flasks and they were closed with glass stopper and properly shaken. The flasks were left in the dark for 1 hour. After that, 20 ml of potassium iodide and 150 ml of distilled water were added to release the iodine from non-reacted iodine monochloride. Finally, the mixtures were all titrated with sodium thiosulphate solution until yellow colour nearly disappeared before

1-2 ml of starch solution was added as indicator and titration continued. This process ended when blue color of starch solution totally disappeared (AOCS method Cd 1d-92, 1993b).

Slip Melting Point (SMP). SMP was measured according to AOCS Method Cc.3.25 (1993). Capillary tubes were filled with a 1 cm high column of melted fat. The capillary tubes were then rolled against a piece of ice before being chilled in a refrigerator at 10°C for 16 h to solidify the fat. The tubes were subsequently attached with a rubber band to a thermometer and suspended in a 600-mL beaker of boiled distilled water. The bath temperature was adjusted to 8-10°C below the SMP of the sample, and heat was applied using a heating coil element to increase the bath temperature at a rate of 1 C/min. The temperature at which the fat column rises was reported as the SMP.

XRD Analysis. The polymorphic forms of fat crystals were determined with an FR592 Enraf-Nonius Diffractis X-ray generator (Delft, The Netherlands) and an Enraf-Nonius model FR 552 Guinier camera equipped with a customized single-compartment cell with the temperature controlled by an external-circulating thermostatic bath. The melted sample at 60°C was placed in the cell, which was set at the crystallization temperature. Sample was held isothermally until all the polymorphic phases were fully observed.

RESULTS AND DISCUSSIONS

Triacylglycerols (TAGs)

The initial POS level was obtained at 15% after enzymatic interesterification process whereby the conditions were determined to be optimum at 9.5% TLIM enzyme with the temperature of 62.75°C and reaction time of 172.50 minutes. The ratio of palm oil to hydrogenated palm oil used is 1:1. The interesterified fats were then subjected to fractionation process to further purify the CBE.

Table 1 illustrates the yield of POS produced using different temperatures and retention times. It is observed that the yield of POS from fractionation done at 12 hours showed insignificant difference compared to 36 hours of process. The highest TAGs obtained were CBE 32 and CBE 34, with approximately the same percentage of POS yield, of 31.46±0.20% and 31.23±0.70% respectively. The crystal formation temperature during crystallization increases the melting point, which meant that POS increases when temperature decreases (Rodriguez, 2002). As shown in the result obtained, lower crystallization temperature produced higher POS yield. Since POS is dominant in these CBEs, hence they are compatible with CB. Similarly, Mutia et al., 2015 reported that by interesterifying palm mid fraction with stearic acids, the yield of POS increased from 8.59% to 20.54%. The mixture of palm mid-fraction and fully hydrogenated soybean oil produces CBE that has TAG composition of 37.7% of POS as stated by Soekopitojo et al.,

2009. CBE produced from enzymatic transesterification of palm olein and saturated fatty acid distillate produced as high as 42% of POS level (Zainal-Abideen et al., 2012). Many more recent studies showed that palm oil and its derivatives were good materials to produce CBE. Reducing the temperature, residence time, and substrate molar ratio is necessary to develop an industrially applicable and cost-effective process for producing CBE.

Table 1
Yield percentage of POS produced using different temperature and retention time

Sample	Temp (°C)	Time (hours)	Yield (%) %POS
CBE 32	32	12	31.46±0.20
	32	24	31.18±0.35
	32	36	30.83±0.15
CBE 34	34	12	31.23±0.70
	34	24	30.91±0.21
	34	36	31.90±0.94
CBE 36	36	12	22.96±0.22
	36	24	29.55±0.06
	36	36	29.47±0.40
CBE 38	38	12	27.38±0.09
	38	24	29.03±0.62
	38	36	28.54±0.03
CBE 40	40	12	12.34±0.30
	40	24	15.84±0.21
	40	36	16.27±0.03
CBE 45	45	12	23.29±0.46
	45	24	27.19±0.23
	45	36	21.97±0.25

Solid Fat Content (SFC)

SFC is another method to evaluate softness and snapping of fat. It is generally performed using pulse nuclear magnetic resonance (pulse NMR) (Hitachi High-Tech, 2008). The hardness of the fat is reflected at 25°C of solid fat content. The higher the value of solid fat content at this temperature, the harder the fat is (Quast et al, 2011). SFC between 25 and 30°C indicates heat resistance, whereas SFC at 35°C or more denotes waxiness, to which fat remains without quickly melting in the mouth (Kim et al., 2012). Figure 1 shows the SFC of CB and CBEs produced. At 25°C, CBE 32 shows the lowest percentage of solid fat content which is approximately 11%, demonstrating that this fat melts more easily than the others. CBE 45 has the highest solid fat content which is about 50%, has waxiness attribute when made into chocolate. CBE 34, 36, 38 and CB have comparable values, around 29-33°C, indicating that their hardness are nearly the same. Between 25°C-30°C, sample

CBE 40, 38, 36, 34 and 32 shared the same steepness profile and sharp melting behaviour as CB. Figure 1 indicates that at 35°C, sample CBE32, CBE34 and CBE36 presented SFC values which are very close to zero. This corresponds to another important attribute of chocolate in CB or Cocoa Butter Alternatives should have no solid left at 35°C. However, only CBE 45 is observed to have high solid content even after 35°C, which is not favoured in chocolate industry. This is because, the presence of solid fats at temperature higher than 35°C, known as the "fatty residue", is easily detected during the sensory evaluation (Quast et al., 2011). Furthermore, of late, there is a trend toward producing chocolate which is soft rather than hard and quickly melts in the mouth without leaving an aftertaste. The soft CBE has a low SFC overall in a temperature range of 20°C to 35°C, and thus it can provide soft-texture chocolate, but does not form solid crystals at room temperature, which may cause a blooming phenomenon (Kim et al., 2012).

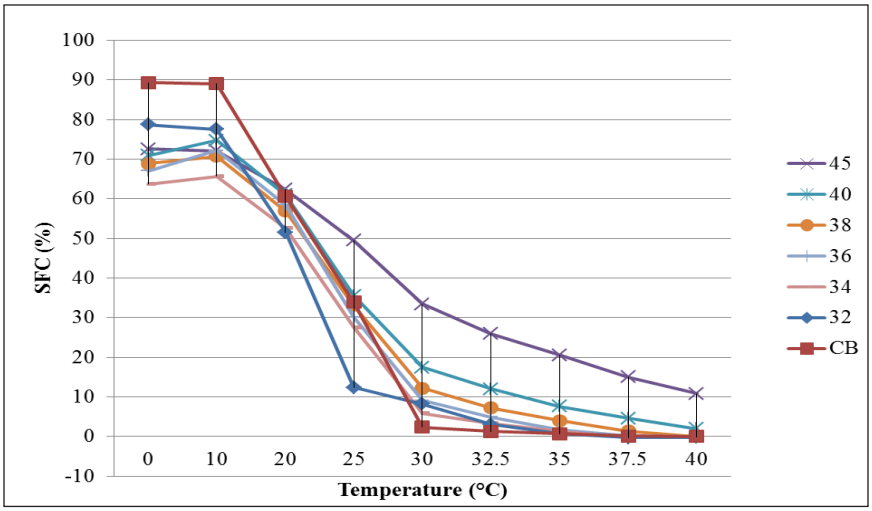

Figure 1. Solid fat content of CB and CBEs after fractional crystallization crystallization

Iodine Value (IV)

One of the important quality measurements of CBE is IV. It is to determine the unsaturation levels in oils and fats. Table 2 shows the IVs of CBE fats produced after fractional crystallization. The higher the unsaturation level (double bond) in the fatty acid chain, the more iodine is being absorbed. Thus the degree of unsaturation in the oil or fat is higher.

The IV values of all samples are higher than CB which is 32.1 indicating that the CBE fats produced are softer. This might be due to the level of oleic in the enzymatically interesterified CBEs, since palm oil is rich in oleic acids. The CBE32 had the lowest level of unsaturation level that was 39.10, while the highest was CBE36 (48.32). CBE from exotic fats such as illepe butter, kokum butter and sal fats IV ranges from 33 to 45. Whereas shea

butter is very soft, which IV is between 52-66 (Gunstone, 2011). IV correlates directly to SMP; when IV of oils or fats is higher, the SMP is lower and vice versa. In other words, the unsaturation levels in oils or fats determine their SMP.

Table 2
The iodine value of CBE32, CBE 34, CBE 36, CBE 38, CBE 40 CBE 45 and CB

CBE	IV
32	39.10 ±0.678
34	43.80 ±0.55
36	48.32 ±2.49
38	42.46 ±0.270
40	42.30 ±0.110
45	41.60 ±3.25
CB	32.10 ±0.14

Slip Melting Point (SMP)

Slip Melting Point (SMP) is where a temperature of fat starts to melt depending on the type of polymorph. This analysis is significant in determining the quality of oils and fats. Stabilization of fat prior to measurement of the slip melting point is extremely important for fats with pronounced polymorphic behaviour, such as cocoa butter (Ranken, 2012). During the changes of fats from solid to liquid phase, the fat crystals network weakens when temperature rises and starts to melt when the SMP of a product is achieved. Table 3 illustrates the SMP of CBEs and CB. It is observed that CBE 45 has the highest SMP, whereas CBE34 has the lowest values, which are 38.96 and 29.05 respectively. The SMP for CBE 32, 34 and 36 are 29.14, 29.05 and 31.30, respectively, which fall in the range as of cocoa butter (29 to 33). The presence of TAG saturated-saturated-saturated (StStSt), saturated-unsaturated-saturated (StUSt), saturated-unsaturated-unsaturated (StUU) and unsaturated-unsaturated-unsaturated (UUU) content in fat and oil affects the value of SMP (Braipson-Danthine & Gibon, 2007). Thus, oleic acids mostly, in the form of POO (palmitoyl-oleoyl-oleoyl) and OOO (oleoyl-oleoyl-oleoyl) present in these samples cause the SMPs to be lower than CB. Consequently, CBE 40 and 45 require higher temperature to melt because they contain higher StUSt and StStSt. Cocoa butter has a sharp melting point which is approximately 31-35 °C and melts completely in the mouth. Its texture is brittle and it fractures readily and does not appear oily (Gunstone, 2011). The SMP of CBEs in Table 4 varies from 28°C to 36°C which are below 37°C except for CBE45. It is essential for CBE to melt in the mouth below body temperature. In many researches done, SMP was recorded approximately from 29.9°C-34.5°C, which consisting of more percentage POS, whereas CBE with higher percentage of SOS triacylglycerols have SMPs at 39°C (Ciftci et al., 2009; Soekopitojo et al., 2009).

Table 3
The SMP of CBE32, CBE 34, CBE 36, CBE 38, CBE 40, CBE 45 and CB

CBE	**SMP (°C)**
32	29.14 ±0.650
34	29.05±0.870
36	31.30 ±1.03
38	35.98 ±2.50
40	36.45 ±0.289
45	38.96 ±0.990
CB	33.01 ±0

Subcell Packing and Polymorphs

CB can exist in different crystal forms, each with different thermodynamic stability. The main three polymorphic forms are α (alpha), β (beta) and β' (beta prime) (Rousseau, 2016). β-crystals have the highest melting point and have a more compact crystal structures than β'-form (Rousseau, 2007). CBE fats exhibit polymorphism similar to that of CB and they CBE should have a similar melting and crystallization behaviour. Thus, the fatty acids and triacylglycerol composition do not affect eutectic formation with CB (Rousseau, 2016). The subcell packing and polymorphs of CBEs (CBE45, CBE40, CBE38, CBE36, CBE34 and CBE32) and CB are shown in Table 4. CBE fats display polymorphism similar to cocoa butter which consist mostly β-form. Hence, it must be tempered as such. Since CBE crystallizes like cocoa butter, therefore, the physical properties of the chocolate like the sharp melting, gloss, shrinkage on solidifying and snap upon breaking are hardly affected (Akoh & Min, 2008). X-ray diffraction, used to identify crystal polymorphs by determining the long and short spacing of crystal (Rousseau, 2016). According to Table 4, only CBE45 has β form, whereas the rest have the same polymorphs as CB. The most preferred polymorph for chocolate products is for β form due to desired gloss and snapping attributes (Rasor & Ducan, 2014). This is because only β crystals contribute to a high melting point (Naik & Kumar, 2014). A study by Solıs-Fuentes and Duran-de-Bazua, 2003, confirmed that β is a stable form of mango seed almond fat as CBE is compatible to CB.

Table 4
Subcell packing and polymorphs of CBE (CBE45, CBE40, CBE38 and CBE36) and CB

CBE	**Subcell packing**	**Polymorphic form**
CBE32	O^ + T//	β′+β
CBE34	O^ + T//	β′+β
CBE36	O^ + T//	β′+β
CBE38	O^ + T//	β+
CBE40	O^ + T//	β′+β
CBE45	O^ + T//	β′
CB	O^ + T//	β′+β

CONCLUSION

Similar physiochemical characteristics of CBE34 fat to that of CB indicate that it could be utilized in confectionery industry as CBE. The CBE produced is slightly softer compared to CB which fulfils the ever-growing trend of softer chocolates that quickly melts in the mouth (Kim et al., 2012). The traits of the CBE produced, having high yield of POS contributed to its high the compatibility with CB. Since according to EU regulations, only 5% of CBE can be used in chocolate formulations, CBE34 can be easily blended with CB. The crystallization profile for CBE34 fits cocoa butter profile (β′+β), suggesting CBE34 the best suited product in this study. Nonetheless, more trials can be done to determine the fractionation yield percentage.

ACKNOWLEDGEMENT

The authors would like to thank Pertanika UPM for the opportunity to publish this paper.

REFERENCE

Akoh, C. C., & Min, D. B. (2008). *Food Lipids: Chemistry, Nutrition and Biotechnology.* New York: CRC Press.

AOCS Official Method Cd 1d-92. (1993b). Iodine Value of Fats and Oils, Cyclohexane-Acetic Method. The American Oil Chemists' Society.

AOCS Official Method Cd 3-25. (1993). Slip Melting point, AOCS Standard open Tube Melting Point. The American Oil Chemists' Society.

Braipson-Danthine, S., & Gibon, V. (2007). Comparative analysis of triacylglycerol composition, melting properties and polymorphic behavior of palm oil and fractions. *European Journal of Lipid Science and Technology, 109*(4), 359-372.

Chiel Jedang Corp. (2012). Fat and Oil Composition for Chocolate and Confectionery. EP 2 508 077 A1. European Patent Application. Munich, Germany: European Patent Office.

Çiftçi, O. N., Fadıloğlu, S., & Göğüş, F. (2009). Conversion of olive pomace oil to cocoa butter-like fat in a packed-bed enzyme reactor. *Bioresource Technology, 100*(1), 324-329. doi: 10.1016/j.biortech.2008.05.035.

Deffense, E. (2008). Dry Fractionation A Booming Technology. Crystallization and Degumming. Retrieved January 15, 2018, from https://webcache.googleusercontent.com/search?q=cache:1l3BM8XjNloJ:https://www.soci.org/-/media/Files/Conference-Downloads/2008/OFI-Abu-Dhabi-Apr-08/Defennse.ashx%3Fla%3Den+&cd=1&hl=en&ct=clnk&gl=my

Desmet Ballestra. (2015). Specialty fats fractionation: new possibilities Statoliser® Technologies. Desmet Ballestra Fractionation with Statoliser® Technology.

Gunstone, F. D. (2011). *Vegetable Oils in Food Technology: Composition, Properties and Uses*. Chichester: John Wiley & Sons.

Hassim, N. A. M., & Dian, N. L. H. M. (2017). Usage of Palm oil, palm kernel oil and their fractions as confectionary fats. Journal of Oil Palm Research, 29(3), 301–310. https://doi.org/10.21894/jopr.2017.2903.01

Hitachi Hi-Tech. (2008). Estimating the Solid Fat Content of Chocolate and Butter Products using DSC to Investigate the Taste of Edible Fats. Hitachi High Tech Corporation. Retrieved January 15, 2018, from https://www.hitachi-hightech.com/file/global/pdf/products/science/appli/ana/thermal/application_TA_085e.pdf

Kellens, M., Gibon, V., Hendrix, M., & De Greyt, W. (2007). Palm oil fractionation. *European Journal of Lipid Science and Technology, 109*(4), 336-349.

Kim, M. J., Lee, S. B., Lee Y. J., Park, S. W., & Lee K. P. (2012). Fat and Oil Composition for Chocolate and Confectionery. European Patent No. EP 2508077A1. Munich, Germany: European Patent Office.

Lawler, P. J., & Dimick, P. S. (2008). Crystallization and Polymorphism of Fats. In C. C. Akoh & D. B. Min (Eds.), Food Lipids: Chemistry, Nutrition, and Biotechnology (pp. 293-318). New York: CRC Press.

MPOB Test Method. (2004). P4.8. Ministry of Plantation Industries and Commodities Malaysia. Kuala Lumpur: Malaysian Palm Oil Board.

Mutia, R., Zaidel, D. N. A., & Muhamad, I. I. (2015). Synthesis of Cocoa Butter Equivalent from Formulated Hard Palm Oil Mid-Fraction and Canola Oil Blends. *Advanced Materials Research, 1113*, 453-458.

Naik, B., & Kumar, V. (2014). Cocoa butter and its alternatives: A review. *Journal of Bioresources Engineering and Technology, 2*(1), 1-11.

Norizzah, A. R., Norsyamimi, M., Zaliha, O., Azimah, N., & Hazirah, S. (2014). Physicochemical properties of palm oil and palm kernel oil blend fractions after interesterification. *International Food Research Journal, 22*(4), 1390–1395. http://www.ifrj.upm.edu.my

Normah, I., Cheow, C. S., & Chong, C. L. (2012). Crystal habit during crystallization of palm oil: Effect of time and temperature. *International Food Research Journal, 20*(1), 417–422.

Oracz, J., Zyzylewicz, D., Budyryn, G., Nebesny. (2015). Cocoa Butter Alternative Fats. *Plant Lipids Science, Technology, Nutritional Value and Benefit to Human Health, 2015*, 87-106.

Quast, L. B., Luccas, V., & Kieckbusch, T. G. (2011). Physical properties of pre-crystallized mixtures of cocoa butter and cupuassu fat. *Grasas Y Aceites, 62*(1), 62-67.

Ranken, M. D. (Ed.). (2012). Food industries manual. New York: Springer Science & Business Media.

Rasor, A. S., & Duncan, S. E. (2014). Fats and Oils – Plant Based. In S. Clark, S. Jung & B. Lamsal (Eds.), *Food Processing: Principles and Applications*, (pp. 457-480). Chichester: John Wiley & Sons.

Rodriguez, A. R. (2002). Literature Review. *American Journal of Medical Quality, 17*(3), 118–123. https://doi.org/10.1177/106286060201700307

Rousseau, D. (2007). The microstructure of chocolate. In D. J. McClements (Ed.), *Understanding and controlling the microstructure of complex foods* (pp. 648-690). Cambridge: Woodhead Publishing Limited.

Rousseau, D. (2016). Microstructural Imaging of Chocolate Confectionery. In N. Sozer (Ed.), *Imaging Technologies and Data Processing for Food Engineers* (pp. 311-333). Heidelberg: Springer.

Soekopitojo, S., Hariyadi, P., Muchtadi, T. R., & Andarwulan, N. (2009). Enzymatic interesterification of palm oil midfraction blends for the production of cocoa butter equivalents. *Asian Journal of Food and Agro-Industry, 2*(4), 807-816.

Solís-Fuentes, J. A., & Durán-de-Bazúa, M. C. (2004). Mango seed uses: thermal behaviour of mango seed almond fat and its mixtures with cocoa butter. *Bioresource Technology, 92*(1), 71-78.

Zainal-Abideen, M., Aris, A., Yusof, F., Abdul-Majid, Z., Selamat, A., & Omar, S. I. (2012). Optimizing the coagulation process in a drinking water treatment plant–comparison between traditional and statistical experimental design jar tests. *Water science and Technology, 65*(3), 496-503.

Zaliha, O., Chong, C. L., Cheow, C. S., Norizzah, A. R., & Kellens, M. J. (2004). Crystallization properties of palm oil by dry fractionation. *Food Chemistry, 86*(2), 245-250.