J Am Oil Chem Soc (2010) 87:1013–1018
DOI 10.1007/s11746-010-1585-y

ORIGINAL PAPER

Performance of a Cocoa Butter-Like Fat Enzymatically Produced from Olive Pomace Oil as a Partial Cocoa Butter Replacer

Ozan Nazım Çiftçi · Fahrettin Göğüş ·
Sibel Fadıloğlu

Received: 3 February 2010 / Revised: 22 March 2010 / Accepted: 30 March 2010 / Published online: 6 May 2010

Abstract Olive pomace oil is a by-product of olive oil processing and it is considered a low-quality oil. Considering its suitable triacylglycerol (TAG) composition, this work aimed to convert refined olive pomace oil (ROPO) to a cocoa butter (CB)-like fat using *sn*-1,3 specific lipase, and to investigate its performance as a partial CB replacer. CB-like fat was produced from olive pomace oil by *sn*-1,3-specific lipase-catalyzed acidolysis in a packed bed reactor. Binary blends of CB and CB-like fat (CB:CB-like fat) were prepared in different proportions, and their physicochemical characteristics [TAG content, melting profile, solid fat content (SFC) and microstructure] were investigated. The contents of 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1(3)-palmitoyl-3(1)stearoyl-2-oleoyl-glycerol (POS) and 1,3-distearoyl-2-oleoyl-glycerol (SOS) in the 100:0 blend were 18.9, 33.1 and 24.7%, respectively. These contents decreased to 11.0, 20.0 and 11.7%, respectively, in the 0:100 blend. Although the melting point (28.5 °C) did not change significantly above 30% CB-like fat addition, the shape of the melting peak became wider and irregular. An isothermal solid diagram of SFC showed that better compatibility was observed at temperatures above 35 °C for all blends. Addition of over 30% CB-like fat caused significant difference in the microstructure.

Keywords Olive pomace oil · Cocoa butter · Cocoa butter-like fat · Enzymatic acidolysis · Binary blend · Thermal properties · Microstructure · Packed bed enzyme reactor

Introduction

Cocoa butter (CB) is the most expensive fat and the premium ingredient of chocolate confectionery products because of its unique physical characteristics. CB has a narrow melting point range (27–33 °C), it is hard and brittle at room temperature, and melts in the mouth completely with a cooling sensation [1, 2]. These distinctive physical properties are the result of its special triacylglycerol (TAG) composition, which consists of three major TAGs, namely, 1,3-dipalmitoyl-2-oleoyl-glycerol (POP), 1(3)-palmitoyl-3(1)stearoyl-2-oleoyl-glycerol (POS), and 1,3-distearoyl-2-oleoyl-glycerol (SOS), with oleic acid at the *sn*-2 position of the glycerol backbone [3]. Numerous factors such as uncertainties in supply and demand, variable quality and high cost compared to other fats have driven an industrial search for alternatives to CB [2].

Conversion of cheap commercial oils to CB-like fat is a good example of the production of value-added products by enzymatic acidolysis. These conversions can be done using *sn*-1,3 specific lipases that catalyze the incorporation of palmitic acid (PA) and stearic acid (SA) at the *sn*-1,3 positions of the source oil containing oleic acid at the *sn*-2 position until a similar TAG composition to CB is obtained [4].

Refined olive pomace oil (ROPO) can be considered a good potential cheap source of oil for CB-like fat production by *sn*-1,3-specific lipase-catalyzed acidolysis because of its high *sn*-2 oleic acid content. Olive pomace oil is a by-product of olive oil processing and considered a low-quality oil because of the long storage periods of pomace and its high-temperature drying applications. Thus, it is generally used for soap-making [5].

Blending oils is a physical way of increasing the thermal stability of oils. It can help to meet the consumer's flavor and

O. N. Çiftçi (✉) · F. Göğüş · S. Fadıloğlu
Department of Food Engineering, Faculty of Engineering,
University of Gaziantep, 27310 Gaziantep, Turkey
e-mail: oznciftci@gmail.com

nutritional expectations in foodstuffs [6], and to decrease production costs. The most important role of CB in confectionery products is to provide the desired physical properties, such as melting, microstructure, and solid fat content. Hence, the fat used to partially replace CB should not change the unique physical characteristics of CB significantly.

The objective of this study was to produce a CB-like fat from ROPO, and to determine the compatibility limit of enzymatically produced CB-like fat addition to CB by investigating the physicochemical characteristics [TAG content, melting properties, solid fat content (SFC) and microstructure] of binary blends of CB and CB-like fat combined at different proportions.

Materials and Methods

Production of CB-Like Fat

CB-like fat was produced from ROPO by performing enzymatic acidolysis reactions on a lab-scale packed-bed reactor using immobilized *sn*-1,3 specific lipase (Lipozyme IM, immobilized from *Mucor miehei*, 140 U/g). Reactions were conducted under optimized reaction conditions: enzyme load 40% (based on weight of pooled substrates), substrate mole ratio of 1:2:6 (ROPO:PA:SA), reaction temperature 45 °C, flow rate 7.5 mL/min, and reaction time 3 h. The TAGs (CB-like fats) produced were then isolated from the reaction mixture. Details of the production of CB-like fat and its isolation from the reaction mixture and the analyses have been reported previously [4].

Blend Preparation

CB and CB-like fat were melted at 80 °C and blended at proportions of 100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 30:70 and 0:100 (wt% CB:wt% CB-like fat).

TAG Analysis

Reaction products and blends were analyzed for the content of target TAGs (POP, POS and SOS) by a reversed-phase HPLC. The HPLC system consisted of a quadratic pump (model LC-10ADVP; Shimadzu, Kyoto, Japan) equipped with a column [Sphereclone 5 μ ODS (2), 250 × 4.6 mm; Phenomenex, Torrance, CA, USA], an accompanying guard column (40 × 3 mm id) of the same phase, and an ultraviolet (UV) detector (Hewlett Packard Series 1100). Elution was monitored by UV absorbance at 215 nm. The mobile phase consisted of acetone and acetonitrile (50:50, v/v) at a flow rate of 1.0 mL/min. The column temperature was set at 50 °C with a column heater (Eppendorf CH-30 column heater). All TAG contents were given in percentage area.

Thermal Characteristics

The melting profiles and SFCs of their blends were analyzed by a differential scanning calorimeter (DSC) (Perkin Elmer DSC-6, Norwalk, CN, USA). The DSC procedure of Siew and Faridah [7] was followed. The samples were completely melted at 80 °C before being weighed. Molten samples (10 ± 0.5 mg) were hermetically sealed in an aluminum pan, with an empty pan used as reference. Samples were initially heated to 80 °C and held at this temperature for 10 min in the DSC instrument to erase the previous thermal history. The samples were then cooled to −60 °C at 40 °C/min. At the end of the cooling, the samples were heated from −60 to 80 °C at 10 °C/min. The SFC was calculated at various temperatures from the DSC heating thermogram data by partial integration according to Tieko and Aparecida [8]. The partial areas were obtained directly from calculations made using the DSC software (Pyris version 7.0).

Microstructure

The crystal network microstructures of the blends were examined by polarized light microscopy using a polarized light microscope (PLM) (Olympus BX51, Olympus Optical Co., Ltd., Tokyo, Japan) equipped with a Pixera color video camera (model PVC 100C, Los Gatos, CA, USA). A static crystallization method similar to the method of Narine and Marangoni [9] was used. Samples were molten at 80 °C for 15 min in order to erase the crystal memory, and 20 µL of melt were placed on a glass microscope slide heated to the same temperature. A glass coverslip at the same temperature as the sample was placed on top of the samples. All samples were then allowed to crystallize simultaneously for 48 h at room temperature (23 °C). A 40× lens was used to image the grayscale photograph of the samples.

Statistical Analysis

Statistical analysis of the obtained data was carried out using the SPSS software package (version 10.0), with a 95% confidence interval employed [10]. Although no standard errors are shown in Table 1 and Fig. 2, the standard deviations in the results varied between 0.1 and 2.8 for all of the experiments conducted.

Results and Discussion

TAG Composition

The TAG composition is one of the most important chemical characteristics that determines the physical

Table 1 Changes in the contents of the major TAGs in the blends with CB-like fat addition

Blend (CB:CB-like fat)	TAG (%)		
	POP	POS	SOS
100:0	18.9	33.1	24.7
90:10	18.6	32.7	24.2
80:20	18.3	32.0	23.0
70:30	17.2	31.0	21.3
60:40	16.3	29.0	20.2
50:50	15.2	27.6	18.3
30:70	13.3	24.3	14.6
0:100	11.0	20.0	11.7

properties of fats and oils. Hence, determining the TAG content is crucial to investigating the compatibility of a fat blend. A CB replacer fat should not cause a significant change in TAG content when blended with CB. Table 1 shows the contents of the major TAGs (POP, POS, SOS) of the blends. CB-like fat obtained from the enzymatic conversion of ROPO contained 11.0% POP, 20.0% POS and 11.7% SOS, while commercial CB contained 18.9% POP, 33.1% POS and 24.7% SOS. The other two major TAGs present in the product were POO (13.7%) and SOO (9.0%).

It is also important to control the content of monoacylglycerols (MAGs) and diacylglycerols (DAGs) at the production stage, because they have a strong effect on fat crystallization. The total content of MAGs and DAGs in the product was 5.3%. It has been reported that MAGs have a retarding effect on crystallization. In general, micelles of MAGs are thought to act as templates for crystallization and may induce heterogeneous nucleation [11]. Sambuc et al. [12] showed that the addition of 4% of a mixture of monopalmitin and monostearin decreases the induction time of vegetable fats. However, the presence of MAGs at low concentrations will not affect the CB crystallization much [13]. DAGs may either promote or delay crystallization, depending on composition and concentration. Shukla [14] and Ziegleder [15] noticed that CBs with a higher DAG level exhibit a slower crystallization. Cebula and Smith [16] reported that DAGs in Coberine (a CB-equivalent fat) reduce the fat's crystallization induction time but subsequently slow down the velocity of growth. Siew and Ng [17] have reported that dipalmitoyl glycerol caused rapid palm olein crystallization, palmitoyl oleoyl glycerol retarded crystallization, and dioleoylglycerol had no significant effect.

It has been observed that CB-like fat addition up to 30% did not cause a significant decrease ($P > 0.05$) in TAGs of CB. Further increasing the proportion of CB-like fat in the blend caused a gradual decrease in the contents of major TAGs. Thirty percent CB-like fat addition (blend 70:30) (17.2% POP, 31.0% POS and 21.3% SOS) was found to be the upper limit for obtaining the most compatible blend formulation in terms of TAG content.

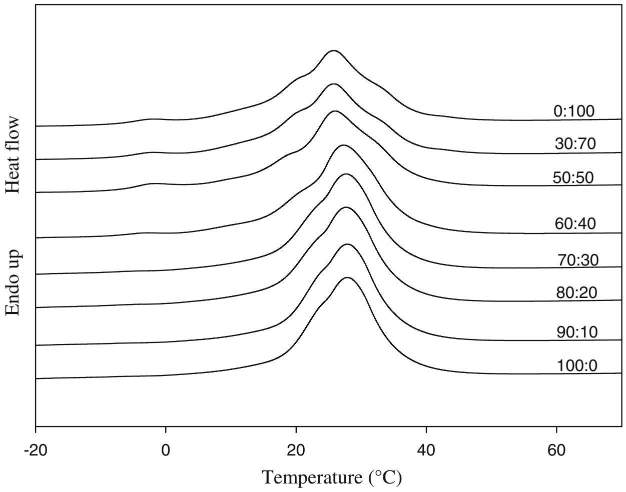

Fig. 1 Melting thermograms of CB and CB-like fat blends

Melting Characteristics

Melting thermograms of the blends are depicted in Fig. 1. CB (100:0) has a sharp and narrow melting peak at 28.5 °C. Three major TAGs (POP, POS and SOS) and their contents are responsible for this unique melting behavior. The melting characteristics of the blend changed as the proportion of CB-like fat increased. Although the melting peak temperature did not change significantly, increasing the proportion of CB-like fat in the blend made the shape

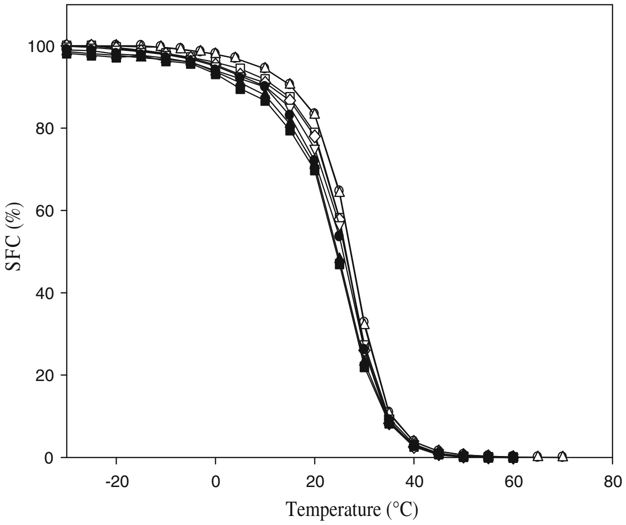

Fig. 2 SFCs of CB and CB-like fat blends as a function of temperature. *Open circles*, 100:0; *open triangles*, 90:10; *open squares*, 80:20; *open diamonds*, 70:30; *inverted triangles*, 60:40; *filled circles*, 50:50; *filled triangles*, 30:70; *filled squares*, 0:100

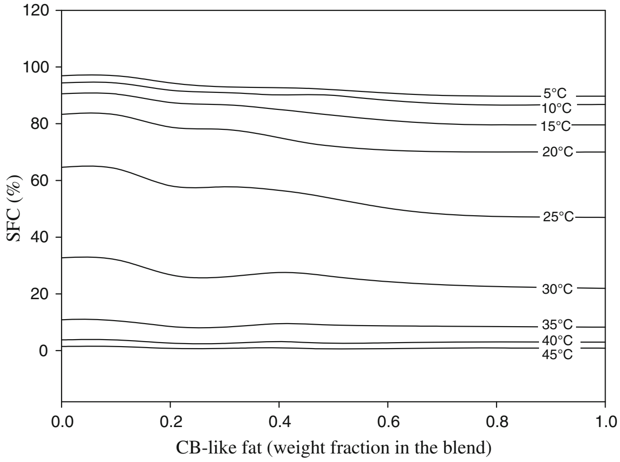

Fig. 3 Isothermal solid diagram of SFC for CB and CB-like fat blends

and range of the melting peak wider and more irregular. Also, new, small melting peaks appeared and became more pronounced as the CB-like fat proportion increased. This can be largely attributed to the decrease in the contents of POP, POS and SOS, and also the presence of unconverted TAGs of ROPO, which have low melting points. It was thus observed that the addition of CB-like fat to CB should be limited to 30% in order to save the sharp and narrow melting range of CB.

Solid Fat Content

The performance of fats in food products depends on their melting behavior. SFC is a good indicator of the melting behavior of fats. The SFC profile of the oils and fats or their blends is important and is used to determine whether a

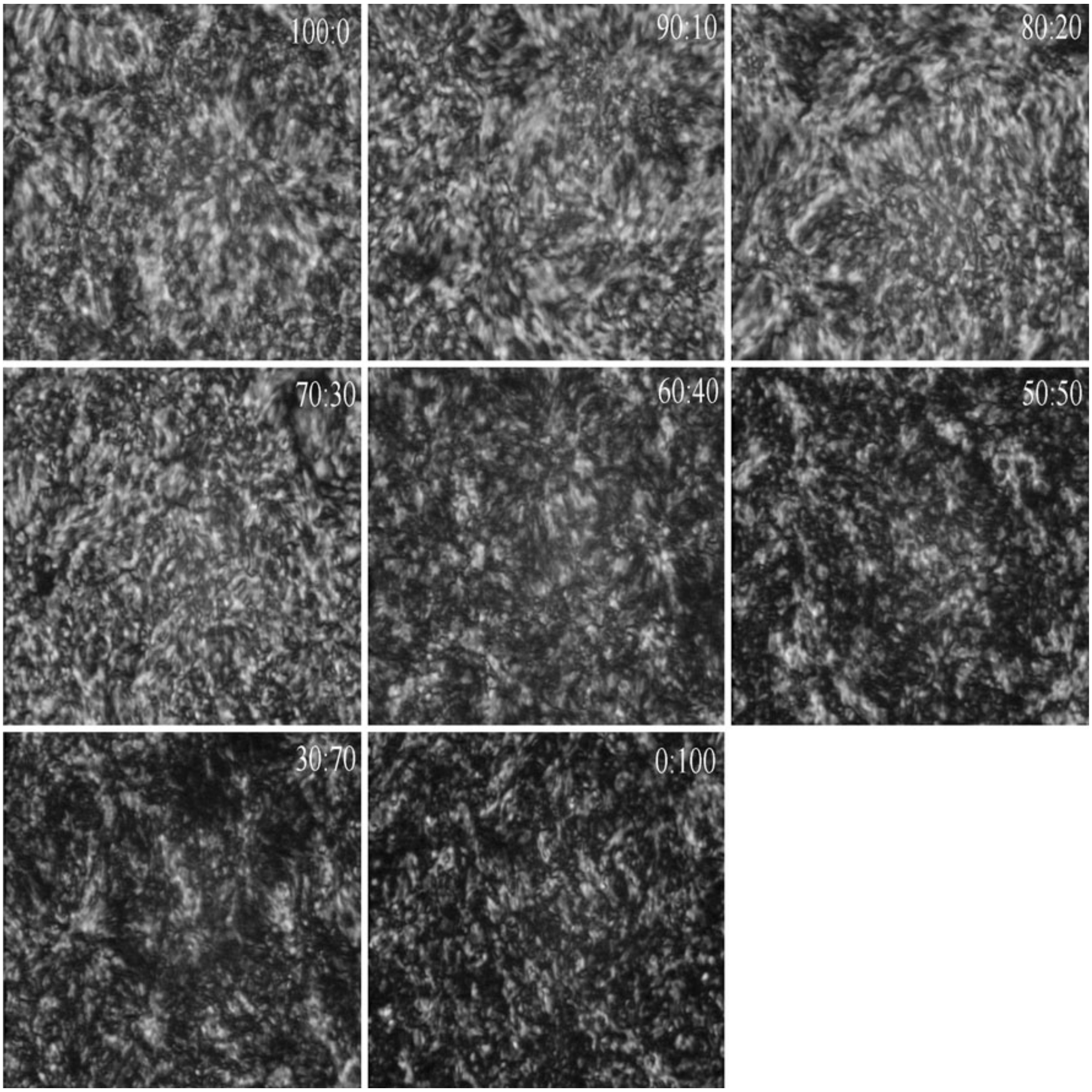

Fig. 4 Polarized light micrograph grayscaled images of CB and CB-like fat blends (CB:CB-like fat)

certain oil, fat or blend is suitable for a particular application [18]. Changes in the SFCs of the blends as a function of temperature are given in Fig. 2. None of the blends changed the melting features of CB. However, it was observed that SFC decreased with increased ROPO proportion in the blend. These variations in the SFC are mainly due to differences in the TAG contents of CB and CB-like fat.

Figure 3 shows the isothermal solid diagram of SFC for the blends. For an ideal mixing compatibility, straight lines should be observed for each isothermal solid line of SFC. A significant decrease ($P < 0.05$) in SFC was observed at all temperatures for blends in which the ROPO ratio was above 10%. Better compatibility between CB-like fat and CB was observed at temperatures of 35 °C and over for all CB-like fat proportions. This means that, in the mouth (at body temperature), the blends will not affect mouthfeel significantly. According to Shukla [14], a softer CB would contain about 63% solid fat at 20 °C and 20% solid fat at 30 °C. The SFC contents of all blends at 20 and 30 °C are above 63 and 20%, respectively. In general, the interactions in the present blends were more noticeable at room temperature. No eutectic effect was observed in the blends. The absence of a eutectic effect highlights the similarity in molecular volume, shape and polymorphs between CB and CB-like fat [19].

Microstructure

The microstructures of fat crystal networks have an important effect on macroscopic properties [20]. Hence, in order to determine the compatibility of fat blends, their microstructures should also be investigated in addition to their chemical and physical characteristics. PLM images of the blends are depicted in Fig. 4. It was observed that the proportion of CB-like fat in the blend affected the microstructure significantly above 30% CB-like fat addition. Under polarization, the anisotropic solid phase refracts light differently than the isotropic liquid phase, so crystals are seen as white or gray [9]. As seen from the images, the addition of up to 30% CB-like fat had no effect on the crystal network. Increasing the CB-like fat proportion in the blend still further decreased the number of crystals and caused crystal shape and distribution in the network to become relatively irregular. This change in the microstructure is due to the decrease in the major TAGs of CB and also the presence of other low melting point TAGs from ROPO. The highly ordered nature of the TAGs in CB results in a regular network. A well-defined crystal network occurs due to strong and defined interactions between microstructural elements, leading to a three-dimensional arrangement with similar intermicrostructural distances [9].

Conclusion

The results of this study suggest that CB-like fat, enzymatically obtained from ROPO, can be used in confectionery products to replace up to 30% of the CB without altering the physical and chemical properties of the product significantly. Replacement of CB up to a certain level with this product may reduce the costs of confectionary manufacture. It is also a good way to utilize ROPO.

Acknowledgments This research was supported by the Scientific Research Projects Governing Unit (BAPYB) of the University of Gaziantep. We would like to thank Bilginoğlu Marbil Yağ San. ve Tic. AŞ., İzmir, Turkey for supplying refined olive pomace oil.

References

1. Smith KW (2001) Cocoa butter and cocoa butter equivalents. In: Gunstone FD (ed) Structured and modified lipids. Marcel Dekker, New York, pp 401–422
2. Lipp M, Anklam E (1998) Review of cocoa butter and alternative fats for use in chocolate—part A. Compositional data. Food Chem 62:73–97
3. Liu KJ, Chang HM, Liu KM (2007) Enzymatic synthesis of cocoa butter analog through interesterification of lard and tristearin in supercritical carbon dioxide by lipase. Food Chem 100:1303–1311
4. Çiftçi ON, Fadıloğlu S, Göğüş F (2009) Conversion of olive pomace oil to cocoa butter-like fat in a packed bed enzyme reactor. Bioresour Technol 100:324–329
5. Kiritsakis APK, Lenart EB, Willet WC, Hernandez RJ (1998) Olive oil from the tree to the table. Food and Nutrition Press, Trumbull
6. Pavithra NR, Prakash M, Kodangala KB (2006) Quality assessment of oil blends by electronic nose technique and sensory methods. J Sens Stud 21:322–332
7. Siew WL, Faridah MDJ (2000) Compositional and differential scanning calorimetry (DSC) studies of crystals of palm olein. J Palm Oil Res 12(2):1–13
8. Tieko NR, Aparecida L (1995) Solid fat content determination by differential scanning calorimetry. Grasas Aceites 46:337–343
9. Narine SS, Marangoni AG (1999) The difference between cocoa butter and salatrim lies in the microstructure of the fat crystal network. J Am Oil Chem Soc 76:7–13
10. SPSS Inc. (1999) SPSS 10.0 for Windows. SPSS Inc., Chicago
11. Gerson T, Escher WL (1966) The effect of monoglycerides on the spreadability of butter. NZ J Sci 9:528–533
12. Sambuc E, Dirik Z, Reymond G, Naudet M (1980) Etude de la cristallisation de corps gras plastiques. VI. Influence des glycérides partiels et des phosphatides en absence et présence d’ eau. A. Cas des monoglycérides stéaropalmitiques. Rev Fr Corps Gras 27:505–512
13. Chaiseri S, Dimick PS (1995) Dynamic crystallization of cocoa butter. I. Characterization of simple lipids in rapid- and slow-nucleating cocoa butters and their seed crystals. J Am Oil Chem Soc 72:1491–1496
14. Shukla VKS (1995) Cocoa butter properties and quality. Lipid Technol 5:54–57
15. Ziegleder G (1988) Kristallisation von Kakaobutter unter statischen und dynamischen Bedingungen (DSC, Rheometer). Suesswaren 12:487–493

16. Cebula DJ, Smith KW (1992) Differential scanning calorimetry of confectionery fats: part II: effects of blends and minor components. J Am Oil Chem Soc 69:992–998
17. Siew WL, Ng WL (1996) Effect of diacylglycerols on the crystallization of palm oleins. J Sci Food Agric 71:496–500
18. NorAini I, Embong MS, Aminah A, Ali AR, Che Maimon CH (1995) Physical characteristics of some shortenings based on modified palm oil, milkfat and low melting milkfat fractions. Fat Sci Technol 97:253–260
19. Noor Lida HMD, Sundram K, Idris NA (2006) DSC study on the melting properties of palm oil, sunflower oil, and palm kernel olein blends before and after chemical interesterification. J Am Oil Chem Soc 83:739–745
20. De Man JM, Beers AM (1987) Fat crystal networks: structure and rheological properties. J Texture Stud 18:303–318