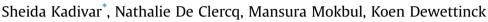
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# Influence of enzymatically produced sunflower oil based cocoa butter equivalents on the phase behavior of cocoa butter and guality of dark chocolate



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#### ABSTRACT

The two enzymatically produced sunflower oil based cocoa butter equivalents (CBEs) were blended with cocoa butter (CB) at varying concentrations (g/100 g). The peak maximum decreased significantly upon the addition more CBE due to the presence of the low-melting TAGs. However, the melting heat and the melting onset temperature were comparable to CB up to 25 g CBE/100 g blend. The iso-solid diagram showed monotectic behavior with softening effect proportional to CBE increase. A significant decrease in SFC was observed at all temperatures for the blends in which the CBE ratio was above 25 g/100 g blend. The second part of this study was set out to determine the performance of CBE replacement in chocolates and compounds at 20 °C. All the chocolates and compounds even in the level of 5 g CBE/100 g blend had significantly lower Casson yield stress than reference chocolate. However, the viscosity of the chocolate with 5 g HSHO CBE/100 g blend was comparable with the reference chocolate. Higher amounts of CBE slightly decreased the peak maximum however up to 5 g CBE/100 g blend, the melting behavior did not significantly change compared to the reference chocolate without CBE. The hardness of the reference chocolate was comparable with chocolate with 5 g CBE/100 g blend up to 2 weeks.

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## 1. Introduction

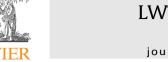
Enzymatic modification of fats and oils for the production of specific structured triacylglycerols (TAGs) is showing lots of attention for a long time (Rozendaal & Macrae, 1997). Nowadays, different industries are using enzymatic interesterification techniques with sn-1,3 specific lipases for the production of cocoa butter equivalents (CBE), human milk fat substitutes, and so on. Specific structured TAGs are TAGs that are stereospecifically modified or reformulated to obtain specific functions for nutrition. food, and pharmaceutical applications. Using chemical interesterification in the production of these types of TAGs is usually not applicable because of deficiency in positional specificity (Xu, 2000).

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Functional and nutritional properties of modified oils and fats depend not only on fatty acid (FA) profiles but also on FA distributions in the glycerol backbone. Cocoa butter (CB) for instance, is an essential ingredient in chocolate as it forms the continuous phase and is responsible for the gloss, texture and typical melting behavior of chocolate. These distinctive physical properties are the result of its special 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 (POSt), and 1,3-distearoyl-2oleoyl-glycerol (StOSt), with oleic acid at the sn-2 position of the glycerol backbone. The melting point of CB is between 32 and 35 °C and it can re-crystallize during tempering process to a stable crystal form (Foubert, Vanrolleghem, Thas, & Dewettinck, 2004).

Although CB is the ideal ingredient in chocolate and other confectionary products, an increasing demand for chocolate products, high price of CB and uncertainty in the supply leads the researchers and manufacturer to look for alternatives such as CBE (Tchobo et al., 2009). A CBE is a non-lauric fat that can replace CB with preservation of its chemical and physical characteristics (Verstringe, De Clercq, Nguyen, Kadivar, & Dewettinck, 2012). By using lipase-catalyzed reactions, all the three main TAGs can be





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Abbreviations: CB, cocoa butter; CBE, cocoa butter equivalent; DAG, diacylglycerols; FA, fatty acids; FFA, free fatty acids; HOSO, high oleic sunflower oil; HSHO, high stearic high oleic sunflower oil; POP, 1,3-dipalmitoyl-2-oleoyl-glycerol; POSt, 1(3)-palmitoyl-3(1)stearoyl-2-oleoyl-glycerol; PLM, Polarized light microscope; SOO, saturated-oleoyl-oleoyl; SOS, saturated-oleoyl-saturated; SSS, Trisaturated; SFC, solid fat content; TAG, triacylglycerols.

produced with oils in which the sn-2 position is mainly occupied by oleic acid (Rozendaal & Macrae, 1997).

# While vegetable fats, other than illipe, palm, sal, shea, kokum and mango are not allowed for use as CBE in chocolate (Union, 2000), the improved physical properties of enzymatically produced CBE may make it a suitable partial replacement ingredient for CB in chocolate and other confectionary products. However, when developing a structured lipid for this purpose, it is important to consider how the novel fat will compare physically with CB. Melting, crystallization, and microstructure are the main properties that are used for the evaluation of an enzymatically produced CBE (Çiftçi, Kowalski, Göğüş, & Fadıloğlu, 2009).

High Oleic Sunflower Oil (HOSO) contains high levels of oleic acid (~80 g/100 g), mainly in the form of triolein (000) (>60 g/ 100 g). It has a very low solid content at room temperature. Instead, High Stearic High Oleic (HSHO) sunflower oil contains considerable amount of stearic acid (~15 g/100 g) but it is still liquid at high temperature. These types of oils are promising sources to produce CBE through enzymatic acidolysis by 1,3 specific enzyme since their TAGs are rich in oleic acid in the sn-2 position of their glycerol backbones. The present work involves formulating several potential CBEs by blending the stearin fraction of two enzymatically produced sunflower oil rich in SOS TAGs with CB and further studying their compatibility with CB through iso-solid diagrams. Subsequently, the formulated CBEs were used as fat phase of dark chocolate in order to evaluate their influence on quality parameters of dark chocolate. The results from this study could help the fats and oils industries to extend their knowledge on the compatibility of two enzymatically produced sunflower oil based CBEs with CB and their influence on the quality of chocolate products.

#### 2. Materials and methods

#### 2.1. Materials

Refined HOSO and FAs were provided from Oleon (Oelegem, Belgium). FAs consisted of a mixture of stearic acid (53.4 g/100 g), palmitic acid (44.8 g/100 g), myristic acid (1.2 g/100 g) and arachidic acid (0.6 g/100 g). HSHO was provided by Nutrisun Business Unit of Advanta Seeds. Immobilized lipase from *Rhizo-mucor miehei* (Lipozyme RMIM, Novozymes, immobilized on ion-exchange resin, sn-1,3 specific) was purchased from Sigma--Aldrich (Schnelldorf, Germany). All other reagents and solvents were of analytical or chromatographic grade.

## 2.2. Methods

#### 2.2.1. CBE production

2.2.1.1. Enzymatic acidolysis. Acidolysis reaction with lipase was carried out in a 1 kg batch reactor under optimized conditions. Details of the production of CBE and its isolation from the reaction mixture and the analyses have been reported previously (Kadivar, De Clercq, Van de Walle, & Dewettinck, 2014). The acidolyzed product was distilled in a short path distillation unit (VTA, Deggendorf, Germany) at P = 0.3 Pa and T = 200 °C. At these conditions, free fatty acids were almost removed (FFA < 0.3 g/100 g).

2.2.1.2. Fractionation. The method of Chong, Hoh, and Wang (1992) was adopted for the fractionation. The distilled product was dissolved in hexane (1:10 g/ml) and left at 4 °C for 24 h. The precipitated fat was filtered off, and the filtrate was evaporated to dryness. The mother liquor was then dissolved in acetone (1:10 g/ml) and cooled at 4 °C for 24 h. The second precipitated fat fraction was obtained and dried under nitrogen for 4 h at 60 °C.

#### 2.2.2. Blend preparation

CB and enzymatically produced CBEs were melted at 70  $^{\circ}$ C and blended at proportions of 100:0, 75:25, 50:50, 95:05 and 0:100 (g/ 100 g).

#### 2.2.3. Fatty acid composition

The AOCS official method Ce 2-66 (Firestone, 1998) was used for preparing methyl esters for further analysis by GC according to the AOCS Official Methods Ce 1-62 (Firestone, 1998).

### 2.2.4. Triacylglycerol composition

TAGs were analyzed with an optimized method developed by Rombaut, De Clercq, Foubert, and Dewettinck (2009). Separation of TAG species was performed on a Shimadzu HPLC system (Shimadzu, Japan). The details of the system was reported in our previous study (Kadivar et al., 2014). The results of TAG composition are presented in Table 1.

#### 2.2.5. Pulsed nuclear magnetic resonance (pNMR)

Solid fat content (SFC) was measured by pulsed NMR (pNMR) with a Bruker Minispec pc 20 (Bruker, Karlsruhe, Germany). Melted CB and CBEs were placed in NMR tubes (three replicates) and submitted to the tempering treatments of the IUPAC 2.150 serial tempering method. SFC was determined in the range of 5-40 °C at 5 °C intervals following 60 min incubations at each temperature.

#### 2.2.6. Differential scanning calorimetry (DSC)

The DSC experiments were performed with a Q1000 DSC with a refrigerated cooling and an auto-sampler system (TA Instruments, New Castle, USA). Nitrogen was used to purge the system. Samples were sealed in hermetic pans and an empty pan was used as a reference. Non-isothermal crystallization and melting experiments were performed. Samples were initially heated from room temperature to 65 °C and held at this temperature for 10 min to destroy crystal memory; cooled to -20 °C at 5 °C/min and held for 5 min; and heated to 65 °C at 5 °C/min to determine the melting profile. Results were presented using the TA Universal Analysis 2000 software (TA Instruments).

#### 2.2.7. Chocolate production

Standard dark chocolate products with the formulation of 48 g fine sugar/100 g chocolate (BarryCallebaut Belgium, Wieze, Belgium) with particle size distribution of around 0.1 mm, 30.90 g CB/100 g chocolate, 20.5 g cocoa powder/100 g chocolate and 0.6 g soy lecithin/100 g chocolate (Unimills, Zwijndrecht, the Netherlands) were prepared at UGent Cacaolab. The cocoa powder was lightly alkalized dark brown with pH in the range of 6.8–7.2. The fat and moisture content were 11 g/100 g cocoa powder and 5 g/100 g cocoa powder, respectively. The soy lecithin had the moisture content of 0.19 g/100 g with acetone insolubles value of 64.25 g/100 g. Different chocolate and compound products with different amounts of CBEs (5 g, 25 g, 50 g and 100 g in 100 g fat blend) were produced and stored at 20 °C. Chocolate production started with mixing all the ingredients. The mixture of sugar, cocoa powder and CB/CBE needed to be refined to a particle size <35 µm. After refining the mixture was transferred to the conche. This process is carried out in two different stages: dry conching and liquid conching following the tempering process.

#### 2.3. Quality parameters of chocolate

#### 2.3.1. Flow behavior

The flow behavior of chocolate and compounds were analyzed using TA Instruments AR 2000 (TA Instruments, New Castle, Delaware, USA). Chocolate and compound samples were melted at 50  $^\circ$ C

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