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## 7 **Characterization of sunflower stearin-based confectionary fats in bulk** 8 **and in compound coatings**

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25 **Abstract**

26 Sunflower stearins obtained by fractionation of high oleic-high stearic sunflower oil  
27 can be used for cocoa butter equivalents (CBEs). The main objectives of this work  
28 were: 1) Compare the performance of two sunflower stearin-based CBEs (palm-free  
29 and palm-containing) with commercial CBEs and cocoa butter in terms of heat stability,  
30 hardness and microstructure; and 2) Test these sunflower stearin-based CBEs in milk  
31 chocolate and compound coating formulations in order to understand the evolution of  
32 their crystalline structure and the bloom development during storage. Sunflower  
33 stearin-based CBEs exhibited different polymorphic behavior, as well as a smaller and  
34 more compact microstructure than commercial CBEs and cocoa butter. Although  
35 sunflower stearin-based CBEs contain less saturated fatty acids than commercial ones,  
36 they had more solids at high temperature and higher hardness and could work as a  
37 cocoa butter improver. On the other hand, the use of increasing levels of sunflower  
38 stearin-based CBEs formulated and tested in this work on chocolate and compound  
39 coating formulations favored a faster crystallization into more stable polymorph, and  
40 influenced the bloom evolution of these confectionery products.

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43 **Keywords:** *High oleic-high stearic sunflower oil, sunflower hard stearin, cocoa butter*  
44 *equivalent, milk chocolate, compound coating, heat resistance, hardness, fat bloom.*

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## 49 **Introduction**

50 Cocoa butter (CB) is the fat extracted from the beans of the cocoa fruit (*Theobroma*  
51 *cacao* L.), and it is the most important ingredient in chocolate and confectionery  
52 production. Chocolate is essentially cocoa mass and sugar suspended in a cocoa butter  
53 matrix, the latter constituting the continuous phase [1]. Since CB is the main  
54 structuring material in chocolate and confections, their physical properties are largely  
55 determined by those of the underlying fat phase [2]. Thus, CB is responsible for the  
56 functional and organoleptic attributes of chocolate products, such as gloss, snap  
57 (hardness and brittleness at room temperature), contraction during demolding,  
58 complete melting and the release of its flavor at the temperature of the mouth [3, 4,  
59 5]. CB is the most expensive ingredient in chocolate manufacturing, corresponding to  
60 25-35% of the final product's cost [6]. Therefore, confectionery manufacturers have  
61 been looking for cheaper alternatives that can partially replace CB in chocolate  
62 formulations. Among these alternatives, CB equivalents (CBE) are vegetable fats with  
63 similar chemical and physical properties to CB [7]. CBEs are fully miscible with CB (no  
64 eutectic behavior) and thus, they can be used interchangeably with CB in any recipe  
65 [7]. CBEs with high levels of solids can be used to harden soft CBs. This special class of  
66 CBEs are called generally cocoa butter improvers (CBIs) [8]. Alternatively, CB

67 substitutes (CBSs) and replacers (CBRs) are more affordable fats, however they are not  
68 fully compatible with CB as they have an eutectic softening effect and can therefore  
69 not be mixed with CB in any proportion [4]. CBSs are based on lauric fats (palm kernel  
70 or coconut oil), whereas CBRs are generally obtained from soft and liquid oils that have  
71 been partially hydrogenated (the major source of *trans* fatty acids) [7, 9].

72 CBEs are based in blends of palm mid fractions and stearins derived from tropical  
73 butters rich in stearic acid like shea, illipe or sal [10]. In this regard it would be  
74 interesting to have alternative sources of the latter fats in countries with a temperate  
75 climate. High oleic-high stearic (HOHS) sunflower oil (commercialized under the brand  
76 *Nutrisun*) is a convenient source of stearic-rich fat, free of *trans* fatty acid and  
77 cholesterol-raising saturated fatty acids [11, 12]. HOHS sunflower oil contains high  
78 melting point triacylglycerols (TAGs) but due to certain limitations in the assembly of  
79 saturated fatty acids in sunflower TAGs, dry or solvent fractionation techniques need  
80 to be applied to obtain stearins enriched in stearic acid [13]. The solvent fractionation  
81 of HOHS sunflower oil yields hard stearins containing 40% to 55% stearic acid and  
82 levels of disaturated TAGs (SUS) up to 95%, that make them suitable to be used in  
83 confectionery formulations [14, 15, 16, 17].

84 Chocolate manufacturers are actively working to meet nutrition goals by reformulating  
85 chocolate recipes, lowering the amount of unhealthy saturated fats and eliminating  
86 *trans* fatty acids [18]. However, this reduction in saturated fat can be quite challenging,  
87 as the final product must retain its functionality, organoleptic properties and physical  
88 characteristics. Various attempts have been made to develop heat resistant chocolate  
89 that can withstand high temperatures during its shelf life [19]. As can be found in the

90 patent literature, to date most efforts along these lines have focused on modifying the  
91 formulations by adding ingredients like surfactants (polyglycerol polyricinoleate) [20],  
92 polyols or thermal structuring components (dextrose) [21]. Alternatively, a tropicalized  
93 shell comprising chocolate, a humectant liquid and a non-tropicalized chocolate core  
94 have also been contemplated [22].

95 The main objectives of this study were to assess the performance of HOHS sunflower  
96 stearins in the production of CBEs with improved heat resistance and to test these  
97 stearin-based CBEs on chocolate and compound coatings formulations. Two CBEs  
98 containing sunflower stearins (one containing palm oil and one palm oil free) were  
99 formulated and compared with commercial CBEs and CB with regards their chemical  
100 composition, heat stability, hardness and microstructure. Afterwards, these sunflower  
101 stearin-based CBEs were tested in milk chocolate and compound coating production in  
102 order to study the evolution of their crystalline structure and bloom development  
103 during storage.

104

## 105 **Materials and methods**

106

### 107 **Materials**

108 Two types of sunflower stearins were used in this work: a sunflower soft stearin  
109 obtained by dry fractionation of refined, bleached, deodorized and partially dewaxed  
110 HOHS sunflower oil reported elsewhere [23]; and a sunflower hard stearin produced  
111 by solvent fractionation of the sunflower soft stearin at 15 °C using an oil: acetone  
112 ratio of 4:1 [17]. Two CBEs containing sunflower stearins were formulated: sunflower-

113 palm CBE (SP-CBE) was obtained by blending a palm mid-fraction (PMF) with sunflower  
114 hard stearin in a ratio 1:4 (as previously reported by Bootello *et al.* [15]); whereas  
115 sunflower CBE (S-CBE) was prepared by mixing sunflower hard stearin with 20% of  
116 sunflower soft stearin. The characteristics of sunflower hard stearin, palm mid fraction  
117 and sunflower soft stearin are shown in the supplementary data file. All the  
118 ingredients used in the chocolate making trials (Ivory Coast CB, cocoa liquor, full cream  
119 milk powder and soy lecithin) and the commercial CBEs (CBE 1 and CBE 2) were kindly  
120 supplied by Nestle-PTC York (UK), whereas the PMF was provided by Lípidos Santiga  
121 (Barcelona, Spain).

122

### 123 **Chocolate and compound coating**

124 Two compound coatings (with 5% and 100% CB replacement) and a milk chocolate  
125 were prepared according to the recipes shown in Table 1. Each sample (a 2 kg batch of  
126 each formulation) was produced by mixing the melted fat components (10% of the  
127 total CB and CBE) with dry powders (sucrose, milk powder and cocoa liquor) and  
128 simultaneously warming them to 45°C in a Hobart mixer (Hobart Ltd, Troy, OH, USA).  
129 The resulting mass was refined in a three roll refiner (Model SDX 600, Buhler Ltd,  
130 Switzerland), adjusting the gap size/pressure between the rollers. The refined  
131 chocolates were stored in plastic containers and conditioned at 55 °C for 24 hours  
132 prior to conching. Thereafter, dry conching was performed at a low speed for 140 min  
133 at 60 °C. Soy lecithin was added to the remaining CB/CBE (90% of the total) and this  
134 mixture was conched at a higher speed for 30 minutes. The samples were then melted  
135 at 50 °C for 4 hours and afterwards, they were tempered. Milk Chocolate and  
136 compound coatings were hand tempered using a conventional tempering process for

137 chocolate containing milk-fat fractions [24]: 50 °C, 30 min; 27.7 °C, 4 min; and 31 °C, 2  
138 min. Hand tempering was performed by pouring 2/3 of the melted chocolate mass on  
139 a tempering marble cold table, spreading and scraping with a bench spatula until  
140 cooling up to the lowest tempering temperature and finally by adding the remaining  
141 1/3 melted chocolate in order to increase the temperature up to 31 °C. Chocolate bars  
142 were molded in plastic molds and allowed to cool in a cold room (12 °C) for 2 hours.  
143 Finally, they were extracted from the molds onto plastic trays and stored at 18 °C.

#### 144 **TAG and fatty acid composition**

145 TAGs were analyzed by gas-liquid chromatography (GLC), achieving high-resolution  
146 separation on an Agilent 6890 gas chromatography apparatus (Palo Alto, CA, USA) with  
147 hydrogen as the carrier gas. The injector and detector temperatures were both 370 °C,  
148 the oven temperature was 335 °C, and a head pressure gradient from 70 to 120 kPa  
149 was applied. The gas chromatography column was a Quadrex Aluminium-Clad 400-  
150 65HT (30 m length, 0.25 mm i.d., 0.1 µm film thickness: Woodbridge, CT, USA), and a  
151 linear gas rate of 50 cm/s, a split ratio 1:80 and a flame ionization detector (FID) were  
152 used. The samples were dissolved in heptane at approximately 2.7 mg/mL and the  
153 TAGs were analyzed by injecting 1 µL aliquots of these solutions into the GC system.  
154 The TAG species were identified as described elsewhere [25] and quantified by  
155 applying the correction factors reported previously by Carelli and Cert [26].

156 The fatty acid moieties of TAGs were transmethylated into fatty acid methyl esters  
157 (FAMES) by treating 10 mg of oil samples for 1 h at 80 °C with 3 mL of  
158 methanol/toluene/sulfuric acid (88/10/2 v/v/v). The resulting methyl esters were then  
159 extracted twice with 1 mL of heptane and analyzed by GC in a Hewlett-Packard 6890  
160 gas chromatography apparatus (Palo Alto, CA, USA). The column used was a Supelco

161 SP-2380 fused silica capillary column (30 m length, 0.25 mm i.d., 0.20  $\mu\text{m}$  film  
162 thickness: Bellefonte, PA, USA) with hydrogen as the carrier gas at  $28\text{ cm}\cdot\text{s}^{-1}$ . The  
163 detector and oven were maintained at a temperature of  $200\text{ }^{\circ}\text{C}$  and  $170\text{ }^{\circ}\text{C}$ ,  
164 respectively. The different methyl esters were identified by comparing their retention  
165 times with those of known standards.

166

#### 167 **Solid fat content (SFC) assessed by p-NMR**

168 The solid fat content was determined by pulsed nuclear magnetic resonance (p-NMR)  
169 using an Oxford Instruments MQC23 NMR Analyzer equipped with on-board software  
170 for data processing (Oxford Instrument plc, Abingdon, UK). NMR tubes of 10 mm  
171 diameter were filled with approximately 2.0–2.5 g of completely melted fat and the  
172 samples were tempered according to the AOCS official method Cd 16–81 for stabilizing  
173 confectionery fats [27]: melting and storage for 15 min at  $100\text{ }^{\circ}\text{C}$ ; then at least 5 min at  
174  $60\text{ }^{\circ}\text{C}$ ; followed by 90 min at  $0\text{ }^{\circ}\text{C}$ , 40 h at  $26\text{ }^{\circ}\text{C}$ , 120 min at  $0\text{ }^{\circ}\text{C}$ ; and finally, 60 min at  
175 each chosen temperature. The SFC was measured in  $5\text{ }^{\circ}\text{C}$  increments from 0 to  $40\text{ }^{\circ}\text{C}$ .

176

#### 177 **Thermal analysis by differential scanning calorimetry (DSC)**

178 The melting profiles of CB and CBEs were determined by DSC in a Q2000 V23.5  
179 calorimeter (TA instruments, New Castle, DE, USA). This instrument was calibrated  
180 prior to use with indium, azobenzene and undecane (Sigma-Aldrich, Madrid, Spain),  
181 and nitrogen was used to purge the system. Samples were prepared by transferring  
182 approximately 6 mg of the melted oils to aluminum pans that were then hermetically  
183 sealed, with an empty pan serving as a reference. The exact weight of the pans and the

184 sample was determined in an electronic Sartorius M2P microbalance (Sartorius AG,  
185 Goettingen, Germany). The results were processed using the TA Universal Analysis  
186 software provided by the manufacturer. The following program was used for samples  
187 tempered in DSC pans outside the calorimeter according to the AOCS official method  
188 Cd 16-81 and stored for 40 h prior to DSC measurement: cooling at 100 °C/min to -  
189 30 °C; holding for 10 min; and heating to 80 °C at a rate of 5 °C/min.

190 The melting profiles of milk chocolate and compound coatings were measured by  
191 weighing about 10 mg of the finished product in aluminum pans after different  
192 numbers of days in storage and then quickly transferring them to the calorimeter. The  
193 following temperature program was run: cooling at 1 °C/min to -10 °C; holding for 2  
194 min; and finally heating to 60 °C at a rate of 5 °C/min.

195

#### 196 **Crystal morphology by polarized light microscopy (PLM)**

197 The microstructure of the samples tempered according to the AOCS official method  
198 Cd 16-81 was observed by PLM. A drop of each melted sample was placed on a pre-  
199 heated glass slide (80 °C) and covered with a coverslip. After being tempered by the  
200 aforementioned AOCS method, the slides were incubated at the temperature of  
201 analysis (20 °C) for 180 minutes. The crystal morphology was evaluated on an Olympus  
202 BX61 polarized light microscope (Olympus, Tokyo, Japan) with a 10x objective lens.  
203 Sample images were acquired with a Nikon DS-Ri2 digital video camera (Nikon  
204 Corporation, Tokyo, Japan) coupled to the microscope and processed using Cellsens  
205 imaging software (Olympus Corporation, Japan).

206

### 207 **Texture measurements**

208 The hardness of the tempered samples was measured using a penetration test,  
209 whereby 20 mL of completely melted fat was placed in a plastic beaker, crystallized  
210 following the AOCS official Cd 16–81 method and stored at 20 °C for 48 h. Hardness  
211 was evaluated using a TA-HD Plus Texture Analyzer (Stable Micro Systems, Godalming,  
212 Surrey, England) with a penetration probe (needle P/2) and a 50 kg load cell. Hardness,  
213 defined as the maximum penetration force (N), was determined in five replicates by  
214 penetrating the sample at a pre-test speed of 2.0 mm/seconds, a test speed of  
215 1mm/seconds and a post-test speed of 10.0 mm/seconds, each to a distance of 7 mm  
216 at 20 °C.

217

### 218 **Bloom evolution**

219 The bloom evolution of milk chocolate and the compounds coating was studied after 5  
220 heating/cooling cycles at 37 °C. The experiment involved 5 cycles of heating to 37 °C  
221 and cooling to 21 °C, with an interval of 72 h at 21 °C between cycles 3 and 4. The  
222 bloom surface was measured using a DigiEye System cabinet (VeriVide, Leicester, UK)  
223 equipped with a Nikon D80 digital camera (Nikon Corporation, Tokyo, Japan) and  
224 quantified with the DigiPix software provided by the manufacturer.

225

### 226 **Statistical analysis**

227 Origin Pro 8 software (OriginLab Corporation, Northampton, USA) was used for the  
228 statistical analysis. The number and nature of replicates for each figure and table are

229 indicated in their corresponding legends. The standard deviations of the fatty acid and  
230 triacylglycerol compositions can be found as supplementary material. An analysis of  
231 variance (ANOVA) and a Tukey's test were used to determine the significant  
232 differences of the means at a probability level of 5% ( $p < 0.05$ ).

233

## 234 **Results and discussion**

### 235 **Chemical characterization of cocoa butter and cocoa butter equivalents**

236 The fatty acids and TAGs composition of CB and CBEs are shown in Table 2 and 3. A CB  
237 from Ivory Coast was used as a reference, in which the main fatty acids were stearic  
238 (36.3%), oleic (31.9%) and palmitic (27.1%) acids. Commercial CBEs did not mimic the  
239 fatty acid composition of CB, containing more palmitic, and less stearic and oleic acid  
240 (Table 2). Moreover, medium chain fatty acids were found in commercial CBEs, such as  
241 lauric (2.2%) and myristic acid (1.4%) in CBE1. No traces of behenic acid were found in  
242 either the CB or commercial CBEs. The fatty acid composition of the sunflower stearin-  
243 based CBEs was closer to that of CB, with similar or higher levels of stearic and oleic  
244 acid, as well as lower amounts of palmitic acid. Furthermore, sunflower stearin-based  
245 CBEs contained long chain fatty acids (up to 2.7 % of arachidic and 3.2% of behenic  
246 acid for S-CBE). Finally, the proportions of total saturated fatty acids were significantly  
247 lower for both SP-CBE and S-CBE than CB ( $p < 0.05$ ), whereas commercial CBEs had the  
248 highest levels of saturated fatty acids.

249 In terms of TAG composition (Table 3), CB and CBEs mainly contained symmetrical  
250 disaturated TAGs (SUS), such as POP, POST and StOSt. While CB was richer in POST

251 (38.3%), commercial CBEs contained more POP (ranging from 31.2 to 41.4%).  
252 Sunflower stearin-based CBEs were enriched in StOSt (up to 49.2% for S-CBE) and they  
253 had less POP (0.9%) due to the lower levels of palmitic acid. Moreover, SP-CBE and S-  
254 CBE contained remarkable amounts of high melting point SUS TAGs, in the form of  
255 StOA and StOB, reflecting the very long chain fatty acids naturally found in HOHS  
256 sunflower oil [28]. The total proportion of SUS in CB was significantly higher than in  
257 CBEs ( $p < 0.05$ ). For the sunflower stearin-based CBEs, the lower levels of SUS were  
258 counterbalanced by the significant amounts of SUU (from 13.4 to 15.3%) and UUU  
259 (from 5.4 to 10.0%:  $p < 0.05$ ). These SUU values for SP-CBE and S-CBE were similar to  
260 those found in literature for soft CB, as reported for Brazilian CB samples [5]. Since S-  
261 CBE was formulated with sunflower soft stearin, it had the highest levels of  
262 triunsaturated TAGs (mainly due to the contribution of triolein). Trisaturated TAGs  
263 ranged from 1.1 to 5.3%, with a significant increase in commercial CBEs ( $p < 0.05$ ).  
264 Commercial CBEs are usually formulated by blending palm mid fraction with a source  
265 of StOSt like shea or sal stearin. CBE 1 and CBE 2 composition fitted well within this  
266 definition excepting for a small amount of lauric TAGs (in the form of 1,2,3-trilauroyl  
267 glycerol -LaLaLa-, 1,2-dilauroyl-3-myristoyl glycerol -LaLaM- and 1,2-dimyristoyl-3-  
268 lauroyl glycerol -LaMM) accounting for 1.9% of CBE 1. Although CBE 1 cannot be  
269 considered as a CBS, the presence of lauric TAGs may influence the crystallization  
270 pattern and phase behavior, particularly given the incompatibility and eutectic effect  
271 between TAG species with very different chain lengths [3].

272

273 **Solid Fat Content (SFC) of CB and CBEs**

274 The SFC curves provide information about the performance of CB and CBEs [5]. There  
275 are three temperature intervals of interest in a SFC profile that serve to assess some of  
276 the important sensory attributes of confectionery fats like hardness, heat resistance,  
277 coolness and waxiness [29]. Thus, hardness and brittleness correspond to high SFC  
278 values at temperatures between 15 and 25 °C. Heat resistance is the resistance to  
279 deform or melt at elevated temperatures and is related with the solid content in the  
280 temperature interval from 25 to 35 °C. A sharp decrease in SFC within the temperature  
281 range between 25 and 35 °C is related to coolness and creaminess [7]. This cooling  
282 sensation is due to the fast melting of the fat phase and the subsequent latent heat  
283 release in the mouth. Finally, waxiness can be detected by measuring the SFC in the  
284 temperature interval from 35 to 40 °C. No solid fat should be present above 37 °C  
285 (mouth temperature) in order to avoid waxy sensations.

286 The SFC profiles of CB, commercial CBEs and sunflower stearin-based CBEs were  
287 studied (Figure 1), and while commercial CBEs had a higher SFC than CB below 20 °C  
288 (Figure 1A), the SFC of both CBEs was lower above 20 °C. This reduction was especially  
289 notable for CBE 1, the fat with highest SSS levels (Table 3). Although SSS TAGs provide  
290 SFC at higher temperatures and they are associated with a higher NMR melting point  
291 (the temperature where the SFC becomes 0%) [30], the presence of low melting point  
292 lauric TAGs significantly ( $p < 0.05$ ) reduced the SFC of CBE 1 respecting CB in the interval  
293 embracing from 25 to 35 °C (in proportions of 11.6%, 13.2% and 8.6%, Figure 1A).  
294 However, the SFC of sunflower stearin-based CBEs followed the opposite trend (Figure  
295 1B), with a lower SFC than CB below 25 °C and higher values above this temperature.  
296 All the CBEs studied here exhibited SFC curves similar to CB, with a drastic drop in SFC  
297 between 25 to 35 °C. Taking into account the SFC profile of CB as reference, the CBEs

298 formulated in this work displayed higher SFC than cocoa butter in the 25 to 35°C  
299 interval (Figure 1B). There was a significant increase ( $p < 0.05$ ) of the solid content in  
300 both sunflower based CBEs. Thus, SP-CBE displayed 15.3% more solids than CB at  
301 32.5°C whereas the CBE elaborated with 100% sunflower derived stearins (S-CBE)  
302 displayed the maximum increase of solid with regard to CB (25.8% more solids than CB  
303 at 32.5°C). The differences for both CBEs were also significant at 35°C. This behavior  
304 can be explained in terms of TAGs composition. Commercial CBEs were richer in  
305 palmitic-based TAGs (mainly POP), whereas SP-CBE and S-CBE contained more stearin-  
306 based TAGs (Table 3). The higher POP concentration in commercial CBEs contributed  
307 to their higher SFC at low temperatures (below 20 °C). However, the predominance of  
308 StOSt, especially in S-CBEs, produced higher SFC between 30 and 35 °C, and therefore,  
309 a higher NMR melting point. The amount of solid fat observed in the temperature  
310 range from 35 to 37 °C may be due to the StOA and StOB in sunflower stearin-based  
311 CBEs. There was no significant SFC above 37 °C and thus, waxiness was kept to a  
312 minimum for SP-CBE and S-CBE. This higher content of solid at these temperatures  
313 pointed to a higher heat resistance of these fats with regard to Ivory Coast CB and to  
314 their possible application as CBI to harden soft CB in chocolates and compounds.

315 Coolness was similar for the three fats, since all CBEs SFC curves exhibited similar sharp  
316 melting profile pattern, with those from sunflower stearin-based CBEs moved towards  
317 higher temperatures. The reduction of the palm fraction in the CBE formulation  
318 enhanced in general their heat resistance, as occurred for sunflower stearin-based  
319 CBEs. In particular, S-CBE (palm-free) could be suitable for use as a cocoa butter  
320 improver in chocolate and compound coatings in tropical climates.

321

## 322 **Melting behavior of CB and CBEs**

323 The melting properties of CB and CBEs were studied by DSC. Due to the complex  
324 nature of the TAG blends in oil samples and to the polymorphic transitions that may  
325 occur during melting, the interpretation of thermal DSC curves is not easy. Here, the  
326 melting curve and polymorphic behavior of CB was used as a reference to study the  
327 thermal properties of commercial and sunflower-based CBEs. The polymorphic forms  
328 of CB have been studied thoroughly [31] and in accordance with the current  
329 nomenclature, six different polymorphs have been identified for CB in order of  
330 increasing stability [32]:  $\gamma$  (sub- $\alpha$ ),  $\alpha$ ,  $\beta_2'$ ,  $\beta_1'$ ,  $\beta_2$  and  $\beta_1$ . As a result of combining the  
331 traditional Roman numbering system, described by Wille and Lutton [31] with the  
332 current nomenclature, the subscripts V and VI are often applied to the most stable  $\beta$   
333 polymorphs, referred to as  $\beta_V$  and  $\beta_{VI}$ , respectively [29]. Thus,  $\beta_V$  is the desirable  
334 polymorph of CB in a well-tempered chocolate obtained from the liquid state under  
335 proper cooling conditions. The polymorphic transition from  $\beta_V$  and  $\beta_{VI}$  is mediated only  
336 by a solid-solid transformation, and it takes place during the storage of chocolate [33].

337 As explained in the Materials and Methods, samples were tempered outside the  
338 calorimeter according to the AOCS official method Cd 16–81 (AOCS, 2009), the aim  
339 being to crystallize the samples as their most stable polymorph prior to DSC analysis.  
340 When the DSC melting thermograms of CB and CBEs obtained after this tempering  
341 protocol were compared (Figure 2), CB exhibited two peaks at  $20 \pm 0.4$  (peak A) and  $34$   
342  $\pm 0.1$  °C (peak B). According to the melting ranges reported previously by Ribeiro et al.  
343 [5] for CB polymorphs, these corresponded to the  $\beta'$  and  $\beta$  polymorphs:  $\beta'$  (20.0-

344 28.0 °C),  $\beta_V$  (27.4-35.0 °C) and  $\beta_{VI}$  (29.0-36.2 °C). This result was consistent with the  
345 time-temperature state diagram for the polymorphs of statically crystallized CB  
346 reported by Marangoni and McGauley [34], where a blend of  $\beta'$  and  $\beta$  was obtained  
347 after crystallization of CB for 40 h at 26 °C. The thermal behavior of CBE 1 and CBE 2  
348 was similar to that of CB, with two melting peaks (A and B) corresponding to different  
349 polymorphic forms. Since CBEs exhibit the same polymorphic behavior as CB, these  
350 peaks should correspond to the  $\beta'$  and  $\beta$  forms.

351 The DSC parameters (Table 4) were obtained from the thermograms. The  
352 temperatures of the melting peak A and the corresponding ranges in the transition  
353 phase for commercial CBEs were not significantly different to those of CB ( $p < 0.05$ ),  
354 whereas significantly lower  $T_{peak}$  values ( $p < 0.05$ ) were found for peak B of CBE 1 and  
355 CBE 2. Although the ranges for peak B were significantly larger than for CB ( $p < 0.05$ ),  
356 they were within the melting range limits of the CB  $\beta_V$  polymorph (dashed lines in  
357 Figure 2). With regard to the sunflower-based CBEs, the  $T_{peak}$  and melting range of  
358 both peak A and B of the palm-containing CBE did not differ significantly from CB  
359 ( $p < 0.05$ , Table 4). However, the melting behavior of the palm-free CBE did differ and S-  
360 CBE only exhibited peak B, with the highest  $T_{peak}$  value and melting range (Table 4).  
361 Rincón-Cardona et al. [35] studied the polymorphic forms of hard and soft fractions of  
362 HOHS sunflower oils by XRD and DSC. These authors reported  $T_{peak}$  values for the  $\beta$   
363 polymorph of 32 °C (for soft stearins) and 37 °C (for hard stearins). Since S-CBE was  
364 prepared by mixing hard stearin with 20% of soft stearin, a  $T_{peak}$  value of  $34.9 \pm 0.1$  °C  
365 was consistent with previous data [35], confirming the crystallization of the  $\beta$   
366 polymorph. Conversely, the melting enthalpies of the sunflower-based CBEs turned  
367 out to be significantly lower than those of CB and the commercial CBEs ( $p < 0.05$ , Table

368 4). This difference in  $\Delta H_m$  can be explained by the TAG composition. As stated by  
369 Himawan et al. [36], TAGs containing mainly saturated fatty acids that can easily align  
370 to form compact structures that are more difficult to melt. However, TAGs containing  
371 unsaturated fatty acids have kinks in their aliphatic chains and the disrupted packing of  
372 unsaturated TAGs hampers the formation of crystals, decreasing the melting enthalpy  
373 relative to saturated TAGs with the same chain length. Thus, the lower values of  
374 melting enthalpies of SP-CBE and S-CBE could be due to their higher unsaturated TAG  
375 (SUU and UUU) content (Table 3).

376

### 377 **Microscopy Analyses**

378 The microstructure of the crystal network of CB and the CBEs tempered following the  
379 method used for DSC analysis were studied by PLM at 20 °C (Figure 3). For CB, large  
380 microstructures with a granular center surrounded by feather-like crystallites were  
381 observed. The phase transition from the  $\beta'$  form to the  $\beta$  polymorph usually leads to  
382 the formation of this kind of large microstructure [34]. A similar microstructure was  
383 found for the commercial CBE 1, although a compact bulk of crystals was observed  
384 between the large microstructures. CBE 2 exhibited a morphology consisting of crystals  
385 with a small granular center and a needle-like periphery that coexisted with the  
386 feather-like structures, again surrounded by a compact bulk of smaller crystals.

387 By contrast, SP-CBE gave rise to crystal aggregates with extensive granular centers and  
388 needle-like structures at the border of the crystals. The pattern of crystallization of S-  
389 CBE was different, since it formed a denser and more uniform network, with no  
390 feather-like structures. In fact, this morphology was very similar to the 100% sunflower

391 hard stearin (Figure 3). Kadivar et al. [37] studied the changes in the growth and  
392 morphology of crystals in blends of cocoa butter and sunflower oil based CBEs by PLM.  
393 These authors reported an inhibitory effect in the crystal growth process at increasing  
394 levels of SUU TAGs in the blends. In the present work, both sunflower stearin-based  
395 CBEs contain more SUU than CB and commercial CBEs (Table 3). Therefore, less crystal  
396 formation could occur after the application of the afore mentioned AOCS tempering  
397 procedure to these sunflower containing CBEs. In summary, sunflower stearin-based  
398 CBEs had a smaller and more compact microstructure than commercial CBEs or CB.  
399 This could have important implications on the macroscopic and mechanical properties  
400 of these fats, such as their hardness.

401

#### 402 **Hardness of CB and CBEs**

403 Hardness is a textural property that is crucial to determine the functionality of CB-  
404 based confectionery products. There are several factors that influence the hardness of  
405 fats, including the SFC, chemical composition (fatty acids and TAGs), the polymorphic  
406 behavior of the fat crystals, and the size and shape of the crystals [38]. The hardness of  
407 tempered samples was evaluated by needle penetration test. The relationship  
408 between hardness and SFC at 20 °C was evaluated for samples tempered according to  
409 aforementioned AOCS official method (Figure 4). Although sunflower stearin-based  
410 CBEs were significantly harder ( $p < 0.05$ ) than commercial CBEs and CB, their SFC values  
411 at 20 °C were lower. This fact can be explained since the hardness of a fat crystal  
412 network it is not only determined by the SFC. Apart from the effects of TAG  
413 composition (in general, the lower the degree of unsaturation and the longer the chain  
414 length in the fatty acid, the harder the fat [39]), the polymorphism and microstructure

415 of the crystal network also influence the mechanical properties of fats. As shown  
416 previously, sunflower stearin-based CBEs tended to crystallize into a  $\beta$  polymorph  
417 under these tempering conditions, whereas commercial CBEs and CB did in  $\beta'$  and  $\beta$   
418 polymorphs. The influence of polymorphism on hardness has been reviewed in  
419 literature [40, 41, 42]. The strengthening of the crystal network due to the formation  
420 of solid bridges between crystals (sintering) augments hardness [42].  $\beta'$  crystals are  
421 more prone to sintering than  $\beta$  crystals as the latter are more isometric and hinder the  
422 formation of compound crystals [41]. These data could suggest that  $\beta$ -tending  
423 sunflower stearin-based CBEs should be softer than  $\beta'+\beta$  CBEs. However,  $\beta'$  was not  
424 the predominant polymorph of CB and commercial CBEs, and, on the other hand,  
425 sintering usually takes place in long term post-crystallization stages (which were not  
426 reached by this tempering protocol). Thus, polymorphism could influence mechanical  
427 properties indirectly through its effects on the microstructure, as indicated by Brunello  
428 et al. [40] for CB crystallized statically. According to the PLM analysis, sunflower  
429 stearin-based CBEs produce smaller crystals with a more compact microstructure  
430 compared to CB and commercial CBEs. Since fat crystals with a smaller size generally  
431 lead to fat systems with a higher hardness [30], the microstructure of the fat network  
432 may plausibly explain the increment in hardness under these tempering conditions.

433

#### 434 **Evolution of the crystalline structure of milk chocolate and compound coatings** 435 **during storage**

436 The use of sunflower stearin-based CBEs was tested in the production of milk  
437 chocolate and compound coatings with different amounts of CB. Two compound  
438 coatings for SP-CBE and S-CBE (with 5% and 100% CB replacement) and milk chocolate

439 (used as a reference) were prepared according to the given recipes (Table 1). For the  
440 100% CB replacement compounds, the CB present in the formulation comes  
441 exclusively from cocoa liquor (which contains around 54% CB). The evolution of the  
442 crystalline structure of the milk chocolate and compound coatings was evaluated  
443 during storage by DSC (Figure 5 and Table 5). Samples were tempered using a  
444 conventional tempering process for chocolate containing milk-fat fraction [24] and  
445 stored at 18 °C. After 24h of storage, all melting curves showed an endothermic peak  
446 between 30.2 and 32.8 °C, with the higher peak temperatures corresponding to the  
447 100% replacement compounds. All these melting peaks were within the melting  
448 interval corresponding to the  $\beta_v$  polymorph of CB. After 1 week of storage, milk  
449 chocolate and the 5% replacement compounds experienced a stronger increment in  
450 the melting enthalpies, whereas the melting enthalpies for the 100% replacement  
451 compounds remained almost unchanged (Table 5). Hence, shorter storage time would  
452 be necessary for compound coatings containing more sunflower stearin-based CBEs,  
453 since they reached more stable crystalline structures earlier. In summary, using  
454 increasing amounts of sunflower stearin-based CBEs could enhance the tempering  
455 process, favoring faster crystallization into more stable polymorphs.

456

#### 457 **Bloom evolution of milk chocolate and compound coatings**

458 Accelerated shelf-life tests are frequently performed by confectionery manufacturers  
459 to monitor the formation of fat bloom in chocolate products. These tests usually  
460 involve a number of temperature cycling programs where time/temperature  
461 parameters are modified according to the storage conditions. In this work, the bloom

462 evolution of milk chocolate and the compound coatings was studied after 5  
463 heating/cooling cycles at 37 °C. This temperature was selected in order to study the fat  
464 bloom in heat resistant chocolates that can withstand high temperatures during shelf  
465 life. The bloom surface area for cycles 1 to 3, and 72 h after cycle 3 was quite similar  
466 for SP-CBE and S-CBE compounds (Figure 6). For both CBEs, the 5% replacement  
467 compound developed less bloom than the milk chocolate. However, this was not the  
468 case when CB was totally replaced: although the 100% compounds produced a similar  
469 bloom surface in the first 3 temperature cycles, yet after cycles 4 and 5, the behavior  
470 of the 100% S-CBE and SP-CBE compounds differed as they produced a larger bloom  
471 surface. This difference could reflect the strong stability of the crystals formed in these  
472 CBEs, since the aim of cycles 4 and 5 was to remove the bloom formed in the 72h  
473 interval at 21 °C. Different bloom patterns were observed for each formulation, even  
474 for those samples with similar bloom levels (Figure S1, supplementary material). This  
475 data would suggest that different mechanisms underlying the development of bloom  
476 take place in function of the amount and type of CBE fat used in the formulation the  
477 compound coatings. The experiments concerning to blooming have been run as single  
478 determination. So, more research would be necessary to reach definitive conclusions  
479 on the effect of these new fats on the crystallization of milk and black chocolate.

## 480 **Conclusions**

481 The results of this study have shown that sunflower stearin-based CBEs can be  
482 successfully used in the formulation of chocolate and compound coatings. Two CBEs  
483 (palm-free and palm-containing) based on sunflower stearin were formulated in order  
484 to compare their performance with commercial CBEs and CB in terms of chemical

485 composition, crystallization, microstructure of the crystal network and mechanical  
486 properties (hardness). Despite the sunflower stearin-based CBEs contained lower  
487 saturated fatty acids levels than commercial CBEs, they showed higher contents of  
488 solids at high temperatures. The reduction of the palm fraction in the sunflower  
489 stearin-based CBEs enhances this effect, with higher SFC values above 25 °C compared  
490 to CB due to the contribution of high melting point TAGs, making these fats suitable as  
491 CBIs. The sunflower stearin-based CBEs tended to crystallize in  $\beta$  polymorph, with a  
492 smaller and compact microstructure and higher hardness. In particular palm-free CBE  
493 crystallized in a highly stable polymorphic form with a melting point close to the  $\beta_{VI}$   
494 polymorph of CB. Both sunflower CBEs were tested in the production of milk chocolate  
495 and compound coating to study the evolution of the crystalline structure during  
496 storage and the bloom after thermal cycles. The use of increasing amounts of  
497 sunflower stearin-based CBEs in the formulation of these confectionery products could  
498 enhance the tempering, favoring a faster crystallization into more stable polymorphs.

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506

## 507 **References**

- 508 1. Andrae-Nightingale LM, Lee SY, Engeseth NJ (2009) Textural changes in  
509 chocolate characterized by instrumental and sensory techniques. *J Texture Stud*  
510 40:427–444
- 511 2 Marangoni AG (2005) In Marangoni AG (Ed) *The Functionality of Milk Fat*  
512 *Fractions in Confectionery and Plastic Fats*. Marcel Dekker Inc, New York
- 513 3 Quast LB, Luccas V, Ribeiro APB, Cardoso LP, Kieckbich TG (2013) Physical  
514 properties of tempered mixtures of cocoa butter CBR and CBS fats  
515 International. *J Food Sci Tech* 48:1579-1588
- 516 4 Smith KW (2001) In: Gunstone FD (Ed) *Cocoa Butter and Cocoa Butter*  
517 *Equivalents*. Marcel Dekker Inc, New York
- 518 5 Ribeiro APB, Claro da Silva R, Gioielli LA, De Almeida Gonçalves MI, Gimaldi R,  
519 Gonçalves LAG, Kieckbich TG, (2012) Physico-chemical properties of Brazilian  
520 cocoa butter and industrial blends Part I- Chemical composition solid fat  
521 content and consistency. *Grasas Aceites* 63:79-88
- 522 6 Timms RE (2003) In: Timms RE (Ed) *Raw Materials*. The Oily Press, Bridgwater  
523 UK
- 524 7 Talbot G (2009a) In: Becket S (Ed) *Industrial Chocolate Manufacture and Use*,  
525 4<sup>th</sup> ed. Willey-Blackwell, York UK
- 526 8 Lipp EM, Anklam, E (1998) Review of cocoa butter and alternative fats for use in  
527 chocolate—part A. Compositional data. *Food Chem* 62: 73-97
- 528 9 Foubert I, Vereecken J, Smith K, Dewettinck K (2006) Relationship between  
529 crystallization behavior microstructure and macroscopic properties in trans

530 containing and trans free coating fats and coatings. *J Agr Food Chem* 54:7256-  
531 7262

532 10 Lipp M, Simoneau C, Ulberth F, Anklam E, Crews C, Brereton P, de Greyt W,  
533 Schwack W, Wiedmaier C (2001). Composition of genuine cocoa butter and  
534 cocoa butter equivalents. *Journal of Food Composition and analysis* 14:399-408

535 11 Garcés R, Martínez-Force E, Salas JJ, Venegas-Calero M (2009) Current  
536 advances in sunflower oil and its applications. *Lipid Technol* 21:79–82

537 12 Hunter JE, Zhang J, Kris-Etherton PM (2010) Cardiovascular disease risk of  
538 dietary stearic acid compared with trans other saturated and unsaturated fatty  
539 acids: A systematic review *Am J Clin Nutr* 91 1–18

540 13 Salas JJ, Bootello MA, Garcés R (2015) In: Martínez-Force E, Salas JJ, Dunford  
541 NT, (Eds) *Sunflower: Chemistry Production Processing and Utilization*. AOCS  
542 Press, Champaign IL

543 14 Salas JJ, Bootello MA, Martínez-Force E, Garcés R (2011) Production of stearate-  
544 rich butters by solvent fractionation of high stearic-high oleic sunflower oil.  
545 *Food Chem* 124:450–458

546 15 Bootello MA Hartel RW Garcés R, Martínez-Force E, Salas JJ (2012) Evaluation  
547 of high oleic-high stearic sunflower hard stearins for cocoa butter equivalent  
548 formulation. *Food Chem* 134:1409-1417

549 16 Bootello MA, Hartel RW, Levin M, Martínez-Blanes JM, Real C, Garcés R,  
550 Martínez-Force E, Salas JJ (2013) Crystallization properties and polymorphic  
551 behavior of sunflower hard stearin-based confectionery fats. *Food Chem*  
552 139:184-195

- 553 17 Bootello MA, Garcés R, Martínez-Force E, Salas JJ (2015) Effects of solvents on  
554 the fractionation of high oleic-high stearic sunflower oil. Food Chem 172:710-  
555 717
- 556 18 Talbot G (2011) In: Talbot G (Ed) Reducing saturated fats in foods. Woodhead  
557 Publishing, Cambridge
- 558 19 Stortz TA, Marangoni A (2011) Heat resistance chocolate. Trends Food Sci  
559 Technol 22:201-214
- 560 20 Paggios K, Thiele M, Balzer H, Zsigmond I (2015) Method of producing  
561 chocolate. US Patent 0024110
- 562 21 Wentzel J, Hausman D, Glazier BD, Tweedie GC (2014) Heat resistant chocolate.  
563 Patent WO 2014052421
- 564 22 Althaus TO, Palzer S, Niederreiter G, Chisholm H, Bovet N (2013) Chocolate  
565 product with tropicalised shell. Patent WO 2013083641
- 566 23 Bootello MA, Garcés R, Martínez-Force E, Salas JJ (2011) Dry fractionation and  
567 crystallization kinetics of high-oleic high-stearic sunflower oil. J Am Oil Chem  
568 Soc 88:1511-1519
- 569 24 Yella Reddy S, Full N, Dimick PS, Ziegler GR (1996) Tempering method for  
570 chocolate containing milk-fat fractions. J Am Oil Chem Soc 73:723-727
- 571 25 Fernández-Moya V, Martínez-Force E, Garcés R (2000) Identification of  
572 triacylglycerol species from high-saturated sunflower (*Helianthus annuus*)  
573 mutants. J Agr Food Chem 48:764–769
- 574 26 Carelli A, Cert A (1993) Comparative study of the determination of  
575 triacylglycerol in vegetable oils using chromatographic techniques. J  
576 Chromatogr A 630:213-222

- 577 27 AOCS official method Cd 16–81 (2009) In Official methods and recommended  
578 practices of the American Oil Chemists' Society. AOCS Press, Champaign IL
- 579 28 Salas JJ, Martinez-Force E, Garces R (2005) Very long chain fatty acids synthesis  
580 in sunflower kernels. *J Agr Food Chem* 53:2710–2716
- 581 29 Talbot G (2009b) In: Talbot G (Ed) Technology of coated and filled chocolate  
582 confectionery and bakery products. CRC Press, Boca Raton FL
- 583 30 De Graef V, Vereecken J, Smith KW, Bhaggan K, Dewettinck K (2012) Effect of  
584 TAG composition on the solid fat content profile microstructure and hardness  
585 of model fat blends with identical saturated fatty acid content. *Eur J Lipid Sci*  
586 *Technol* 114:592-601
- 587 31 Wille RL, Lutton ES (1966) Polymorphism of cocoa butter. *J Am Oil Chem Soc*  
588 43:491-496
- 589 32 Van Malssen K, Van Langevelde A, Peschar R, Schenk H (1999) Phase behavior  
590 and extended phase scheme of static cocoa butter investigated with real time  
591 X-ray powder diffraction. *J Am Oil Chem Soc* 76:669-676
- 592 33 Loisel C, Keller G, Lecq G, Bourgaux C, Ollivon M (1998) Phase transitions and  
593 polymorphism of cocoa butter. *J Am Oil Chem Soc* 75:425-439
- 594 34 Marangoni AG, McGauley SE (2003) Relationship between crystallization  
595 behavior and structure in cocoa butter. *Cryst Growth Des* 3:95-108
- 596 35 Rincon-Cardona JA, Martini S, Candal RJ, Herrera ML (2013) Polymorphic  
597 behavior during isothermal crystallization of high stearic high oleic sunflower  
598 oil stearins. *Food Res Int* 51:86-97
- 599 36 Himawan C, Starov VM, Stapley AG (2006) Thermodynamic and kinetic aspects  
600 of fat crystallization. *Adv Colloid Interfac* 122:3-33

601 37 Kadivar S, De Clercq N, Danthine S, Dewettinck K (2016) Crystallization and  
602 polymorphic behavior of enzymatically produced sunflower oil based cocoa  
603 butter equivalent. *Eur J Lipid Sci Technol* 118:1521-1538

604 38 de Man JM, de Man L, (2002) In: Marangoni AG (Ed) *Physical properties of*  
605 *lipids*. Marcel Dekker Inc. New York

606 39 Birkett J (2009) In: Talbot G (Ed) *Technology of coated and filled chocolate*  
607 *confectionery and bakery products*. CRC Press, Boca Raton FL

608 40 Brunello N, McGauley SE, Marangoni AG (2003) Mechanical properties of cocoa  
609 butter in relation to its crystallization behavior and microstructure. *Food Sci*  
610 *Technol* 36:525-532

611 41 Foubert I, Dewettinck K, Van de Walle D, Dijkstra AJ, Quinn PJ (2007) In:  
612 Gunstone F, Harwood J, Dijkstra AJ, (Eds) *The Lipid Handbook*, 3<sup>rd</sup> ed. CRC Press,  
613 Boca Raton FL

614 42 De Graef V, Foubert I, Smith KW, Cain FW, Dewettinck K (2007) Crystallization  
615 behavior and texture of trans-containing and trans-free palm oil based  
616 confectionery fats. *J Agr Food Chem* 55:10258-10265

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639 **Figure captions**

640 **Figure 1.** Solid fat content profiles of cocoa butter and cocoa butter equivalents as  
641 measured by NMR: (A) commercial CBEs and (B) sunflower stearin-based CBEs. Data  
642 represent the average of two experimental replicates: CB (-□-), CBE 1 (-▲-), CBE 2 (-  
643 ▼-), SP-CBE (-◆-) and S-CBE (-●-). Increments of solid fat content for each CBE  
644 regarding CB in the temperature range from 25°C to 35°C were shown in the inner  
645 table. The asterisks indicates significant difference with regard to cocoa butter solid  
646 curve ( $p < 0.05$ ). For abbreviations see Table 2.

647

648 **Figure 2.** DSC thermograms for cocoa butter and cocoa butter equivalents tempered  
649 according to the AOCS official method Cd 16-81. Dashed lines indicate the melting  
650 range of the  $\beta_v$  polymorph of cocoa butter that serves as a reference. The data  
651 represent the averages of three experimental replicates. For abbreviations see Table 2.  
652

653 **Figure 3.** PLM crystal morphology at 20 °C for cocoa butter and cocoa butter  
654 equivalents tempered according to the AOCS official method Cd 16-81. For  
655 abbreviations see Table 2.

656

657 **Figure 4.** Relationship between hardness and solid fat content at 20 °C obtained  
658 through the penetration test for cocoa butter and cocoa butter equivalents tempered  
659 according to the AOCS official method Cd 16-81. The data represent the averages of  
660 five independent experimental determinations and the points followed by the same  
661 letter indicate significant difference ( $p < 0.05$ ) as assessed with a Tukey's test. For  
662 abbreviations see Table 2.

663

664 **Figure 5.** DSC melting curves of milk chocolate and chocolate compounds showing the  
665 evolution of the crystalline structure of the samples after storage for 24h (A) and 1  
666 week (B) at 18 °C. The data represent the averages of two experimental replicates.

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668 **Figure 6.** Bloom evolution of milk chocolate and compound coatings after 5  
669 heating/cooling cycles at 37 °C. Single determination.

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682 **Tables**

683 **Table 1.** Recipes used for the formulation of milk chocolate and compound coatings  
684 with different levels of cocoa butter.

<i>Ingredients (%)</i>	<b>Milk Chocolate (Reference)</b>	<b>Compound 5% CB replacement</b>	<b>Compound 100% CB replacement</b>
Sucrose	47.0	47.0	47.0
Full cream milk powder	23.0	23.0	23.0
Cocoa liquor	12.0	12.0	12.0
Cocoa butter	17.5	12.5	0.0
<b>CBE</b>	<b>0.0</b>	<b>5.0</b>	<b>17.5</b>
Lecithin	0.5	0.5	0.5
<b><i>Fat composition (%)</i></b>			
Cocoa Butter	24.0	19.0	6.5
CBE	0.0	5.0	17.5
Milk Fat	6.0	6.0	6.0
<b><i>Total fat (%)</i></b>	<b>30.5</b>	<b>30.5</b>	<b>30.5</b>

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694 **Table 2.** Fatty acid composition of cocoa butter and cocoa butter equivalents.

	Fatty acids (% w/w)								Total Saturated
	12:0	14:0	16:0	18:0	18:1	18:2	20:0	22:0	
CB	0.0	0.0	27.1	36.3	31.9	3.7	1.1	0.0	64.4 <sup>b</sup>
CBE 1	2.2	1.4	41.3	21.9	29.0	3.3	0.8	0.0	67.7 <sup>a</sup>
CBE 2	0.0	0.6	36.1	29.1	29.6	3.6	1.0	0.0	66.9 <sup>a</sup>
SP-CBE	0.0	0.4	20.8	34.4	36.9	3.0	2.1	2.3	60.1 <sup>c</sup>
S-CBE	0.0	0.0	6.6	43.9	41.5	2.0	2.7	3.2	56.5 <sup>d</sup>

695 CB, Ivory Coast cocoa butter; CBE 1 and 2, Commercial cocoa butter equivalents; SP-CBE, Sunflower-  
696 Palm CBE; S-CBE, 100% Sunflower CBE. Peaks accounting for less than 0.1 % of total fatty acids were not  
697 integrated. The data represent the averages of two independent analytical determinations and the  
698 means followed by the same letter within a column indicate significant difference (p<0.05) as assessed  
699 with a Tukey's test. Data are the average of three experimental replicates; Standard deviations are  
700 shown as Supporting Information (Table S1).

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**Table 3.** Triacylglycerol composition of cocoa butter and cocoa butter equivalents.

	Triacylglycerols and triacylglycerol class (% w/w)																								
	Lauric TAGs	PPP	MOP	POP	PLP	POSt	POO	PLSt	POL	StOSt	StOO	StLSt	OOO	StOL	OOL	StOA	OOA	StOB	OOB	LLB	Other	SSS	SUS	SUU	UUU
CB	0.0	0.1	0.0	13.7	1.4	38.3	2.2	3.4	0.3	30.7	3.6	2.3	0.3	0.4	0.0	1.9	0.0	0.0	0.0	0.0	1.4	1.5 <sup>c</sup>	91.8 <sup>a</sup>	6.5 <sup>c,d</sup>	0.3 <sup>d</sup>
CBE 1	1.9	1.7	1.1	41.4	3.6	11.6	2.8	1.1	0.9	25.1	1.8	2.1	0.5	0.4	0.0	1.6	0.0	0.0	0.0	0.0	2.4	5.3 <sup>a</sup>	88.2 <sup>b</sup>	6.1 <sup>d</sup>	0.5 <sup>c</sup>
CBE 2	0.0	1.4	0.8	31.2	3.0	11.0	2.0	1.3	0.6	36.2	3.6	3.4	0.5	0.4	0.1	2.3	0.2	0.2	0.0	0.0	1.8	3.2 <sup>b</sup>	89.5 <sup>b</sup>	6.7 <sup>c</sup>	0.6 <sup>c</sup>
SP-CBE	0.0	1.0	0.4	11.6	1.7	8.8	4.2	0.4	1.3	43.2	4.9	0.3	4.8	0.4	0.6	6.2	0.5	6.6	0.8	1.1	1.3	1.9 <sup>c</sup>	79.3 <sup>c</sup>	13.4 <sup>b</sup>	5.4 <sup>b</sup>
S-CBE	0.0	0.4	0.0	0.9	0.0	8.3	2.4	0.1	0.2	49.2	8.8	0.2	9.0	0.5	0.7	7.2	0.9	7.7	1.1	1.3	1.1	1.1 <sup>d</sup>	73.6 <sup>d</sup>	15.3 <sup>a</sup>	10.0 <sup>a</sup>

705

706 Triacylglycerols were named with 3 letters: M, myristic; P, palmitic; O, oleic; St, stearic; L, linoleic; A, arachidic; B, behenic. The peaks accounting for less than 0.1 % of total  
707 triacylglycerols were not integrated. **SSS**, total content of trisaturated triacylglycerols; **SUS**, total content of disaturated triacylglycerols; **SUU**, total content of monosaturated  
708 triacylglycerols; **UUU**, total content of triunsaturated triacylglycerols. The data represent the averages of two independent analytical determinations and three experimental  
709 replicates the means followed by the same letter within a column indicate significant difference ( $p < 0.05$ ) as assessed with a Tukey's test. Standard deviations are shown as  
710 Supporting Information (Table S2). For other abbreviations see Table 1.

711

**Table 4.** DSC parameters obtained from the thermograms for cocoa butter and cocoa butter equivalents shown in Figure 2.

	Peak A		Peak B		$\Delta H_m$ (J/g)
	$T_{peak}$ (°C)	R (°C)	$T_{peak}$ (°C)	R (°C)	
CB	$20.0 \pm 0.4^{a,b}$	$12.2 \pm 1.3^a$	$34.0 \pm 0.1^b$	$12.7 \pm 0.4^c$	$131.9 \pm 2.0^{a,b}$
CBE 1	$19.6 \pm 0.7^{a,b}$	$11.3 \pm 1.6^a$	$32.9 \pm 0.0^d$	$15.9 \pm 0.3^b$	$129.0 \pm 2.0^b$
CBE 2	$17.8 \pm 0.3^b$	$14.3 \pm 1.7^a$	$33.3 \pm 0.1^c$	$16.7 \pm 1.2^b$	$135.3 \pm 1.4^a$
SP-CBE	$20.2 \pm 1.5^a$	$13.2 \pm 1.0^a$	$34.2 \pm 0.1^b$	$12.0 \pm 0.3^c$	$121.7 \pm 1.7^c$
S-CBE	–	–	$34.9 \pm 0.1^a$	$21.5 \pm 1.1^a$	$122.7 \pm 2.7^c$

–, Peak not detected;  $\Delta H_m$ , melting enthalpy;  $T_{peak}$ , temperature of the melting peak; R, range of the transition phase (difference between the initial temperature and final temperature in the melting phase). The data represent the averages of three independent experimental replicates and the means followed by the same letter within a column indicate significant difference ( $p < 0.05$ ) as assessed with a Tukey's test. For abbreviations see Table 1.

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**Table 5.** DSC parameters obtained from the thermograms for milk chocolate and chocolate compounds shown in Figure 5.

	24 h			1 week		
	T <sub>peak</sub> (°C)	R (°C)	ΔH <sub>m</sub> (J/g)	T <sub>peak</sub> (°C)	R (°C)	ΔH <sub>m</sub> (J/g)
Milk chocolate	30.6 ± 0.2	27.5 ± 2.1	21.8 ± 1.0	31.2 ± 0.3	34.4 ± 0.8	27.9 ± 1.1
SP-CBE Compound 5%	31.1 ± 0.1	29.4 ± 0.9	22.1 ± 1.3	31.1 ± 0.1	31.1 ± 1.3	28.4 ± 0.9
S-CBE Compound 5%	30.8 ± 0.8	28.8 ± 1.0	23.2 ± 1.1	31.1 ± 0.3	31.4 ± 1.2	29.6 ± 1.3
SP-CBE Compound 100%	32.5 ± 0.5	30.3 ± 1.3	25.7 ± 1.8	31.7 ± 0.4	33.3 ± 1.3	27.8 ± 1.6
S-CBE Compound 100%	32.8 ± 0.9	31.7 ± 1.5	25.5 ± 0.5	32.7 ± 0.2	32.2 ± 1.9	26.1 ± 1.0

ΔH<sub>m</sub>, melting enthalpy; T<sub>peak</sub> temperature of the melting peak; R, range of the transition phase (difference between the initial temperature and final temperature in the melting phase). The data represent the averages of two experimental determinations plus minus standard deviations (p<0.05).

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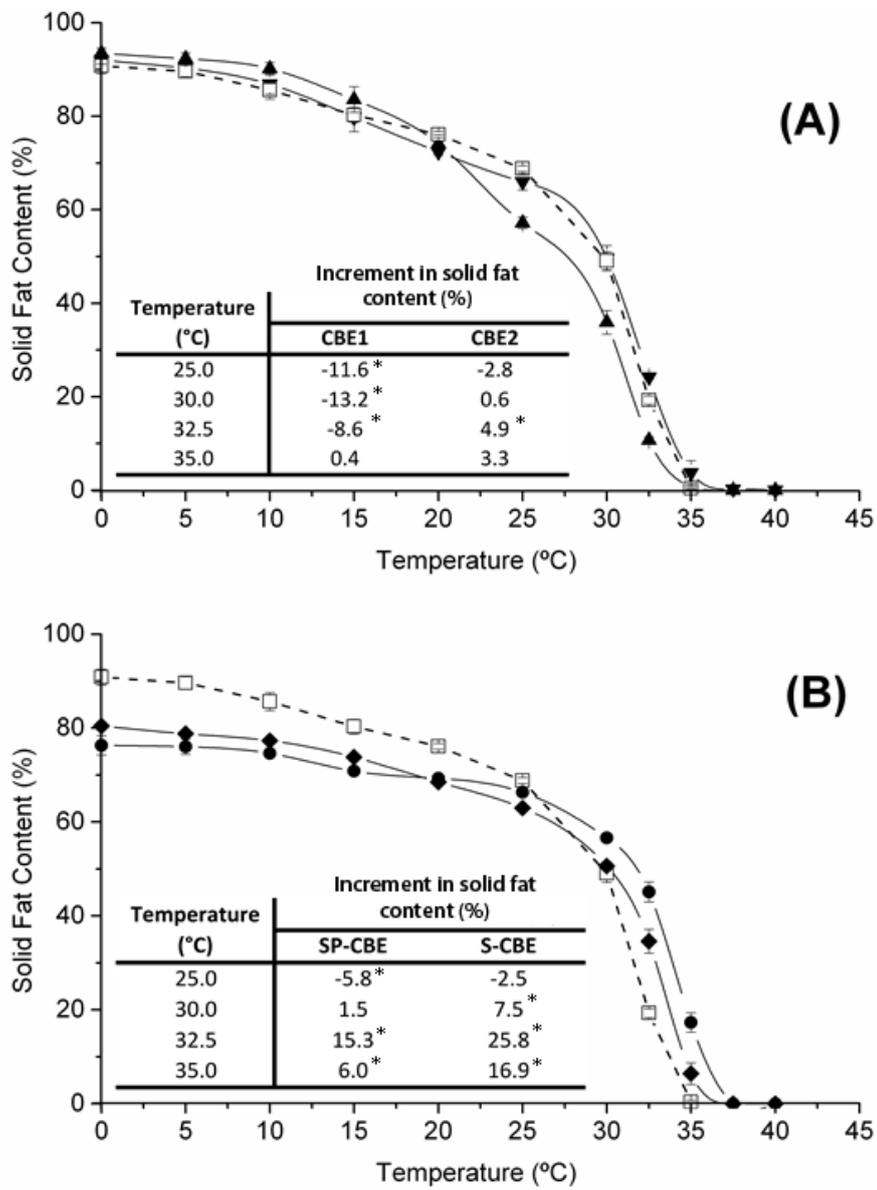
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738 **Figure 1**



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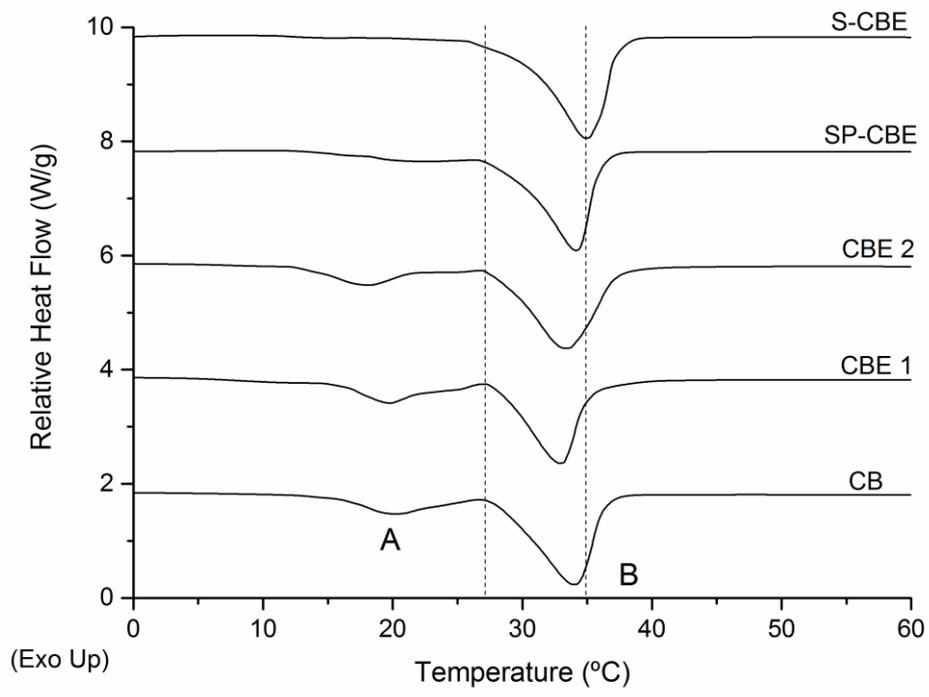
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745 **Figure 2**

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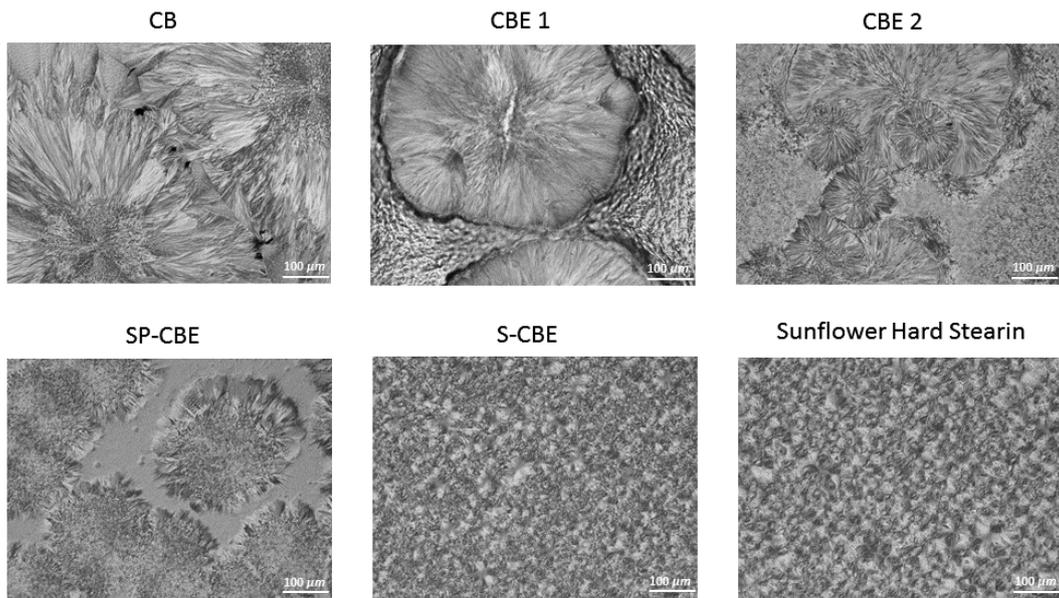
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759 **Figure 3**

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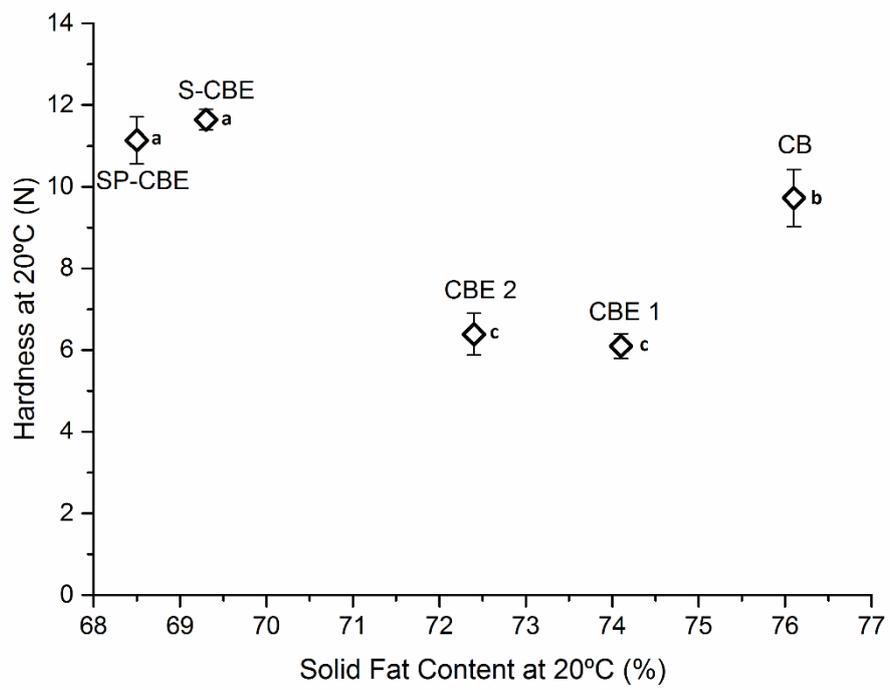
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772 **Figure 4**

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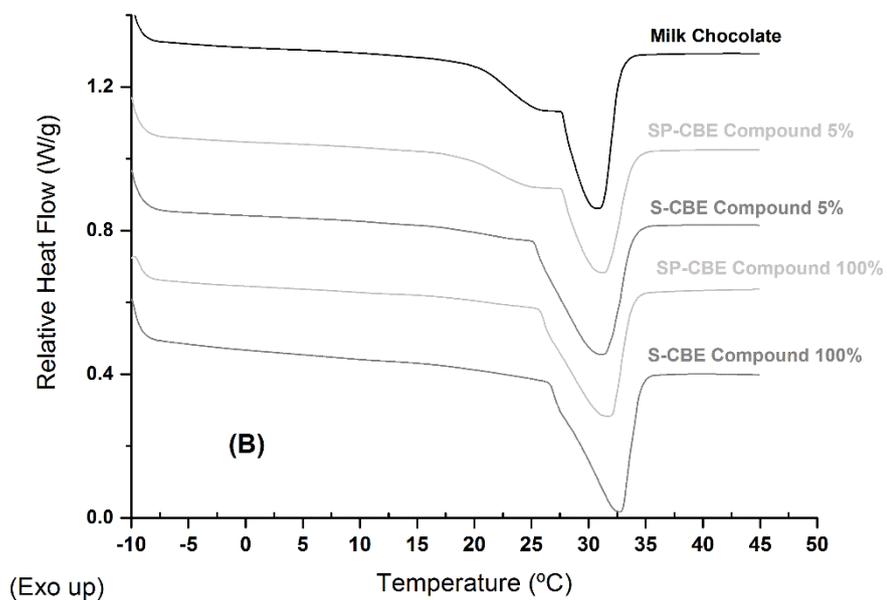
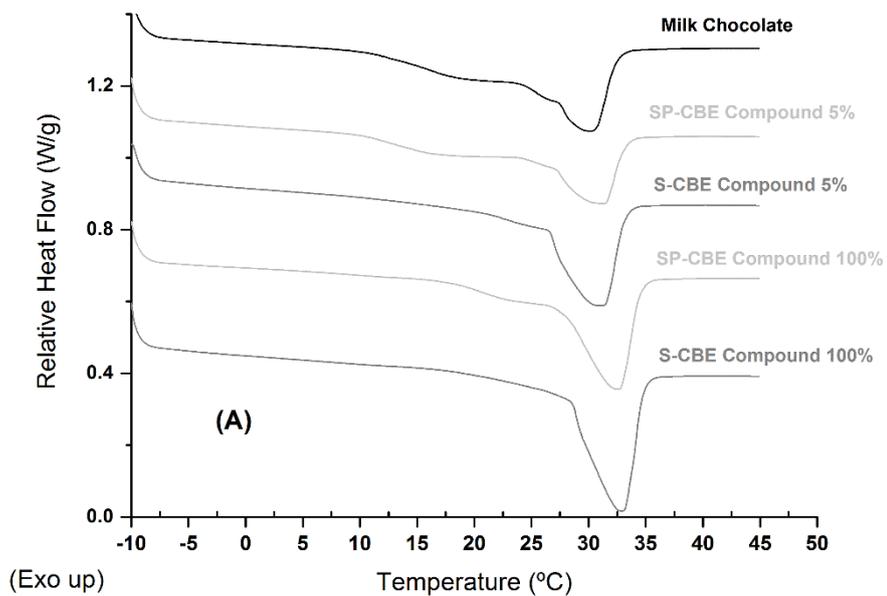
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785 **Figure 5**

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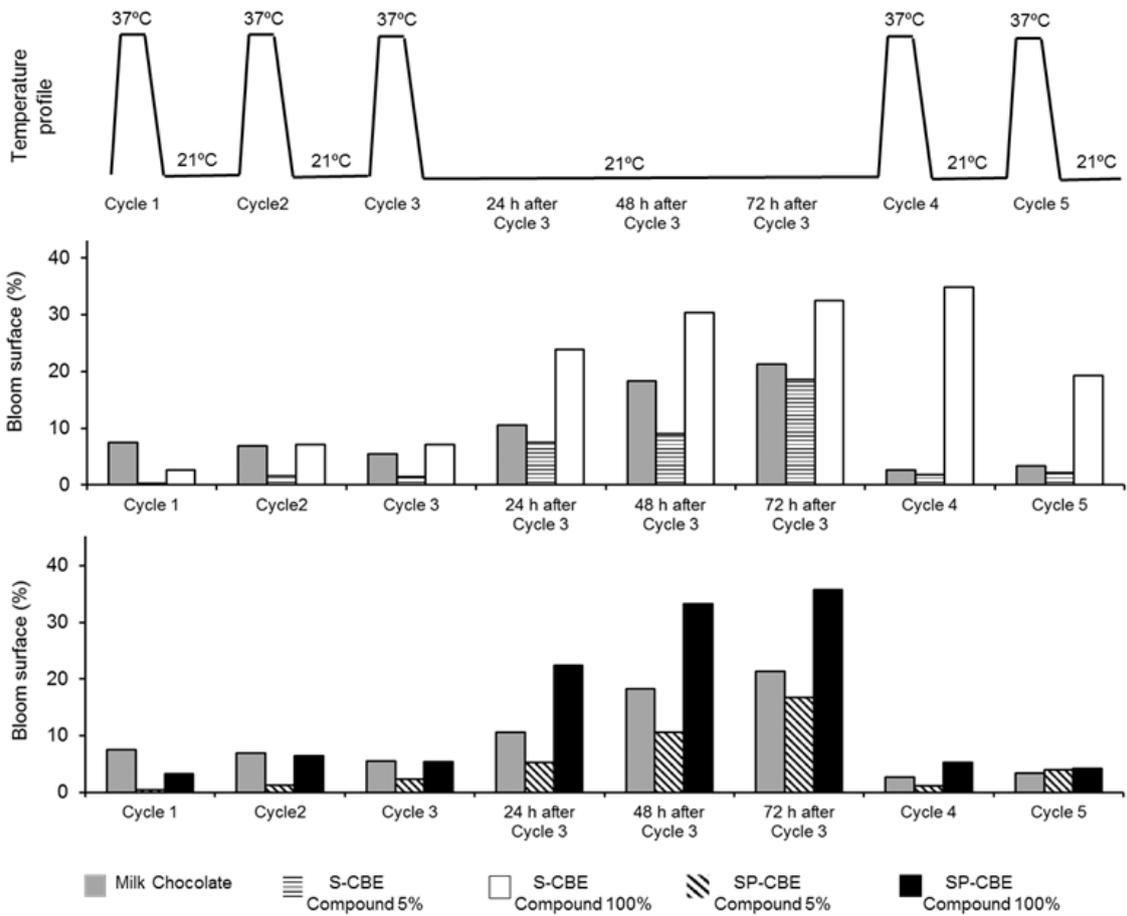
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791 **Figure 6**

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