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1 **The effect of Limonene on Bloom in Cocoa Butter and Seeded Dark Chocolate**

2 **Model**

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12

13 **Abstract**

14 This study concerns the effect of replacing a fraction of cocoa butter with limonene on
15 fat crystallization and bloom in limonene:cocoa butter blends and seeded dark chocolate
16 models prepared with these blends. Bloom is the number one chocolate quality defect in
17 consumer complaints. It is characterized by a whitish appearance of the chocolate
18 surface. One of the mechanisms driving this is the crystallization behaviour of the
19 chocolate fat phase. Samples containing up to 2 g of limonene in 30 g of
20 limonene:cocoa butter blends were stored at 20 and 29 °C changing cyclically every 12
21 h. Samples were analysed at weekly intervals up to three weeks by colour measurement
22 for the whiteness index to detect bloom, by X-ray diffraction (XRD) for crystal phase
23 determination and by DSC to assess the melting behaviour. The white colour of cocoa
24 butter limited bloom detection by colour but a large increase in whiteness index was
25 recorded for chocolate models . XRD and DSC revealed an acceleration of crystal phase
26 transformation and changes in the melting behaviour for both types of samples. Hence,
27 for practical applications it has to be considered that the use of limonene, either as a
28 flavouring or for viscosity reduction in chocolate, can potentially result in increased
29 bloom formation due to its effect on cocoa butter crystallization and polymorphism
30 transformation rate.

31

32

33

34 **Introduction**

35 Chocolate represents a highly-filled composite material formulated from sugar, cocoa
36 liquor and cocoa butter with added surfactant. Other fats may be present in small
37 amounts and milk chocolate also contains milk solids. The fat phase in dark chocolate is
38 normally present at a level of 30-40 g/100 g, which represents a dispersed volume
39 fraction close to its maximum packing fraction, Φ_m . Hence, reducing the amount of the
40 fat phase and thereby increasing the solid fraction is not an option for developing a fat-
41 reduced chocolate. This approach would not only negatively affect the processing
42 properties as viscosity would increase but also eating quality properties such as texture
43 and melting in the mouth (1). One patented approach to reduce cocoa butter without
44 concomitantly increasing the solid fraction while retaining the flow properties of the
45 product relates to replacing up to 5% of the cocoa butter fraction with the zero calorie
46 ingredient limonene (1). Limonene is a terpene found in citrus oil. Of its two isomeric
47 forms, L and D, the D-isomer is most widely found in commercial essential oils. Food
48 products containing limonene will have an orange flavour limiting the amount of
49 limonene feasible to add to chocolate.

50 The aforementioned patent (1) led to several publications on the impact of
51 limonene on the properties of chocolate. The viscosity reducing functionality was
52 validated by Do et al. (2) who demonstrated that the addition of low quantities of
53 limonene to cocoa butter led to a decrease in the liquid fat viscosity. A decrease in
54 chocolate hardness was also reported and linked to the lower solid fat content of these
55 chocolates due to mixing of limonene within the cocoa butter triglycerides. The impact
56 of limonene on the polymorphism of cocoa butter was explored in a follow up

57 publication by Ray et al. (3). Applying the methods of X-ray diffraction (XRD),
58 differential scanning calorimetry (DSC) and polarized light microscopy, they found that
59 in the presence of limonene, lower polymorphs formed on cooling. Their transformation
60 during storage into more stable polymorphs was reported to be accelerated (4, 5).

61 Re-crystallization of cocoa butter during storage is typically associated with fat
62 bloom, a negative quality attribute discernible as a white or greyish appearance of the
63 chocolate surface. It is also normally associated with the loss of gloss and a rougher
64 surface texture (6). Fat bloom occurs when less stable cocoa butter crystals undergo
65 partial melting and the liquid fat diffuses to the surface of the chocolate where re-
66 crystallization into a higher polymorphic form occurs. Cocoa butter has six polymorphs
67 with Form I being the thermodynamically most unstable form and Form VI the
68 thermodynamically most stable form (7). There are also differences in melting
69 behaviour. In commercial chocolate production only Forms IV to VI are important (8)
70 with Form IV found in untempered chocolate. Successful tempering will lead to Form
71 V, the preferred form in commercial chocolate production, since it is the highest
72 polymorph that can be process induced. Transformation to Form VI will slowly occur
73 over time and eventually lead to chocolate bloom (9). Omitting the tempering stage
74 inevitably leads to an earlier appearance of bloom.

75 Since the addition of limonene to chocolate has already been shown to affect
76 cocoa butter polymorphism (10), it can be hypothesized that it also affects bloom
77 formation. Therefore, it was the objective of this study to investigate bloom formation
78 in chocolate formulated with limonene in relation to cocoa butter crystal polymorphism.
79 To achieve this untempered limonene:cocoa butter blends and tempered dark chocolate

80 with added limonene were formulated and exposed to cyclic temperature storage for
81 three weeks in order to accelerate bloom formation. Limonene:cocoa butter blends were
82 not tempered as this was a preliminary study to assess the effect of limonene on the
83 formation of bloom on cocoa butter, without any influence from other ingredients such
84 as sugar and cocoa powder. These ingredients were reported to have the potential to
85 provide nucleation sites during the crystallization process (8). Based on the results of
86 this preliminary study chocolate formulations were tempered. All of the samples were
87 analysed after processing (at time zero) and then at weekly intervals for bloom
88 formation through colour measurement to determine the whiteness index, through
89 acquisition of XRD patterns to establish the type of polymorphism of the cocoa butter
90 and by DSC to assess the melting behaviour. The results of this study outline
91 commercial implications of this fat-reduction strategy for chocolate.

92

93 **Materials and Methods**

94 **Materials**

95 Cocoa butter, cocoa powder and sunflower lecithin were supplied by ADM (Hull, UK).
96 Soy lecithin and MyCryo Form V cocoa butter seed crystals were donated by Barry
97 Callebaut (Banbury, UK). Sunflower lecithin was used in limonene:cocoa butter blends
98 while soy lecithin was used in the chocolate preparations. Food grade limonene (97%
99 pure) was a gift from FD Copeland and Sons Ltd (London, UK). The sugar used was
100 icing sugar due to its smaller particle size compared to granulated or caster sugar, and

101 was bought from a local supermarket. All ingredients were of standard factory product
102 quality and used as received.

103

104 Preparation of Untempered Limonene:Cocoa Butter Blends

105 Untempered limonene:cocoa butter blends were prepared at three levels of limonene
106 substitution; 0%, 3.3% and 6.7% relative to the cocoa butter content on a weight basis,
107 which is equivalent to 0:30, 1:29 and 2:28 blends. The maximum level of substitution
108 (6.7%) corresponds to a level of 2.5% in a chocolate containing 38% fat and was limited
109 to this value due to taste implications. The blends were prepared by initially melting
110 cocoa butter at 50 °C for at least 24 h using an oven to erase all thermal memory.
111 Limonene was then added directly into the cocoa butter, mixed thoroughly with a
112 spatula and immediately transferred into square chocolate moulds (35 mm x 35 mm x 5
113 mm) and rectangular XRD sample holders (10 mm x 15 mm x 1 mm). 2:28
114 limonene:cocoa butter samples were moulded into a small approximately 30 mL
115 aluminium foil cup as the samples were otherwise too difficult to de-mould due to their
116 fragile soft texture. All samples were immediately transferred into an incubator set at 7
117 °C. They were kept at this temperature for 1 h before de-moulding, wrapped in
118 aluminium foil and placed into an airtight plastic container. Samples were then stored at
119 -18 °C for 5 days to minimise any crystal growth. They were then increased to room
120 temperature overnight before being transferred into an incubator set to cycle
121 temperature between 20 and 29 °C changing temperature every 12 h. Analyses were
122 carried out on the day the incubator storage started (Week 0) and then every seven days
123 for a further three weeks of storage. Two batches of all samples were prepared. Analysis

124 for each batch of samples was performed using two replicates, except for whiteness
125 index where four replicates were used for each batch. Data are presented as means and
126 standard deviations.

127

128 Preparation of Seeded Dark Chocolate Model Blends

129 The seeded dark chocolate model blends prepared in this study contained 41.5 g/100 g
130 of icing sugar, 20 g/100 g of cocoa powder and 0.5 g/100 g of lecithin. The remaining
131 38 g/100 g were cocoa butter, including the cocoa butter seed crystal fraction, with
132 limonene substitution at the same three levels as applied to the limonene:cocoa butter.
133 Based on chocolate, the seeded dark chocolate model blends contained 0 g, 1.27 g and
134 2.53 g of limonene/100 g of chocolate. All of the ingredients, including the appropriate
135 amount of cocoa butter allowing for the later addition of cocoa butter seed crystals,
136 were mixed together at 50 °C for 4 h using a household food processor with temperature
137 control (Thermomix TM31, Vorwerk, Ascot, UK). While mixing limonene was added
138 immediately after the temperature controller was switched off. Once the temperature
139 reached between 32-34 °C, depending on sample composition as detailed later, the
140 cocoa butter seed crystals were added at 1 g/100 g chocolate and mixed continuously for
141 4 min at 200-300 rpm to ensure that the seed crystals were uniformly distributed. For
142 the seeding temperature of the 0:30 blend, the seed crystal supplier's recommendation
143 of 34 °C for dark chocolate was followed and seed crystals were added between 33-34
144 °C. As the addition of limonene lowers the viscosity of the mix, the slightly lower
145 temperature window of 32-33 °C was chosen as the seeding temperature for the 1:29
146 and 2:28 blends. The seeded chocolate was then poured into square plastic moulds (38

147 mm x 38 mm x 8 mm). These were placed into an incubator (MIR-153, Sanyo Electric
148 Biomedical Co., Bunkyo, Tokyo, Japan) and kept at 10 °C for 30 min to set. The
149 chocolate model was then de-moulded and the temper status evaluated using DSC. The
150 remaining chocolate model samples were sealed into an aluminium pouch and aged at
151 20 °C for one week to accelerate the formation of higher stable polymorphs before the
152 accelerated storage trial applying the same conditions as for the limonene:cocoa butter
153 blends. “Week 0” in the following tables and graphs refers to the start of this storage
154 trial. All analyses for each batch were carried out on two replicates and data are
155 presented as means and standard deviations. As before whiteness index was performed
156 on four replicates for each batch.

157

158 Determination of Whiteness Index

159 The development of bloom was followed through quantifying the whitish appearance on
160 the surface of the cocoa butter and the chocolate samples by the whiteness index (WI)
161 defined in Equation 1 (11-13). The parameters L , a and b were obtained from measuring
162 the surface colour of the samples with a Hunter colorimeter (Hunter Lab Ultrascan
163 Colorimeter, Hunter Associates Inc., Reston, USA). After calibrating the instrument
164 with white and black glass standards, several spots of each sample were scanned and the
165 whiteness index calculated with the equipment’s software using the following equation:

$$166 \quad WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad (\text{Equation 1})$$

167 The results were statistically analysed to compare the means of the WI between
168 the weeks of storage for each limonene concentration using one-way ANOVA.

169 Significant differences between samples were analysed using Tukey HSD (Honest
170 Significant Difference) multiple comparisons test at 95% significance level.

171

172 Acquisition of X-Ray Powder Diffraction Patterns

173 X-Ray powder diffraction (XRD) patterns were acquired using an X-Ray diffractometer
174 (D5005, Bruker, UK) at room temperature (20-22°C). The radiation was
175 monochromated copper K alpha ($\text{CuK}\alpha$) with a wavelength of 1.5418 Å. A slit focus
176 reflection geometry was used and scans were run over 2θ values between 3 and 38° at
177 0.05° intervals with a scan time of 2.5 s per interval. This protocol has previously been
178 applied to limonene containing cocoa butter (3). The XRD patterns were analysed for d -
179 values using Diffrac Plus V1.01.

180 While cocoa butter samples were directly scanned as moulded into the XRD
181 sample holders, chocolate samples required the removal of the sugar from the chocolate
182 sample as the intense sugar diffraction peaks would overlay the diffraction pattern of the
183 cocoa butter rendering data interpretation difficult. A slight modification of the
184 published method of Cebula and Ziegleder (14) was followed. The chocolate was
185 chopped into small pieces, with largest dimensions of 0.5-1.5 mm or less. These pieces
186 were placed into cold water at a ratio of at least 1:100 (w/v) of chocolate:water. The
187 mixture was vigorously mixed for about 5 min and left to stand for at least 2 h for the
188 sugar to dissolve. The mixture was then filtered to remove the water and the
189 undissolved material was left at room temperature until most of the water had
190 evaporated. Finally, the leftover material was pressed into a rectangular XRD sample

191 holder (10 mm x 15 mm x 1 mm) and the surface levelled with a blade. XRD patterns
192 were acquired every week for the course of the three weeks of cyclic temperature
193 storage.

194

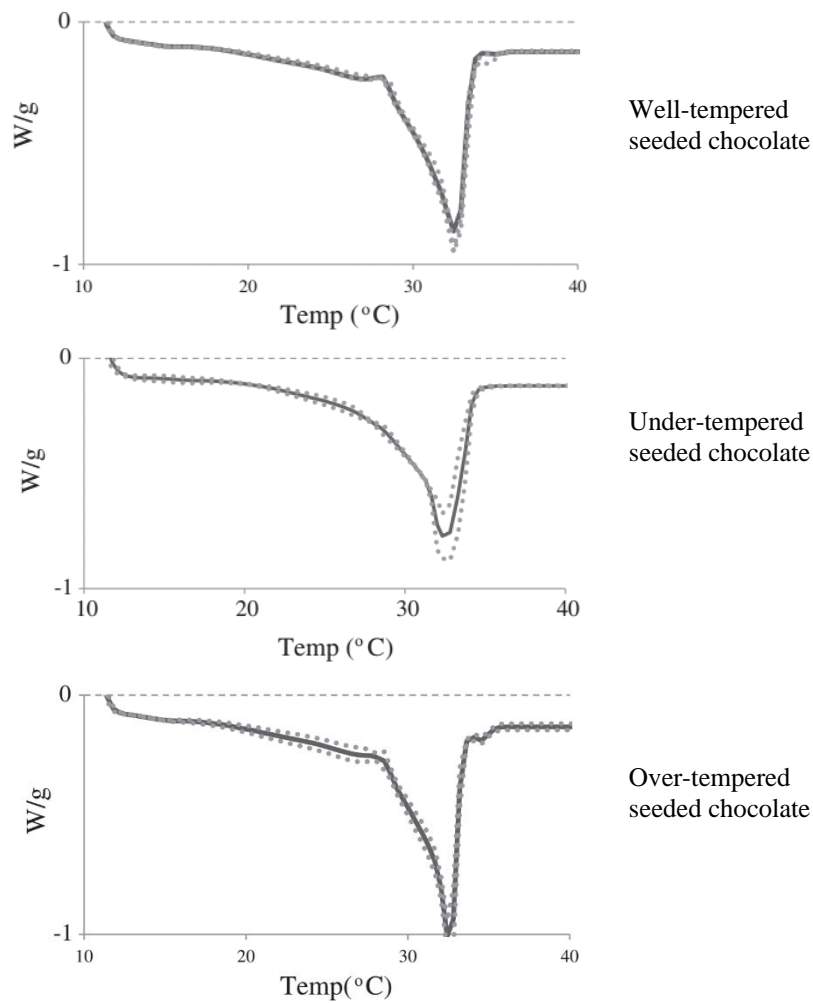
195 Evaluation of Thermal Properties

196 Differential scanning calorimetry measurements were carried out on the chocolate
197 samples to observe the thermal behaviour of the chocolate during cyclic temperature
198 storage and to ascertain the state of temper immediately after chocolate solidification.
199 All DSC analyses were carried out using a Mettler Toledo DSC Model 823e calorimeter
200 (Mettler Toledo, Zurich, CH) fitted with an auto sampler and liquid nitrogen cooling
201 accessory. A sealed empty aluminium pan was used as reference. Results are presented
202 as normalized heat flow (W per g) of sample. The onset temperature (T_{onset}), peak
203 temperature (T_{peak}) and endset temperature (T_{end}) of melting were determined using
204 Mettler Toledo Star software following standard protocols such as that in reference (15).

205 The tempering status of the seeded chocolate was evaluated from a DSC melting
206 curve following published work (16). Since no reference exists of a DSC melting
207 pattern which confirms the tempering status of cocoa butter in the presence of limonene
208 a well-tempered status was judged to be present when the pattern was comparable to the
209 pattern published in the aforementioned reference, see Figure 1. Well-tempered samples
210 showed a peak where the area under the curve was not too broad and not too narrow. A
211 too broad peak indicated an ‘under-tempered’ sample while a too narrow peak indicated
212 an ‘over-tempered’ sample. Here, the DSC evaluation was carried out immediately after

213 chocolate setting at 10 °C for 30 min. Approximately 15 mg of sample were placed into
214 an aluminium pan that was then hermetically sealed. Samples were loaded into the DSC
215 furnace at 10 °C, held for 3 min at this temperature and then heated to 50 °C at 4
216 °C/min.

217



218

219 **Fig. 1** DSC melting curves after solidification of chocolate. Reproduced from (16)
220 with slight modification and permission from the publisher.

221

222 To evaluate the thermal behaviour of the three chocolate model blends during the
223 cyclic temperature storage, the protocol published by Fessas et al. (17) was followed.
224 About 15 mg of sample were hermetically sealed into an aluminium pan and loaded into
225 the DSC furnace at 20 °C. The temperature was then lowered to 15 °C at 10 °C/min,
226 held at this temperature (15 °C) for 5 min followed by an increase to 50 °C at 2 °C/min.

227

228

229 **Results and Discussion**

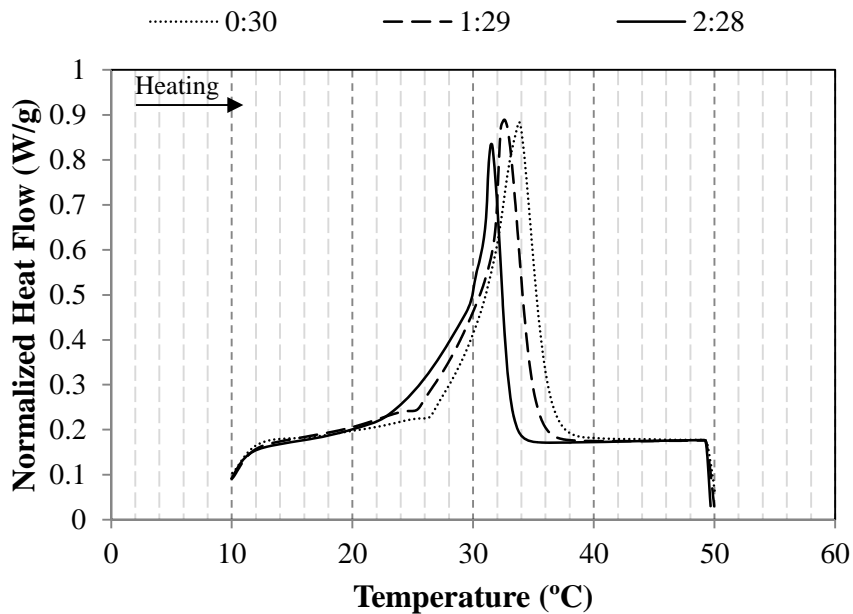
230 The results relating to the tempering status of seeded dark chocolate samples are
231 presented first followed by all other data acquired on both sets of samples.

232

233 **Tempering Status of the Dark Chocolate Model Blends**

234 The tempering status of the seeded dark chocolate model blends containing 0%, 3.3%
235 and 6.7% of limonene in the fat phase was evaluated by comparing their DSC melting
236 curves, see Figure 2, to the published patterns reproduced in Figure 1. As mentioned in
237 the methods section a well-tempered chocolate would show a narrower peak than an
238 under-tempered sample due to the narrower distribution of polymorphic forms (16).
239 Figure 2 shows that the melting curve of the seeded chocolate with 0% limonene
240 appeared to be that of a well-tempered chocolate. The peak temperature of 33.9 °C
241 indicated that the majority of the cocoa butter crystals was in Form V (7). The melting
242 curves of the seeded chocolates containing limonene had a similar profile to the curve of

243 the control sample although they were increasingly shifted to lower temperatures as the
244 limonene substitution increased as previously reported (2). Here, T_{peak} was 32.6 °C and
245 31.5 °C for the chocolate containing 3.3% and 6.7% limonene in the fat phase,
246 respectively. The values for T_{onset} were 25.5 °C, 23.7 °C and 22.0 °C in order of
247 increasing limonene substitution.



248

249 **Fig. 2** Thermal behaviour of the seeded chocolate samples containing different levels
250 of limonene

251

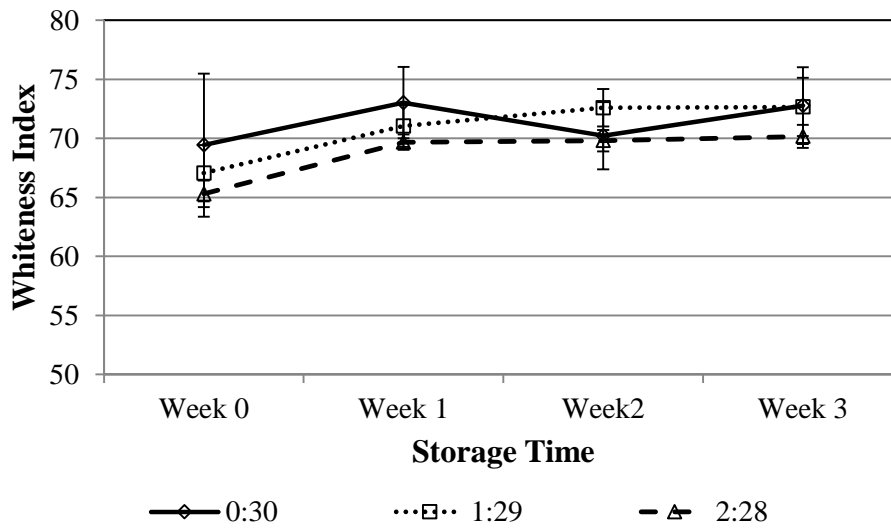
252 Bloom Formation Evaluated Visually and by Whiteness Index

253 Bloom formation on the dark chocolate models were clearly visible to the naked eye. As
254 early as in Week 0, the chocolate samples containing limonene showed a matt surface,
255 which was in stark contrast to the shiny surface of the sample not containing limonene.
256 During the three weeks of cyclic temperature storage bloom visibly increased whereas

257 the appearance of the sample not containing limonene remained the same. Bloom
258 formation on the limonene:cocoa butter blends was hardly visible and none of the
259 samples had a shiny surface. In case of the sample without limonene this was most
260 likely due to the untempered status of the cocoa butter. These visual observations, both
261 on the limonene:cocoa butter blends and the seeded chocolate model samples, were
262 reflected in the data acquired for the whiteness index, WI. WI was measured over the
263 three weeks of cyclic temperature storage and an increase in WI would signify bloom.
264 In case of the limonene:cocoa butter blends, see Figure 3, WI changed little over storage
265 with only a slight increase seen between Week 0 and Week 1 irrespective of the
266 concentration of limonene in the blends. The results were compromised by the naturally
267 white colour of cocoa butter and reflected the visual appearance of the sample surfaces.

268 The data acquired on the chocolate model samples were more meaningful as
269 expected from the visual assessment, see Figure 4. With the exception of the Week 0
270 data, acquired at the beginning of the cyclic temperature storage trial, WI was higher at
271 a higher level of cocoa butter substitution with limonene. The control sample was seen
272 to have a largely unchanged value of WI over the three week course of storage. Upon
273 substituting cocoa butter with 3.3% limonene, WI showed a slight increase between
274 Week 1 and Week 2, followed by a higher increase between Week 2 and Week 3. At the
275 higher level of cocoa butter substitution with limonene a more pronounced increase in
276 WI between Week 1 and Week 2 was observed. Since bloom formation is the result of
277 cocoa butter polymorphism, and in particular re-crystallisation into higher forms, this
278 experimental evidence of limonene addition into cocoa butter and chocolate favouring
279 bloom formation was strengthened by acquiring X-ray powder diffraction patterns.

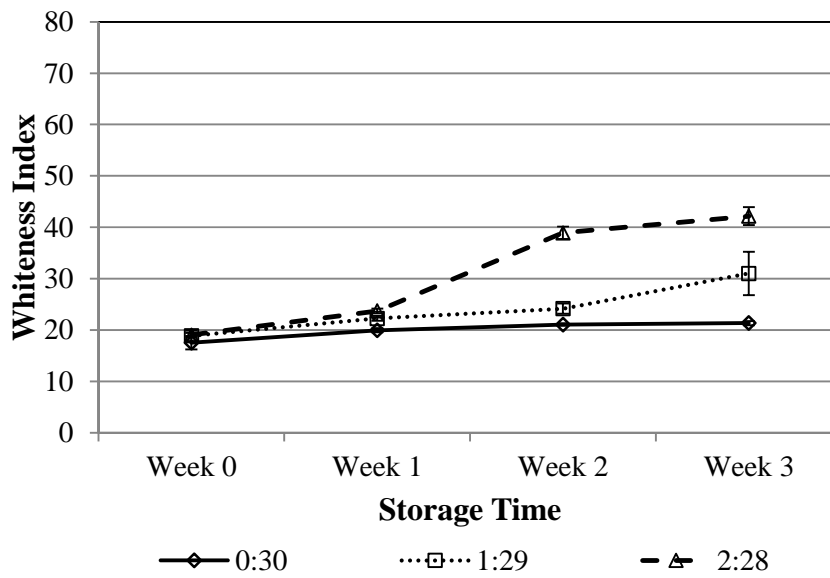
280



281

282 **Fig. 3** Whiteness index of limonene:cocoa butter blends.

283



284

285 **Fig. 4** Whiteness index of seeded dark chocolate samples containing different levels
286 of limonene in the fat phase.

287

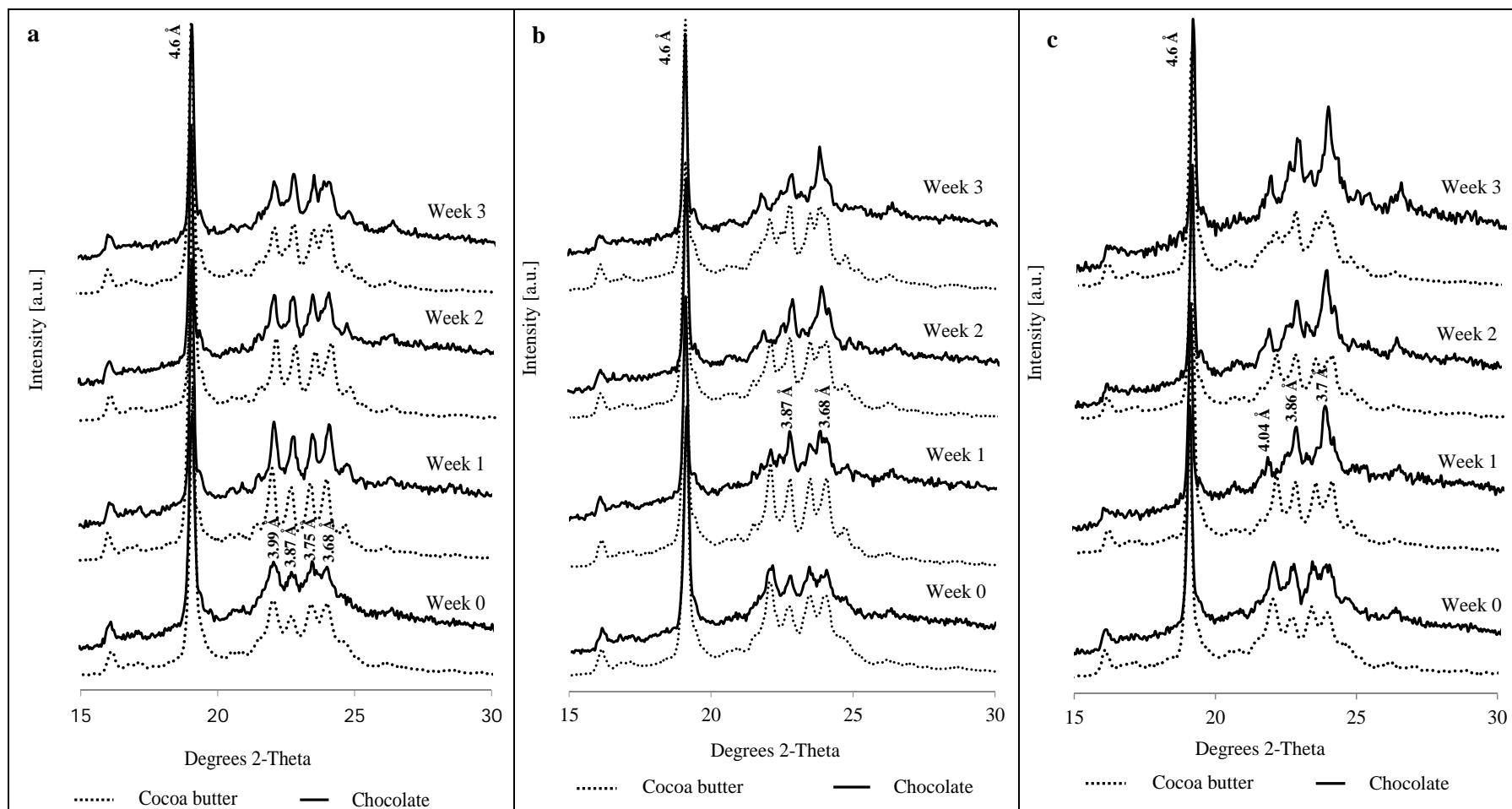
288 XRD Patterns

289 The XRD patterns acquired on the limonene:cocoa butter and the seeded dark chocolate
290 model blends are shown in Figure 5. The identification of the polymorphic forms was
291 undertaken by comparing the values of the *d*-spacing with published work (12, 18). The
292 strong diffraction peak at 4.6 Å and four smaller peaks at 3.99, 3.87, 3.75 and 3.68 Å,
293 featuring in Figure 5a, are evidence for Form V crystals in both types of samples
294 prepared in the absence of limonene. Form V was prevalent throughout the three-week
295 storage period. Both of the limonene containing cocoa butter samples, i.e., the 1:29 and
296 2:28 blends, were initially in Form V, see Figure 5b) and 5c). The XRD patterns
297 acquired at Week 3 show that the diffraction peak at 3.99 Å was reduced in height and
298 shifted towards larger *d*-spacings, while the intensity of the peaks at 3.68 and 3.87 Å was
299 increased. This is evidence for the presence of Form VI crystals.

300 The cocoa butter in both of the limonene containing seeded dark chocolate model
301 samples was in Form V at Week 0. The sample with the 1:29 limonene:cocoa butter
302 blend showed a mixture of Form V and Form VI in Week 1 whereas the crystals in the
303 chocolate sample with the higher limonene substitution in the fat phase (2:28
304 limonene:cocoa butter) appeared by this time to have already fully transitioned into
305 Form VI. By the following Week 2 this transitioning had also occurred for the 1:29
306 limonene:cocoa butter blend.

307

308



309

Fig. 5 XRD patterns acquired at weekly intervals for the three limonene:cocoa butter blends labelled “Cocoa butter” in the graphs and seeded dark chocolate samples labelled “Chocolate” at limonene:cocoa butter blend ratios of: a) 0:30; b) 1:29; c) 2:28.

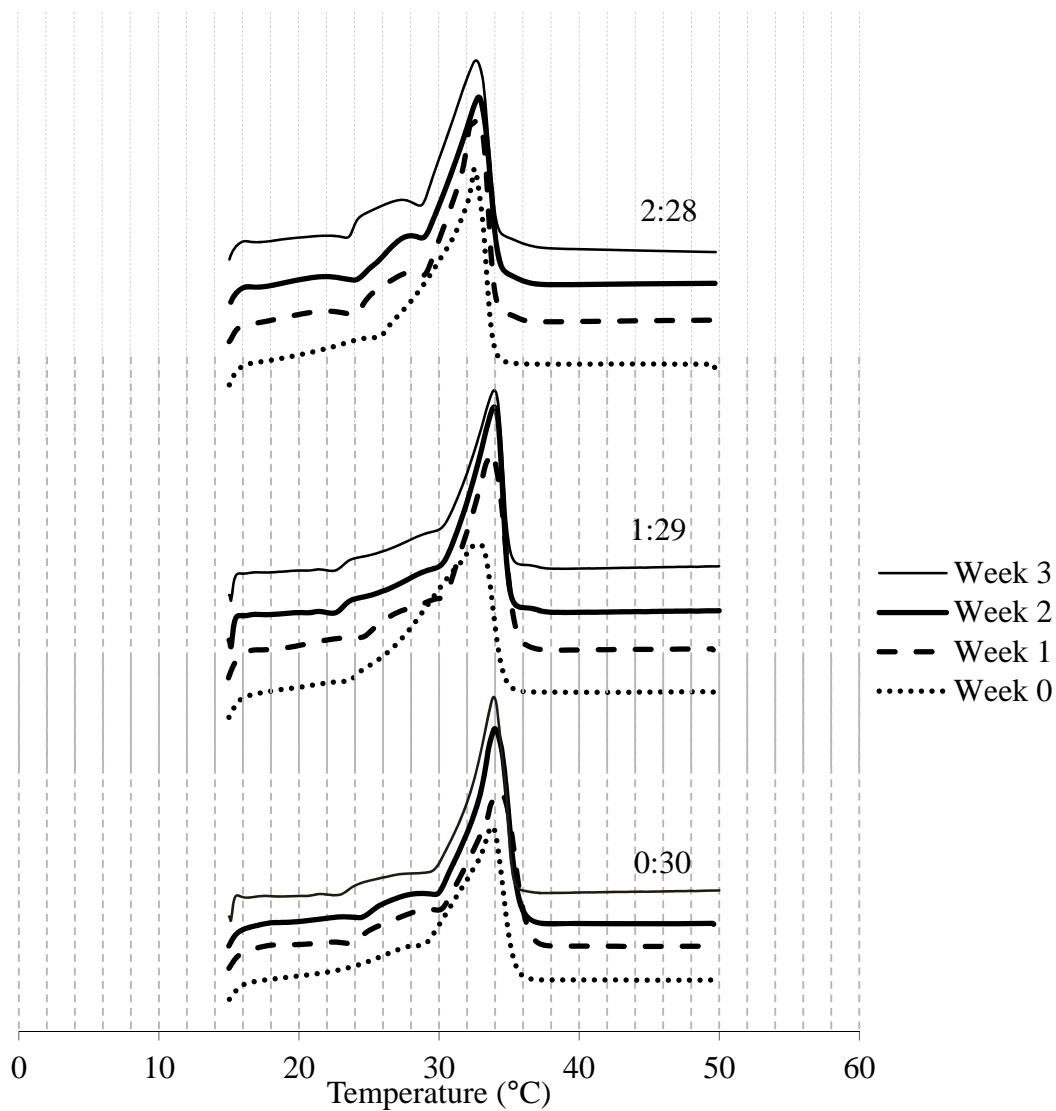
310 It is unusual to find Form V crystals in freshly prepared samples of untempered
311 cocoa butter. One would expect to find Form IV crystals instead (9) as Form V is
312 normally produced through a tempering process. However, Form V can also appear if
313 chocolate is exposed to low temperatures, for example in a cooling tunnel (19). As the
314 preparation of the cocoa butter samples involved a cooling step at 7 °C for 1 h, this may
315 be the reason for the sample to be in Form V at Week 0.

316 Incorporation of limonene into the sample has resulted in a rapid transformation
317 of the cocoa butter crystal from a lower to a higher polymorphic form. The rate of this
318 transition increased with the amount of limonene and in the presence of sugar and cocoa
319 particles. Due to the commercial relevance the chocolate samples were also submitted to
320 thermal analysis for validation of the observations based on the analysis of the XRD
321 patterns.

322

323 Thermal Properties of the Chocolate Model Samples

324 The results of the thermal analysis on the seeded dark chocolate model samples
325 acquired during cyclic temperature storage are depicted in Figure 6. The corresponding
326 characteristic temperature values are reported in Table 1.



327

328 **Fig. 6** DSC melting curves of seeded dark chocolate model samples acquired during
 329 cyclic temperature storage. Blends are identified by their limonene:cocoa butter blend
 330 ratio.

331

332

333 **Table 1** Characteristic temperatures of seeded dark chocolate samples obtained from
 334 the DSC thermograms shown on Figure 6. “Blend ratio” refers to the limonene:cocoa
 335 butter ratio in the fat phase.

Blend ratio	Temperature (°C)	Week 0	Week 1	Week 2	Week 3
0:30	T _{onset}	26.82	23.78	24.58	23.48
	T _{peak}	33.01	34.81	34.34	34.04
	T _{end}	35.68	36.66	35.94	35.84
1:29	T _{onset}	23.64	22.94	22.68	22.81
	T _{peak}	33.14	33.95	34.11	34.11
	T _{end}	34.92	35.75	35.21	34.84
2:28	T _{onset}	24.54	24.04	23.88	23.52
	T _{peak}	32.64	33.03	33.03	32.91
	T _{end}	34.02	33.64	33.80	33.73

336

337 The temperature data demonstrate that in the freshly prepared chocolate model
 338 samples (Week 0) presence of the lower of the two amounts of limonene substitutions
 339 included in this study lead to a decrease of T_{onset}. At the higher substitution level T_{onset}
 340 was slightly higher but still lower than for the chocolate model not containing limonene.
 341 T_{peak} remained about the same whereas T_{end} gradually decreased with increasing
 342 limonene substitution. After Week 1 in cyclic temperature storage the temperature at
 343 which all of the sample had melted (T_{end}) had shifted to a higher value except for the
 344 sample with the highest limonene substitution. An increase in T_{end} is synonymous with

345 the formation of a higher polymorphic form. The lack of temperature increase for the
346 sample with the highest limonene substitution suggests perhaps that Form VI crystals
347 were already present in the freshly prepared sample. The XRD patterns, however,
348 suggested transitioning from Form V to Form VI during the first week of storage. So, it
349 appears that limonene causes two opposite effects in terms of thermal properties.
350 Formation of bloom increases the temperatures whereas incorporation of limonene as
351 liquid substitute for cocoa butter decreases the temperature.

352 The appearance of a small broad peak at the onset of melting, in the region of 24-
353 28 °C, acquired on the seeded dark chocolate model blend with a fat phase
354 limonene:cocoa butter ratio of 2:28 was surprising. It may indicate the separation of
355 lower polymorphic crystals through the presence of limonene. If this phase could be
356 separated from the blend XRD analysis could be applied to test this hypothesis.

357 The effect of limonene in lowering the melting temperature of chocolate samples
358 while driving the fat suspension to a higher polymorphic form of crystals could be due
359 to several factors. Incorporating a low molecular weight hydrophobic compound has
360 previously been claimed to solubilize the solid crystals that had formed in the mixture
361 and has been associated with the reduced amount of solid fat content (SFC) (20, 21).
362 Limonene is a low molecular weight hydrophobic compound and, therefore, following
363 incorporation into chocolate, it could be expected to produce the same effect. This is
364 effectively a colligative lowering of the crystal melting point without changing the
365 polymorphic form. The addition of limonene will increase the proportion of liquid at a
366 given temperature and, thus, reduce the proportion of solid crystals in the mixture. This
367 has been shown in the study of Do et al. (2) where the substitution of cocoa butter with

368 limonene at levels of up to 3% reduced the SFC by over 50% at 25 °C. These
369 observations were explained by the solubilisation effect of limonene dissolving unstable
370 fat crystals which then remain in liquid form in the crystal network of the cocoa butter
371 in the chocolate. Hence, limonene caused the chocolate to have a softer texture and a
372 lower melting temperature (4).

373 The presence of liquid, limonene in this study, has previously been shown to alter
374 the crystallization kinetics of cocoa butter (20-22). So, the liquid limonene appeared to
375 increase the rate of polymorphic transition due to the increased mobility of the
376 crystallisation nuclei (23). The oil-mediated (or liquid-mediated) transformation of
377 crystals has been described as either initiated by spontaneous nucleation in liquid or by
378 heterogeneous nucleation at the surfaces of existing crystals (22). At higher storage
379 temperature (29 °C in this study) the partial melting of cocoa butter would increase the
380 amount of liquid in the sample where triacylglycerol molecules detach from dissolving
381 crystals of Form V and form nuclei of Form VI crystal through volume diffusion in the
382 oil matrix (22). Higher concentrations of limonene promote a higher amount of liquid in
383 the sample at that temperature. Upon lowering the storage temperature (20 °C in this
384 study), heterogeneous nucleation of Form VI may occur as mentioned by Sato and
385 Koyano (22). The growth rate of crystals was observed to be many times faster than the
386 nucleation rate in the presence of liquid (21) hence, a smaller number of large crystals
387 are expected to develop (higher polymorphic form crystals but in a smaller quantity).
388 Large crystal size in the presence of limonene has previously been observed by Ray et
389 al. (3) who showed large distinct feather-shaped spherulites. In relation to bloom the
390 presence of liquid in the microstructure will accelerate the diffusion of fat to the surface

391 promoting recrystallisation and enhancing bloom production. Therefore, the higher the
392 concentration of limonene in the sample, the higher the rate of bloom formation.

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395 **Conclusions**

396 The dark colour of chocolate compared to cocoa butter made the measurement of
397 whiteness index in detecting bloom more reliable. A higher amount of limonene in the
398 sample promoted faster development of bloom. It was also confirmed that in the
399 presence of limonene more stable cocoa butter crystals formed more quickly during
400 cyclic temperature storage. This property of limonene has been explained by a solubility
401 effect where unstable cocoa butter crystals solubilize in the liquid limonene containing
402 fat phase, which co-exists in the cocoa butter fat crystal network. While limonene may
403 be a commercially interesting ingredient to formulate chocolate at a lower level of
404 cocoa butter without compromising viscosity properties (1), the demonstrated
405 accelerated bloom formation makes this a less attractive ingredient for moulded
406 chocolate bars.

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