The effect of limonene on bloom of cocoa butter and seeded dark chocolate model

Mohamad, Nizaha Juhaida; MacNaughtan, Bill; Vieira, Joselio; Wolf, Bettina

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

Nizaha Juhaida Mohamad[1,2*], Olga Smirnova[3], Bill MacNaughtan[2], Josélio Vieira[3], Bettina Wolf[2] ·

[1] Department of Food Sciences, School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

[2] Division of Food Sciences Sutton Bonington Campus, School of Biosciences, The University of Nottingham, Loughborough, Leicestershire LE12 5RD, UK

[3]Nestlé Product Technology Centre York, PO Box 204, Haxby Road, YO91 1XY York, UK

*niezaju@umt.edu.my

Abstract

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

Introduction

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

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

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

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

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

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

Materials and Methods

Materials

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

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

Preparation of Untempered Limonene:Cocoa Butter Blends

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

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

Preparation of Seeded Dark Chocolate Model Blends

The seeded dark chocolate model blends prepared in this study contained 41.5 g/100 g of icing sugar, 20 g/100 g of cocoa powder and 0.5 g/100 g of lecithin. The remaining 38 g/100 g were cocoa butter, including the cocoa butter seed crystal fraction, with limonene substitution at the same three levels as applied to the limonene:cocoa butter. Based on chocolate, the seeded dark chocolate model blends contained 0 g, 1.27 g and 2.53 g of limonene/100 g of chocolate. All of the ingredients, including the appropriate amount of cocoa butter allowing for the later addition of cocoa butter seed crystals, were mixed together at 50 °C for 4 h using a household food processor with temperature control (Thermomix TM31, Vorwerk, Ascot, UK). While mixing limonene was added immediately after the temperature controller was switched off. Once the temperature reached between 32-34 °C, depending on sample composition as detailed later, the cocoa butter seed crystals were added at 1 g/100 g chocolate and mixed continuously for 4 min at 200-300 rpm to ensure that the seed crystals were uniformly distributed. For the seeding temperature of the 0:30 blend, the seed crystal supplier's recommendation of 34 °C for dark chocolate was followed and seed crystals were added between 33-34 °C. As the addition of limonene lowers the viscosity of the mix, the slightly lower temperature window of 32-33 °C was chosen as the seeding temperature for the 1:29 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., Bunkyoku, 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 $$WI = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2} \quad \text{(Equation 1)}$$

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 (CuKα) 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

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

Evaluation of Thermal Properties

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

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

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

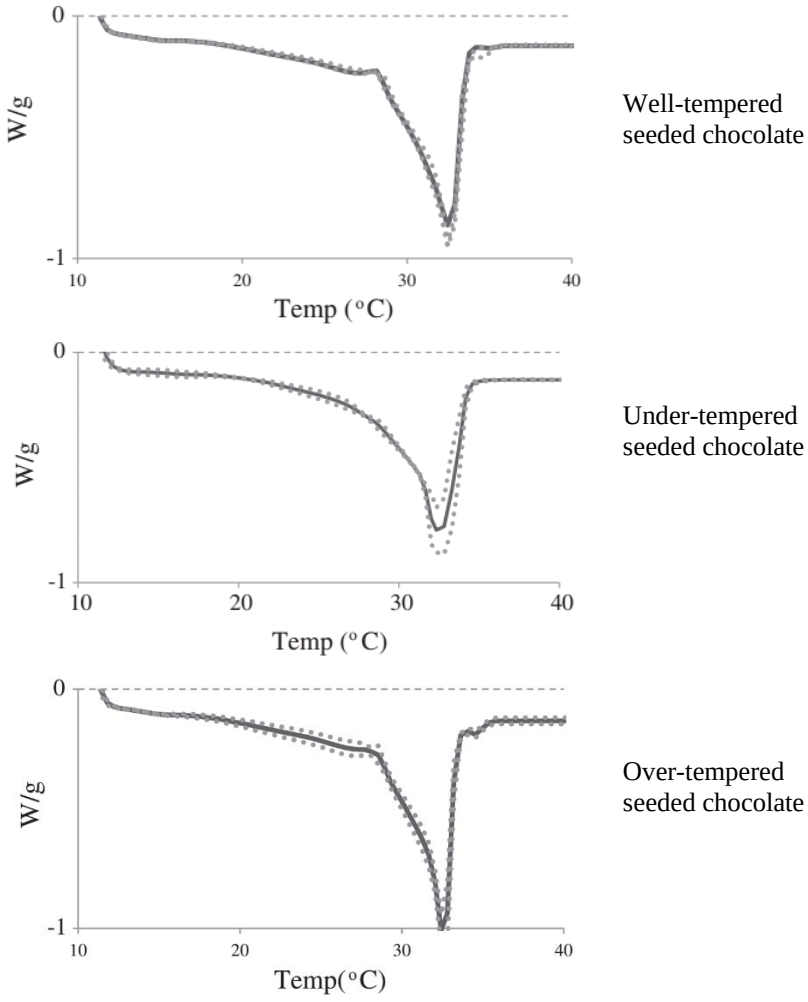


Fig. 1 DSC melting curves after solidification of chocolate. Reproduced from (16) with slight modification and permission from the publisher.

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

Results and Discussion

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

Tempering Status of the Dark Chocolate Model Blends

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

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

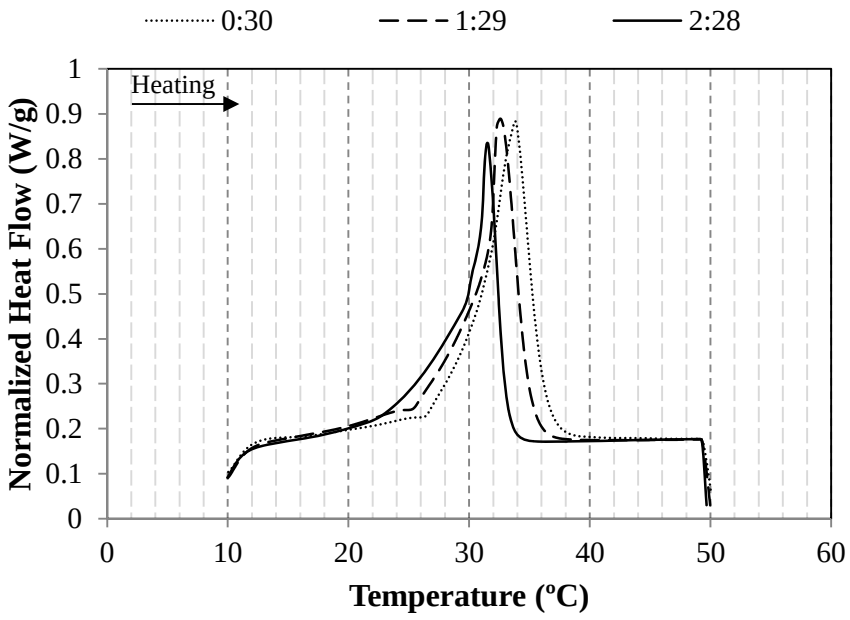


Fig. 2 Thermal behaviour of the seeded chocolate samples containing different levels of limonene

Bloom Formation Evaluated Visually and by Whiteness Index

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

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

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

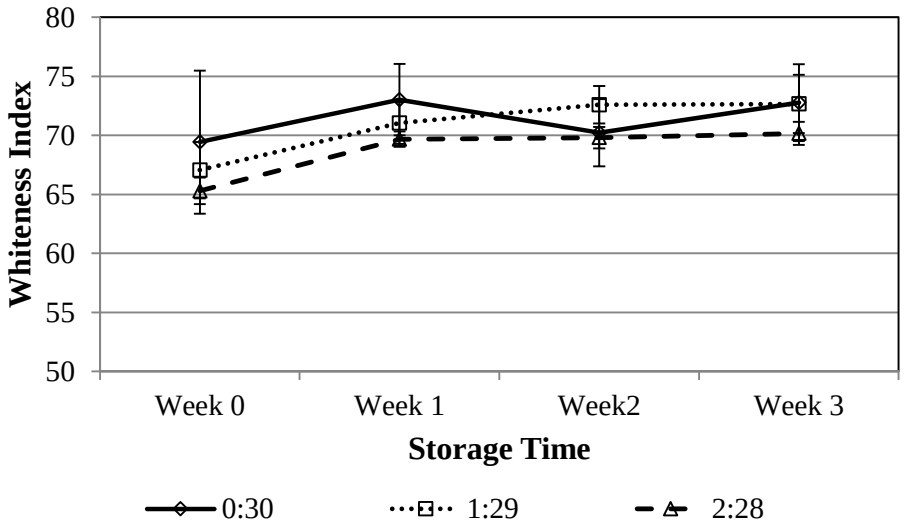


Fig. 3 Whiteness index of limonene:cocoa butter blends.

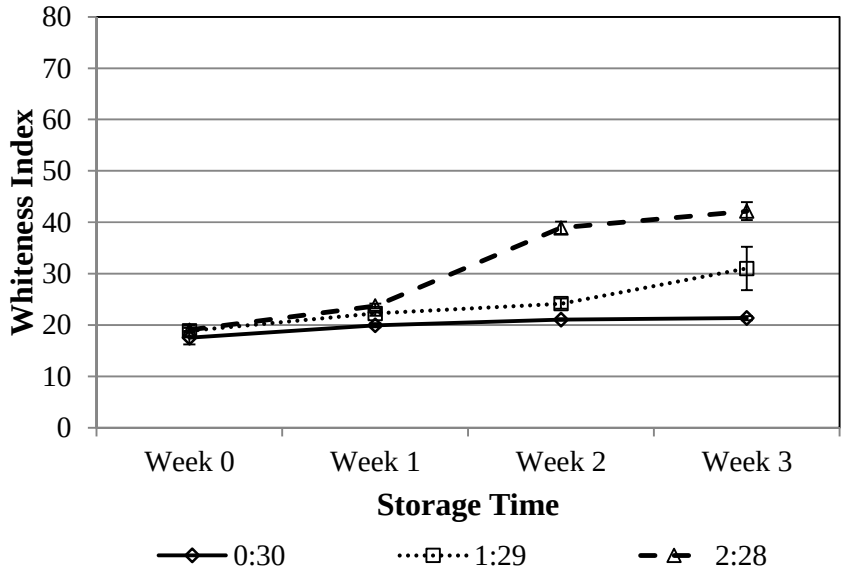


Fig. 4 Whiteness index of seeded dark chocolate samples containing different levels of limonene in the fat phase.

287

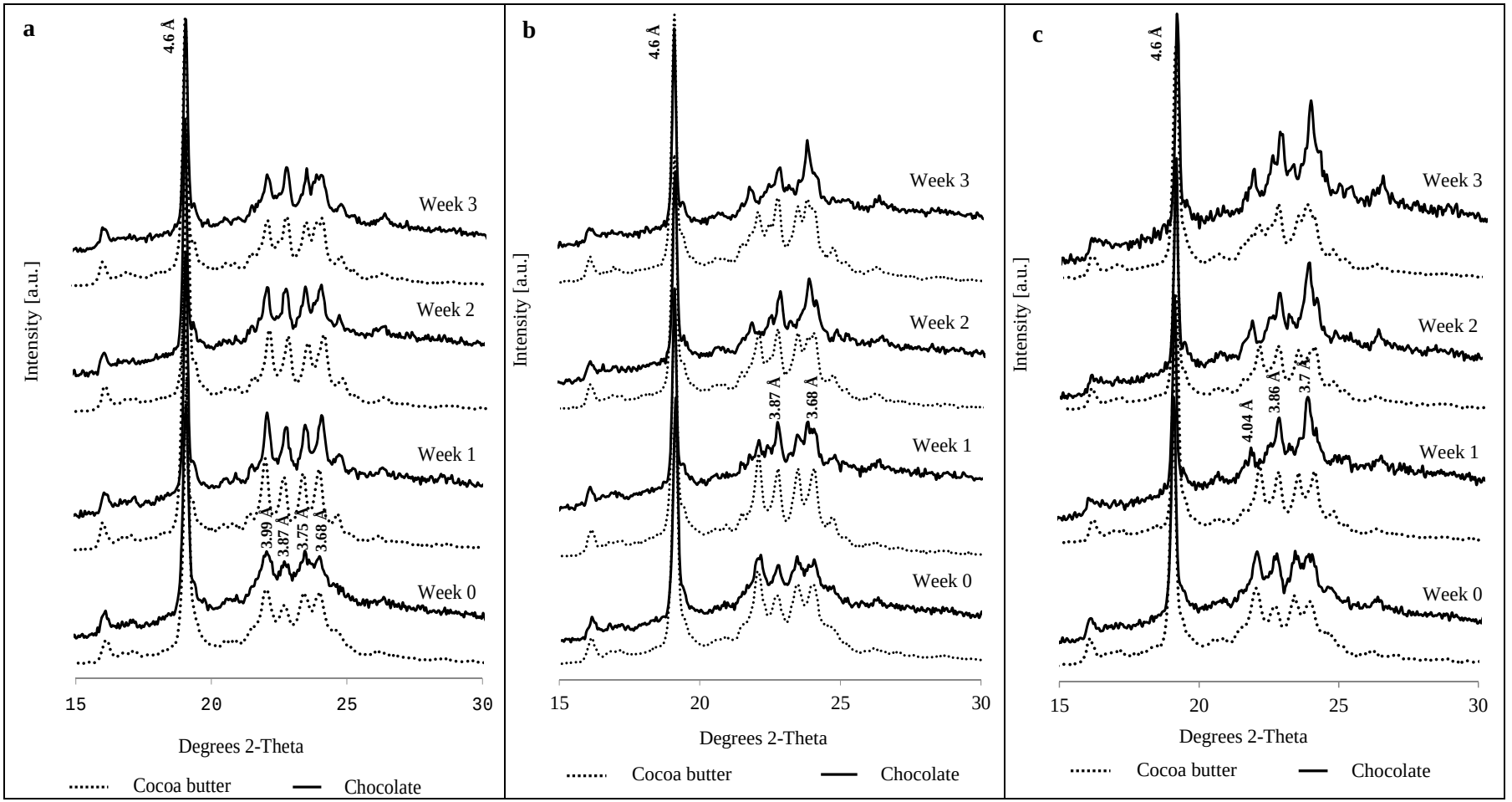
288 XRD Patterns

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 lager *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.

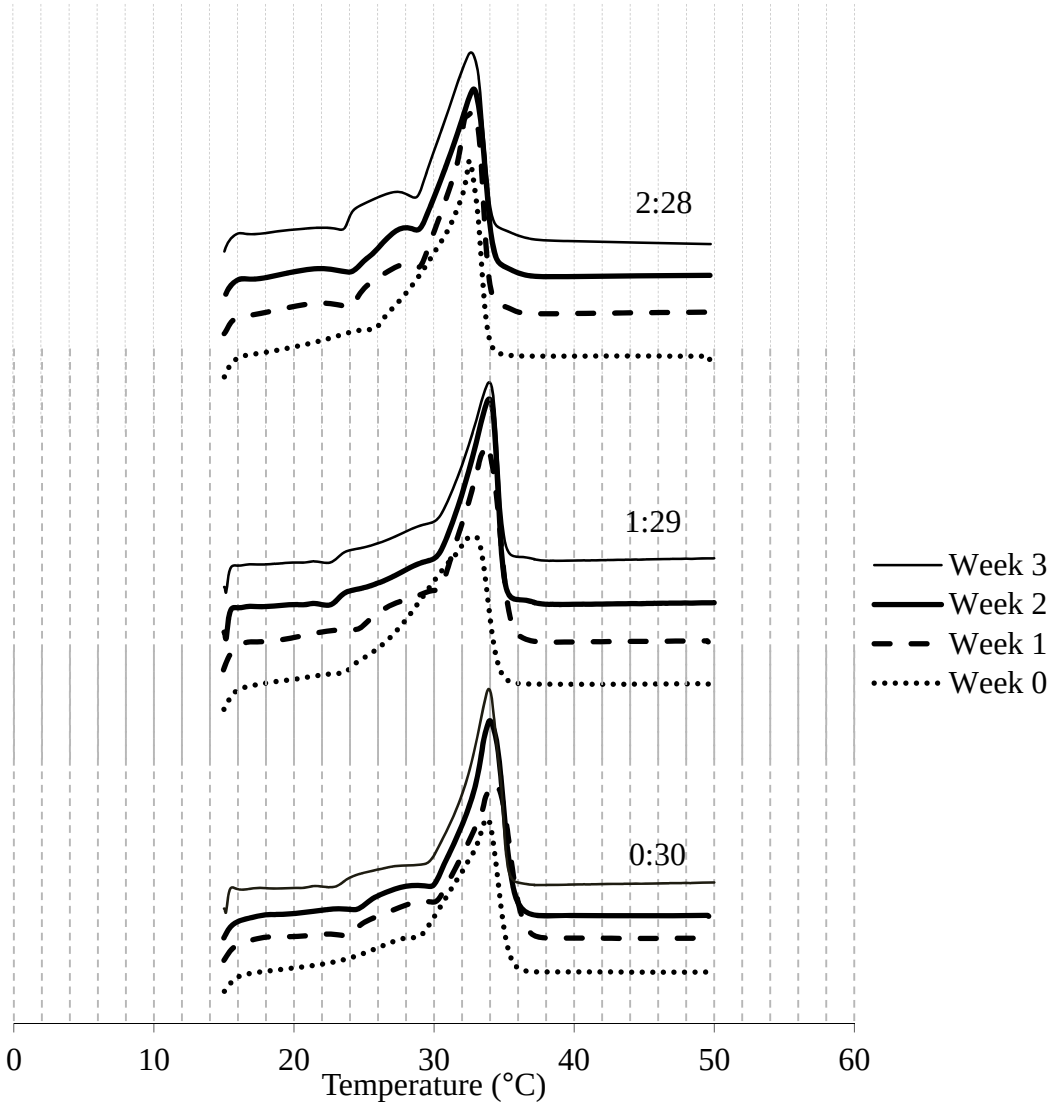
310 It is unusual to find Form V crystals in freshly prepared samples of untempered
311 cocoa butter. One would expect to find Form IV crystals instead (9) as Form V is
312 normally produced through a tempering process. However, Form V can also appear if
313 chocolate is exposed to low temperatures, 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

Table 1 Characteristic temperatures of seeded dark chocolate samples obtained from the DSC thermograms shown on Figure 6. "Blend ratio" refers to the limonene:cocoa 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

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

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

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

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

Conclusions

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

Acknowledgements

This work was part of NJM's PhD project funded by The Ministry of Higher Education, Malaysia and the University of Malaysia Terengganu. Many thanks also to the

University of Nottingham (UK), their Food Sciences laboratory staff and The Nestle PTC York for the technical support.

References

1. Beckett ST (1999) Preparation of chocolate with limonene to reduce fat content, USA Patent
2. Do TAL, Vieira J, Hargreaves JM, Wolf B, Mitchell JR (2008) Impact of limonene on the physical properties of reduced fat chocolate, J Am Oil Chem Soc 85:911-920
3. Ray J, MacNaughtan W, Chong PS, Vieira J, Wolf B (2012) The Effect of Limonene on the Crystallization of Cocoa Butter, J Am Oil Chem Soc 89:437-445
4. Rigolle A, Goderis B, Van Den Abeele K, Foubert I (2016) Isothermal Crystallization Behavior of Cocoa Butter at 17 and 20° C with and without Limonene, J Agr Food Chem 64:3405-3416
5. Miyasaki EK, Santos CAd, Vieira LR, Ming CC, Calligaris GA, Cardoso LP, Gonçalves LAG (2015) Acceleration of polymorphic transition of cocoa butter and cocoa butter equivalent by addition of d-limonene, European Journal of Lipid Science and Technology
6. Lonchampt P, Hartel RW (2006) Surface bloom on improperly tempered chocolate, European Journal of Lipid Science and Technology 108:159-168
7. Wille RL, Lutton ES (1966) Polymorphism of cocoa butter, J Am Oil Chem Soc 43:491-&
8. Svanberg L, Ahrne L, Loren N, Windhab E (2011) Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems, Journal of Food Engineering 104:70-80
9. Timms RE (2002) Oil and fat interactions, Manufacturing Confectioner 82:50-64

10. Ray J, MacNaughtan W, Chong P, Vieira Jl, Wolf B (2012) The Effect of Limonene on the Crystallization of Cocoa Butter, Journal of the American Oil Chemists' Society:1-9
11. Bricknell J, Hartel R (1998) Relation of fat bloom in chocolate to polymorphic transition of cocoa butter, Journal of the American Oil Chemists' Society 75:1609-1615
12. Sonwai S, Rousseau D (2006) Structure evolution and bloom formation in tempered cocoa butter during long-term storage, European Journal of Lipid Science and Technology 108:735-745
13. Sonwai S, Rousseau D (2008) Fat crystal growth and microstructural evolution in industrial milk chocolate, Crystal Growth & Design 8:3165-3174
14. Cebula DJ, Ziegleder G (1993) Studies of Bloom Formation Using X-Ray-Diffraction from Chocolates after Long-term Storage, Fett Wissenschaft Technologie-Fat Science Technology 95:340-343
15. Araújo AAS, Bezerra MdS, Storpirtis S, Matos JdR (2010) Determination of the melting temperature, heat of fusion, and purity analysis of different samples of zidovudine (AZT) using DSC, Brazilian Journal of Pharmaceutical Sciences 46:37-43
16. Svanberg L, Ahrne L, Loren N, Windhab E (2013) Impact of pre-crystallization process on structure and product properties in dark chocolate, Journal of Food Engineering 114:90-98
17. Fessas D, Signorelli M, Schiraldi A (2005) Polymorphous transitions in cocoa butter - A quantitative DSC study, Journal of Thermal Analysis and Calorimetry 82:691-702
18. Himawan C, Starov VM, Stapley AGF (2006) Thermodynamic and kinetic aspects of fat crystallization, Advances in Colloid and Interface Science 122:3-33
19. Talbot G (2009) Chocolate Temper. in Beckett ST (ed) Industrial Chocolate Manufacture And Use. Wiley-Blackwell, United Kingdom, pp. 261-275.
20. Wright A, Batte H, Marangoni A (2005) Effects of canola oil dilution on anhydrous milk fat crystallization and fractionation behavior, Journal of dairy science 88:1955-1965
21. Kaufmann N, Andersen U, Wiking L (2012) The effect of cooling rate and rapeseed oil addition on the melting behaviour, texture and microstructure of anhydrous milk fat, International Dairy Journal 25:73-79

471 22. Sato K, Koyano T (2001) Crystallization Properties of Cocoa Butter. in Garti N,
472 Sato K (eds) Crystallization Processes in Fats and Lipid Systems. Marcel Dekker, New
473 York, pp. 429-456.
474 23. Perez-Martinez D, Alvarez-Salas C, Charo-Alonso M, Dibildox-Alvarado E, Toro-
475 Vazquez JF (2007) The cooling rate effect on the microstructure and rheological
476 properties of blends of cocoa butter with vegetable oils, Food Research International
477 40:47-62

478