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

[1,2*]Nizaha Juhaida, M., [3]Smirnova, O., [2]MacNaughtan, B., [3]Vieira, J. and [2,4]Wolf, B.

*[1]School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*
*[2]Division of Food Sciences, Sutton Bonington Campus, School of Biosciences, The University of Nottingham, Loughborough, Leicestershire LE12 5RD, United Kingdom*
*[3]Nestlé Product Technology Centre York, PO Box 204, Haxby Road, YO91 1XY York, United Kingdom*
*[4]School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, United Kingdom*

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**Abstract**

The present work investigated the effect of replacing a fraction of cocoa butter with limonene on fat crystallisation and bloom in the models of limonene-cocoa butter blends and seeded dark chocolate. Limonene was incorporated at a maximum of 6.7% (w/w) of cocoa butter in both types of samples, and were stored at 20 and 29°C changing cyclically every 12 h. Samples were analysed at weekly intervals up to three weeks, and the analysis was carried out by colour measurement for the whiteness index to detect bloom, and by X-ray diffraction (XRD) for crystal phase determination. DSC analysis was also carried out to assess the melting behaviour in the samples of the dark chocolate model. While the white colour of cocoa butter limited the bloom detection by colour, a large increase in whiteness index was recorded for the chocolate models. The XRD revealed an acceleration of crystal phase transformation in both types of samples. The changes in the melting behaviour for the dark chocolate model showed that the increased amount of limonene had caused the decrease in melting temperature. Hence, for practical applications, it can be suggested that the use of limonene, either as flavouring or for viscosity reduction in chocolate, can potentially result in increased bloom formation due to its effect on cocoa butter crystallisation and polymorphism transformation rate.

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## 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. In dark chocolate, the fat phase is normally present at a level of 30-40 g/100 g, which represents a dispersed volume fraction close to its maximum packing fraction, Φm. Hence, reducing the amount of fat phase and thereby increasing the solid fraction is not an option for developing a fat-reduced chocolate. This measure would negatively affect not only the processing properties (as viscosity would increase) but also the eating quality properties of the chocolate, such as its texture and ability to melt in mouth (Beckett, 2001). One patented approach to reduce cocoa butter without concomitantly increasing the solid fraction yet retaining the flow properties of the product is to replace up to 5% of the cocoa butter fraction with the zero-calorie ingredient limonene (Beckett, 2001). 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 thus limiting the amount of limonene tolerable to be added to chocolate.

The aforementioned patent (Beckett, 2001) led to several publications on the impact of limonene on the properties of chocolate. The viscosity reducing functionality was validated by Do *et al*. (2008), 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 to be linked to the lower solid fat content of these chocolates due to limonene being

*Corresponding author.
Email:niezaju@umt.edu.my

mixed within cocoa butter triglycerides. The impact of limonene on the polymorphism of cocoa butter was explored by Ray *et al*. (2012) by means of X-ray diffraction (XRD), differential scanning calorimetry (DSC) and polarised light microscopy. In their study, they found that in the presence of limonene, lower polymorphs of crystals were formed on cooling. Transformation of the crystals into more stable polymorphs during storage was also reported to be accelerated (Ray *et al*., 2012; Miyasaki *et al*., 2016; Rigolle *et al*., 2016).

The recrystallisation 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. This fat bloom is also normally associated with the loss of gloss and a rougher surface texture (Lonchampt and Hartel, 2006). Fat bloom occurs when less stable cocoa butter crystals undergo partial melting and the liquid fat diffuses to the surface of the chocolate where recrystallisation into a higher polymorphic form occurs. Cocoa butter has six polymorphs with Form I being the thermodynamically most unstable form, and Form VI being the thermodynamically most stable form (Wille and Lutton, 1966). Differences in melting behaviour were also noted. In commercial chocolate production, only Forms IV to VI are important (Svanberg *et al*., 2011) with Form IV being found in untempered chocolate. Successful tempering will lead to Form V, the preferred form in commercial chocolates, because Form V is the highest polymorph that can be process-induced. Transformation to Form VI will slowly occur over time and eventually lead to chocolate bloom (Timms, 2002). 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 (Ray *et al*., 2012), it can be hypothesised that the addition also affects bloom formation. Therefore, the objective of the present work was to investigate bloom formation in chocolate formulated with limonene, particularly in relation to cocoa butter crystal polymorphism. To achieve this objective, the untempered limonene-cocoa butter blends and seeded dark chocolate with added limonene were formulated and exposed to cyclic temperature storage for three weeks in order to accelerate bloom formation. The limonene-cocoa butter blends were not tempered because the focus of this preliminary work was to assess the effect of limonene on the formation of bloom on cocoa butter without any influence from other ingredients such as seeded cocoa butter seed crystals (as one of tempering technique), lecithin, sugar and cocoa powder. These ingredients were reported to have the potential to provide nucleation sites during crystallisation (Svanberg *et al*., 2011). Based on the results of preliminary study, chocolate formulations were tempered by a seeding method. 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 the present work can be used to inform the commercial implications of this fat-reduction strategy for chocolates.

## Materials and methods

### *Materials*

Cocoa butter and cocoa powder were supplied by ADM (Hull, UK). Soy lecithin and MyCryo Form V cocoa butter seed crystals were donated by Barry Callebaut (Banbury, UK). Soy lecithin was used only 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 as compared to granulated or caster sugar, and the icing sugar was purchased from a local supermarket. All the 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, respectively. The maximum level of substitution (6.7%) corresponded to a level of 2.5 g in a chocolate containing 38 g 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, thoroughly mixed with a spatula and immediately transferred into square chocolate moulds (35 mm × 35 mm × 5 mm) and rectangular XRD sample holders (10 mm × 15 mm × 1 mm). The 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 the 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 d to minimise any crystal growth. They were then increased to room temperature overnight before being transferred into an incubator set to a cycle temperature of 20 and 29°C. The temperature was set to alternately change every 12 h. Analyses were carried out on the day the incubator storage started (week 0) and then every 7 d for subsequent 3 w storage.

*Preparation of seeded dark chocolate model blends*

The seeded dark chocolate model blends contained 41.5 g/100 g of icing sugar, 20 g/100 g of cocoa powder and 0.5 g/100 g of soy 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 the total weight of chocolate formulation, the seeded dark chocolate model blends contained 0 g, 1.27 g and 2.53 g of limonene/100 g of chocolate, respectively. All of the ingredients, except the limonene and 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 formulation, and continuously mixed 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. Since the addition of limonene lowers the viscosity of the mix (also reported by Do *et al*. (2008)), 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 mm × 38 mm × 8 mm). The mixture were then placed into an incubator (MIR-153, Sanyo Electric Biomedical Co., Bunkyoku, Tokyo, Japan), and kept at 10°C for 30 min to set. The chocolate model was then de-moulded, and the temper status was evaluated using DSC. The remaining chocolate model samples were sealed into an aluminium pouch, and aged at 20°C for 1 w to accelerate the formation of higher stable polymorphs before the accelerated storage trial was performed by applying the same conditions as for the limonene-cocoa butter blends. "Week 0" in the following tables and graphs refers to the start of this storage trial.

*Determination of whiteness index*

The development of bloom was observed by quantifying the whitish appearance on the surface of the cocoa butter and the chocolate samples by the whiteness index (WI) defined in Eq. 1 (Bricknell and Hartel, 1998; Sonwai and Rousseau, 2006; 2008). The parameters $L^*$, $a^*$ and $b^*$ were obtained by measuring the surface colour of the samples with a Hunter colorimeter (Hunter Lab Ultrascan Colorimeter, Hunter Associates Inc., Reston, USA). After calibrating the instrument with white and black glass standards, several spots of each sample were scanned and the whiteness index was calculated with the equipment's software, using the following equation:

$$W = 100-[(100-L)^2 + a^2 + b^2]^{1/2} \qquad \text{(Eq. 1)}$$

Analyses were performed on four replicates for each batch. The results were statistically analysed to compare the means of the WI between the weeks of storage for each limonene concentration using one-way ANOVA. Significant differences between the samples were analysed using Tukey's HSD (Honest Significant Difference) multiple comparisons test at 95% significance level.

*Acquisition of X-ray powder diffraction patterns*

X-Ray powder diffraction (XRD) patterns were acquired using an X-Ray diffractometer (D5005, Bruker, UK) at room temperature (20-22°C). The radiation was monochromated copper K alpha (CuKα) with a wavelength of 1.5418 Å. A slit focus reflection geometry was used and scans were run over 2θ values between 3 and 38° at 0.05° intervals with a scan time of 2.5 s per interval. This protocol was previously applied to limonene containing cocoa butter (Ray *et al*., 2012). The XRD patterns were analysed for *d*-values using Diffrac Plus V1.01.

The cocoa butter samples were directly scanned as moulded into the XRD sample holders; however, the chocolate samples required the removal of sugar because intense sugar diffraction peaks would overlay the diffraction pattern of the cocoa butter thus causing difficulty in interpretation. A slight modification of the method adopted Cebula and Ziegleder (1993) was followed. The chocolate was chopped into small pieces, with the largest dimensions being 0.5-1.5 mm or less. These pieces were placed into cold water at a ratio of at least 1:100 (w/v) of chocolate to water. The mixture was vigorously mixed for about 5 min

and left to stand for at least 2 h for the sugar to dissolve. The mixture was then filtered to remove the water and the undissolved material was left at room temperature until most of the water had evaporated. Finally, the leftover material was pressed into a rectangular XRD sample holder (10 mm × 15 mm × 1 mm) and the surface levelled with a blade. XRD patterns were acquired every week for the course of 3 w 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 the cyclic temperature storage, and to ascertain the state of temper immediately after chocolate solidification. All the 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. The results are presented as normalised 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 (Araújo *et al*., 2010).

The tempering status of the seeded chocolate was evaluated from a DSC melting curve following the method adopted by Svanberg *et al*. (2013). However, the tempering status of cocoa butter in the presence of limonene could not be confirmed due to the lack of reference on DSC melting patterns, and for this reason, a well-tempered status was judged to be present when the pattern was comparable to the pattern published in the aforementioned reference. The well-tempered samples showed a peak where the area under the curve was neither too broad nor 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 immediately carried out after the chocolate setting step (as described in the preparation of seeded dark chocolate). Approximately 15 mg sample was placed into an aluminium pan which 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.

To evaluate the thermal behaviour of the three chocolate model blends during the cyclic temperature storage, the protocol published by Fessas *et al*. (2005) was followed. Approximately 15 mg 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 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 each of the seeded dark chocolate model blends (containing 0%, 3.3% and 6.7% of limonene, respectively) was evaluated by comparing its DSC melting curves (Figure 1) to the published patterns by Svanberg *et al*. (2013). As mentioned, when compared with an under-tempered

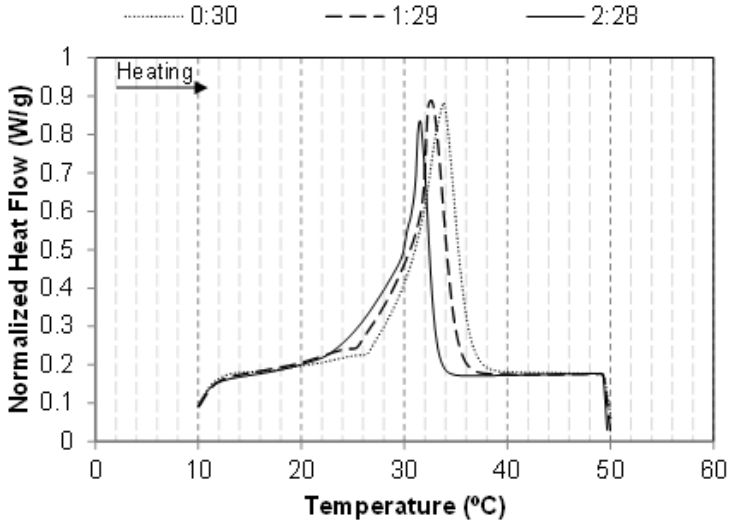


Figure 1. Thermal behaviour of the seeded chocolate samples containing different levels of limonene. Number in ratio refers to limonene-cocoa butter blend in sample.

sample, a well-tempered chocolate would show a narrower peak due to the narrower distribution of polymorphic forms (Svanberg *et al*., 2013). Figure 1 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 the control sample (0% limonene) at 33.9°C indicated that the majority of the cocoa butter crystals were in Form V (Wille and Lutton, 1966). 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 was increased (as previously reported by Do *et al*. (2008)). 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 peak temperature was also close to the seeding temperature used; the temperature was close to 34°C for chocolate without limonene addition, and close to the range 32°C - 33°C for blends with limonene addition. This finding indicates that the samples were in the right polymorphic form of crystals for a tempered chocolate. The values for $T_{onset}$ on the other hand, were 25.5°C, 23.7°C and 22.0°C in order of increasing limonene substitution.

*Bloom formation evaluated visually and by whiteness index*

Bloom formation on the dark chocolate models was clearly visible to naked eyes as compared to the cocoa butter blend samples. As early as in Week 0, the chocolate samples containing limonene showed a matte surface, which was in stark contrast to the shiny surface of the sample not containing limonene. During the 3 w 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.

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 3 w cyclic temperature storage, and an increase in WI would signify bloom. In the case of the limonene-cocoa butter blends (Figure 2a), 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 the cocoa butter, which was reflected in the visual appearance of the sample surfaces.

The data acquired on the chocolate model samples were more meaningful as expected from the visual assessment (Figure 2b). With the exception of the data obtained in the Week 0, which were 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 3 w storage. Upon substituting cocoa butter with 3.3% limonene, WI showed an increasing trend from Week 0 to Week 2, followed by a significant 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

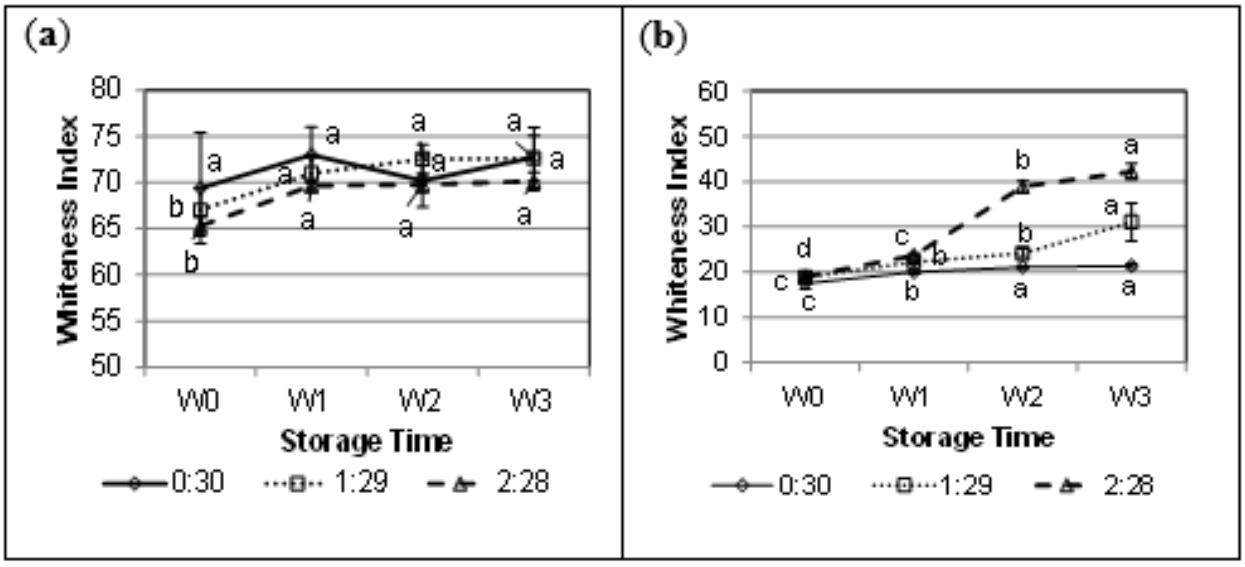


Figure 2. Whiteness index of (a) limonene-cocoa butter blends and (b) seeded dark chocolate during storage from week 0 (W0) to week 3 (W3). Blends were identified by their limonene-cocoa butter ratio. Different letter for each blend ratio showed significant differences between weeks of storage ($p < 0.05$).

was the result of cocoa butter polymorphism and recrystallisation into higher forms, this evidence of the effects of limonene addition into cocoa butter and on chocolate favouring bloom formation was strengthened by acquiring X-ray powder diffraction patterns.

*XRD patterns*

The XRD patterns acquired on the limonene-cocoa butter and the seeded dark chocolate model blends are shown in Figure 3. The identification of the polymorphic forms was undertaken by comparing the values of the d-spacing with those found in previous studies (Himawan *et al*., 2006; Sonwai and Rousseau, 2006). The strong diffraction peak (4.6 Å) and four smaller peaks at (3.99, 3.87, 3.75 and 3.68 Å), as shown in Figure 3a, are evidence of Form V crystals in both types of samples, which were prepared in the absence of limonene. Form V was prevalent throughout the 3 w storage. Both of the limonene-containing cocoa butter samples (the 1:29 and 2:28 blends) were initially in Form V (see Figure 3b and 3c). The XRD patterns acquired at Week 3 showed that the diffraction peak at 3.99 Å was reduced in height, while the intensity of the peaks at 3.68 and 3.87 Å was increased. This observation attested the presence of Form VI crystals, as described by Sonwai and Rousseau (2006).

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

It is unusual to find Form V crystals in freshly prepared samples of untempered cocoa butter (Figure 3a). One would expect to find Form IV crystals instead (Timms, 2002) because Form V is normally produced through a tempering process. However, Form V can also appear when chocolate is exposed to low temperatures, for example, in a cooling tunnel (Talbot, 2008). One reason was that, the preparation of the cocoa butter samples involved a cooling step (at 7°C for 1 h), which might have caused the sample to be in Form V at Week 0.

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

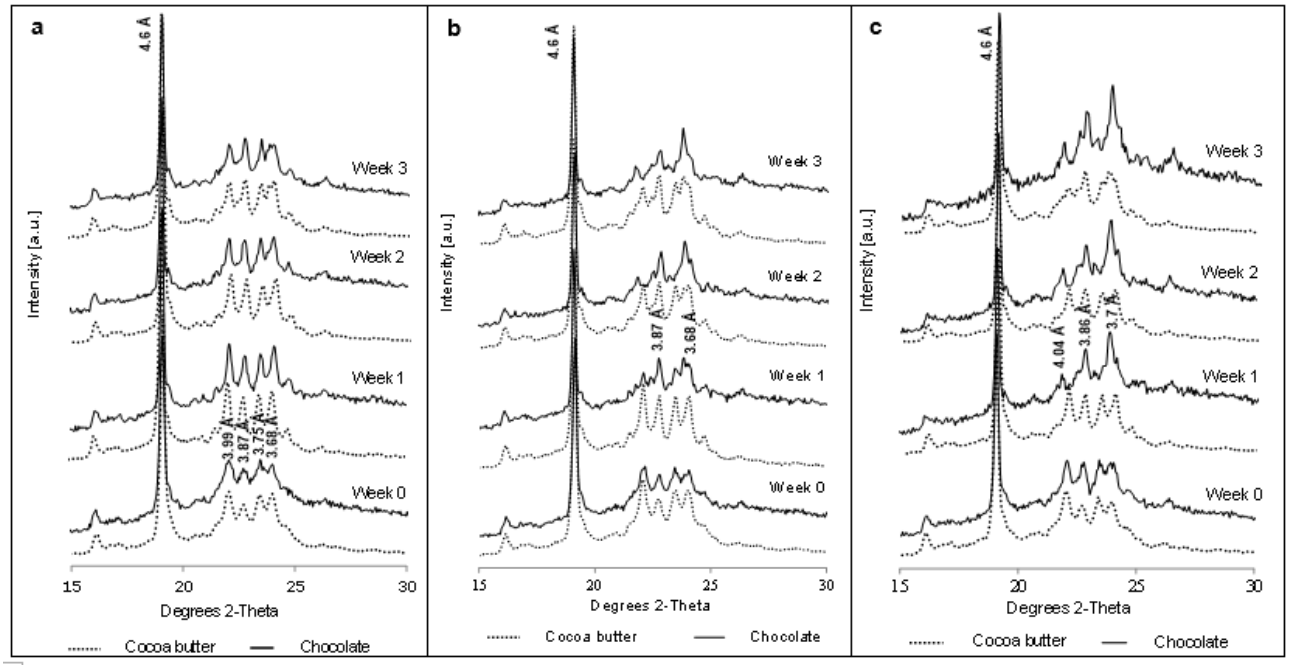


Figure 3. XRD patterns acquired at weekly intervals for the three limonene-cocoa butter blends labelled “cocoa butter” in the graphs and seeded dark chocolate samples labelled “chocolate” at limonene-cocoa butter blend ratios of (a) 0:30, (b) 1:29, and (c) 2:28.

*Thermal properties of the chocolate model samples*

The results of the thermal analysis of the seeded dark chocolate model samples acquired during the cyclic temperature storage are shown in Figure 4. The corresponding characteristic temperature values are reported in Table 1.

Table 1. Characteristic temperatures of seeded dark chocolate samples obtained from the DSC thermograms shown on Figure 4. "Blend ratio" refers to the limonene-cocoa butter ratio.

| 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), the presence of the two amounts of limonene substitutions led 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 samples 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 the formation of a higher polymorphic form. The lack of temperature increase for the sample with the highest limonene substitution suggests that the fat crystals could have fully transformed from Form V to Form VI crystals in the freshly prepared sample, and this finding was parallel to the result observed from the analysis of XRD pattern. So, it appears that limonene causes two opposite effects in terms of thermal properties. Commonly, the formation of bloom increases the temperatures ($T_{onset}$, $T_{peak}$ and $T_{end}$) of chocolate when analysed for its thermal properties. However in this case, the incorporation of limonene as liquid substitute for cocoa butter decreased the temperature values while being in the state of Form VI crystals.

In the region of 24 - 28°C, the appearance of a small broad peak at the onset of melting acquired on the sample with highest limonene substitution was

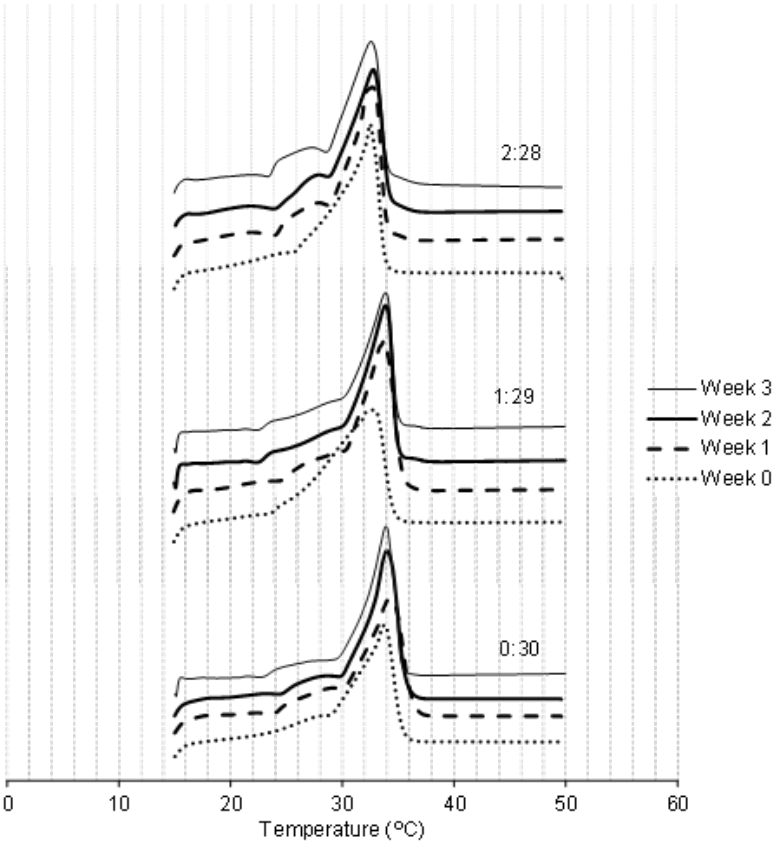


Figure 4. DSC melting curves of seeded dark chocolate model samples acquired during cyclic temperature storage. Blends were identified by their limonene-cocoa butter blend ratio.

surprising. This finding may indicate the separation of lower polymorphic crystals through the presence of limonene. This hypothesis can be tested with the XRD analysis if this phase could be separated from the blend.

Several factors can be attributed to the effect of limonene in lowering the melting temperature of chocolate samples while driving the fat suspension to a higher polymorphic form of crystals. Incorporating a low molecular weight hydrophobic compound has previously been claimed to dilute the anhydrous milk fat (AMF), which consequently reduced the melting temperature and solid fat content (SFC) (Wright *et al*., 2005; Kaufmann *et al*., 2012). Limonene is a low molecular weight hydrophobic compound and, therefore, might produce the same effect following incorporation into chocolate. This is effectively a colligative lowering of the crystal melting point without changing the polymorphic form. The addition of limonene will increase the proportion of liquid at a given temperature and therefore, reduce the proportion of solid fat content in the mixture. This has been shown in a study by Do *et al*. (2008), who found that the substitution of cocoa butter with limonene at levels of up to 3% had reduced the SFC by over 50% at 25°C.

There is also a possibility that the limonene acted as a solvent when being mixed with cocoa butter. Canola oil has been shown to act as a solvent when being mixed with AMF; it solubilised solid fat of AMF as observed through the result of SFC (Wright *et al*., 2005). Similar observation was noted by Do *et al*. (2008), who observed that with the cocoa butter alone when analysed at 10°C, about 90% of solids were detected from the result of SFC while in the blends of cocoa butter-limonene at a ratio of 87:13, only about 65% solids were observed. The reduction of solids was not at the right proportion as it should be 78.3%. Instead, 13.3% of the cocoa butter solids were solubilised in limonene at 10°C. Therefore, some solid fraction from the cocoa butter was pulled out into the liquid low-melting fraction. Hence, limonene caused the chocolate to have softer texture (Do *et al*., 2008) and lower melting temperature.

The presence of liquid limonene in the present work has previously been shown to alter the crystallisation kinetics of cocoa butter (Sato and Koyano, 2001; Wright *et al*., 2005; Kaufmann *et al*., 2012). The liquid limonene appeared to increase the rate of polymorphic transition due to the increased mobility of the crystallisation nuclei (Perez-Martinez *et al*., 2007). 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 (Sato and Koyano, 2001). At higher storage temperature (29°C in the present work), the partial melting of cocoa butter would increase the amount of liquid in the sample where the triacylglycerol molecules detached from dissolving the crystals of Form V to form a nuclei of Form VI crystal through volume diffusion in the oil matrix, as described by Sato and Koyano (2001). At that temperature, the higher concentrations of limonene promoted a higher amount of liquid in the sample. Upon lowering the storage temperature (20°C in the present work), the heterogeneous nucleation of Form VI may have occurred, as also suggested by Sato and Koyano (2001). The growth rate of crystals was observed to be many times faster than the nucleation rate in the presence of liquid (Kaufmann *et al*., 2012) 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*. (2012), who reported the presence of 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 thus promoting recrystallisation and enhancing bloom production. It can be deduced, therefore, that the higher the concentration of limonene in the sample, the higher the rate of bloom formation will be.

## Conclusions

As compared to the colour of cocoa butter, the dark colour of chocolate 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, the transition rate of fat crystals from Form V to Form VI increased during the cyclic temperature storage. This property of limonene can be explained by the dilution and solvent effect; the limonene not only diluted the cocoa butter fat but also solubilised some solid fat into the liquid limonene. Although limonene may be a commercially viable ingredient for formulating chocolate at a lower level without compromising its viscosity properties (Beckett, 2001), the demonstrated accelerated bloom formation makes the substance a less attractive ingredient for moulded chocolate bars.

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