Journal of Food Engineering 104 (2011) 70–80

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng

Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems

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ARTICLE INFO

Article history:
Received 3 May 2010
Received in revised form 14 September 2010
Accepted 25 September 2010
Available online 3 December 2010

Keywords:
Food processing
Crystallisation
Kinetics
Microstructure
Cocoa butter
Seeding

ABSTRACT

The effect of major chocolate ingredients (sugar, cocoa particles and lecithin), in combination with the two pre-crystallization techniques, seeding and non-seeding, was investigated with respect to the kinetics of cocoa butter crystallisation and the resulting microstructure. Confocal laser scanning microscopy (CLSM) was used to monitor microstructural evolution under dynamic thermal conditions. DSC measurements and image analysis were also applied in order to quantify the impacts of processing and formulation on microstructure. All ingredients and pre-crystallisation techniques considered proved to have a large impact on fat crystallisation kinetics and the resulting microstructure. Seeded samples tended to form multiple nucleation sites, inducing rapid growth of a crystal network. The non-seeded samples showed an altering structure, with some domains developing large spherical crystals while in other domains a more heterogeneous microstructure resulted. Lecithin showed a remarkable impact on crystallisation kinetics in both the seeded and non-seeded samples. For the seeded samples, the effect was most noteworthy in samples containing cocoa butter and sugar, where lecithin significantly reduced the induction time. In the absence of seeds, lecithin itself acted as the nucleation site for fat crystallisation.

1. Introduction

Fat bloom, a greyish haze on the chocolate surface, is the leading cause of deterioration of products in the confectionary industry (Rousseau, 2007). Although the exact mechanism behind fat bloom is debated, it is believed to coincide with the migration of less stable triacylglycerols (TAGs) to the surface and the transition between polymorphic forms in the cocoa butter (Bricknell and Hartel, 1998; Lonchampt and Hartel, 2004; Ziegleder, 1997).

Storage temperature (Ali et al., 2001; Guiheneuf et al., 1997), fraction of liquid fat (Lonchampt and Hartel, 2004), and the addition of milk fat (Pajin and Jovanovic, 2005; Tietz and Hartel, 2000) are all factors with a known effect on fat bloom. The relationship between crucial processing steps (i.e., pre-crystallisation and cooling) and chocolate microstructure has also been evaluated for its impact on the shelf life of chocolate, showing that a more dense structure obtained through optimal processing will result in less fat bloom (Afoakwa et al., 2008; Ghosh et al., 2002; Le Révérend et al., 2009; Rousseau, 2007). However, no systematic study exists of the impact of pre-crystallisation process in combination with addition of chocolate ingredients on the cocoa butter crystallisation and microstructure.

Cocoa butter can exist in six different crystalline or polymorphic forms (often denoted by roman numbers I–VI and the Greek letters α, β and β′), each of which exhibit different thermodynamic stability and melting (Rousseau, 2007; Timms, 1984; Wille and Lutton, 1966). However, for commercial chocolate production only forms β'_{IV} to β_{VI} are important. Form β_V is the preferred polymorph, form β'_{IV} is found in untempered chocolate, and form β_{VI} is found in bloomed samples (Timms, 2002). For the final quality of the chocolate product, it is of utmost importance to obtain polymorphic form β_V, as it has the capacity to trap liquid oil within its crystal network, thereby obstructing the migration of liquid TAGs to the surface (Dibildox-Alvarado et al., 2004; Smith et al., 2007). Furthermore, it has a favourable melting point (32–34 °C) well above room temperature and slightly below body temperature (Beckett, 2000).

During chocolate manufacturing, the most frequently applied procedure for obtaining stable form β_V involves subjecting the chocolate to a well-defined temperature programme under the action of shear, which induces the formation of a small proportion (1–3 vol%) of seed crystals. Through this process, the remaining fat solidifies around the seeds, which induces the correct polymorphic form (Seguine, 1991; Stapley et al., 1999; Talbot, 1999). This "conventional" tempering has four key steps: (i) complete melting at 50 °C; (ii) cooling to the point of crystallisation at 32 °C;

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doi:10.1016/j.jfoodeng.2010.09.023

(iii) crystallisation at 27 °C; and (iv) melting out unstable polymorphs at 29–31 °C (Talbot, 1999).

In 2000, Zeng presented a novel pre-crystallisation technique for producing well-tempered chocolate by homogeneously mixing 0.2–2% (w/w) of cocoa butter crystals in the most stable form β_{VI} with pre-cooled chocolate (Zeng, 2000). This process results in a large number of nuclei being present, which provides the basis from which the fat crystals grow. Although the seed crystals are in polymorphic form β_{VI}, the surrounding chocolate solidifies in the preferred form β_V (Zeng et al., 2002).

The remaining chocolate components—sugar, cocoa particles and emulsifier—also affect the chocolate microstructure. Savage and Dimick (1995) found that certain phospholipids, such as the phosphatidylcholine found in the emulsifier soy lecithin, were present in larger quantities in cocoa butter with rapid crystal growth compared to samples with slow crystal growth, indicating the emulsifier influences rate of crystallisation. Similar results were reported by Bowser (2006), where the addition of the emulsifiers soy lecithin and polyglycerolpolyricinoleate (PGPR) resulted in shorter induction times during cocoa butter crystallisation. The author also found that cocoa powder gave rise to additional nucleation sites, which resulted in faster crystallisation. Bricknell and Hartel (1998) reported that model chocolate containing amorphous sugar proved to be more resistant to visual fat bloom compared to samples made with crystalline sugar and explained this by the formation of a more compact microstructure enabled by the small spherical amorphous sugar.

Various microscopy techniques have been applied to study cocoa butter crystallisation. Kinta and Hartel (2010) utilised polarised light microscopy (PLM) to monitor cocoa butter crystallisation in chocolate samples after the addition of different quantities of seeding crystals. The same microscopy technique has also been applied to investigate the effect of incubation temperature on cocoa butter crystallisation, where different temperatures (26–33 °C) yielded large variations in crystal morphology (Dimick and Manning, 1987). Confocal laser scanning microscopy (CLSM) offers the possibility of monitoring microstructural development at different depths and length scales in the bulk under dynamic conditions (Rousseau, 2007). This furthers enables the survey of cocoa butter crystallisation. Bowser (2006) employed both PLM and CLSM to investigate the effect of emulsifiers and solid particles on cocoa butter crystallisation in tempered and non-tempered chocolate samples. The impact of minor lipids present in milk fat has also been evaluated with CLSM and was found, in specific quantities, to generate faster nucleation and crystal growth in cocoa butter (Tietz and Hartel, 2000).

This work aims to elucidate microstructure formation under the CLSM in seeded versus non-seeded samples by studying the effect of chocolate ingredients (sugar, cocoa particles and lecithin) on the kinetics of cocoa butter crystallisation. The crystallisation process was divided into two parts, nucleation and crystal growth, both of which have an impact on the kinetics of cocoa butter crystallisation (Garside, 1987; Walstra, 1998). In order to investigate the effect of each ingredient, different chocolate model systems were developed by adding one ingredient at a time to cocoa butter followed by pre-crystallisation. To estimate the polymorphic forms present in each sample, melting curves of the model systems were also measured with DSC. In this way, the relationship between microstructure, crystallisation process and ingredients was investigated.

2. Materials and methods

2.1. Chocolate model systems

All raw materials were kindly provided by AarhusKarlshamn A/S (AArhus, Denmark). In order to exclude the effects of particle density (as sugar particles are present in larger quantities than cocoa particles in commercial chocolate), the mass ratio between cocoa butter and solid particles were set to 2:1 in all chocolate model systems. Thus, model systems composed of pure cocoa butter (100 wt%) or cocoa butter (66.7 wt%) mixed with either sugar particles (33.3 wt%), cocoa particles (33.3 wt%, whereas 10–12% is cocoa butter) or the combination of sugar and cocoa particles (16.65% and 16.65 wt%, respectively) were prepared. To visualise the crystallisation process a CLSM method was developed. The fluorescent labelled non-polar fatty acid analogue BODIPY FL C_{16} (Invitrogen Ltd., Paisley, UK) was mixed with the chocolate model systems at a final concentration of 37 ppm. The method was first validated with pure cocoa butter, where the liquid phase showed a bright yellow colour compared to the darker crystals. Validation was thereafter performed on samples containing sugar and cocoa particles where the fat phase, both liquid and crystalline, was significantly brighter.

All samples were prepared in the presence or absence of emulsifier (lecithin 0.5 wt%). Samples without emulsifier were produced in triplicate, while samples with emulsifier were produced in duplicate.

2.2. Experimental design

Nucleation of cocoa butter crystals was initiated during pre-crystallisation. Thereafter, crystal growth during cooling was monitored with CLSM, as described in Sections 2.2.1 and 2.2.2 and illustrated in Fig. 1.

The sample preparation and experimental set-up illustrating the approach to the build-up of the complex chocolate microstructure by adding one ingredient at a time is presented in Table 1.

2.2.1. Pre-crystallisation and nucleation

All samples were subjected to one of two pre-crystallisations: *seeded* or *non-seeded*, corresponding to the formation of crystal nuclei either by addition or by temperature treatment. For both regimes, a temperature stage from Linkam Scientific Instruments Ltd., (Surrey, UK) connected to the confocal laser scanning microscope (Leica Microsystems CMS GmbH, Mannheim, Germany) was utilised. To establish the required temperatures, one thermocouple connected to a Logger Testo 177-T4 (Nordtec Instruments AB, Gothenburg, Sweden) was placed in the centre of the samples and temperature was recorded every 10 s.

Seeded: Mixed and stained samples were maintained at 49 °C for 20 min, while being continuously stirred by hand to erase all previous crystal memory and obtain the optimal dissolution of the ingredients and staining dye. Samples were then quenched to 34 °C, and held for 3 min before seeds (1 vol%) were added. Samples were kept at 34 °C for an additional 2 min while being stirred by hand, in order to ensure the seeds completely mixed in the fat phase. Finally, the temperature was reduced to 14 °C and maintained at this level for up to 60 min to induce crystal growth.

Non-seeded: A standard curve for conventional tempered dark chocolate was used as a template for the non-seeded samples. Mixed and stained samples were kept at 47 °C for 10 min. Samples were then quenched to 27 °C and held there for 5 min, before the temperature was raised to 31 °C for 5 min. Throughout the entire pre-crystallisation process, all samples were stirred manually. Finally, the samples were kept at 14 °C for up to 60 min to induce crystal growth.

Due to the microscopy technique and size of the sample holders all model systems were stirred by hand during pre-crystallization. As shear has a significant effect on the cocoa butter crystallisation (Dhonsi and Stapley, 2006; Stapley et al., 1999), the hand-stirring technique was carefully performed and reproducibility was assessed by performing three replicates for each model system.

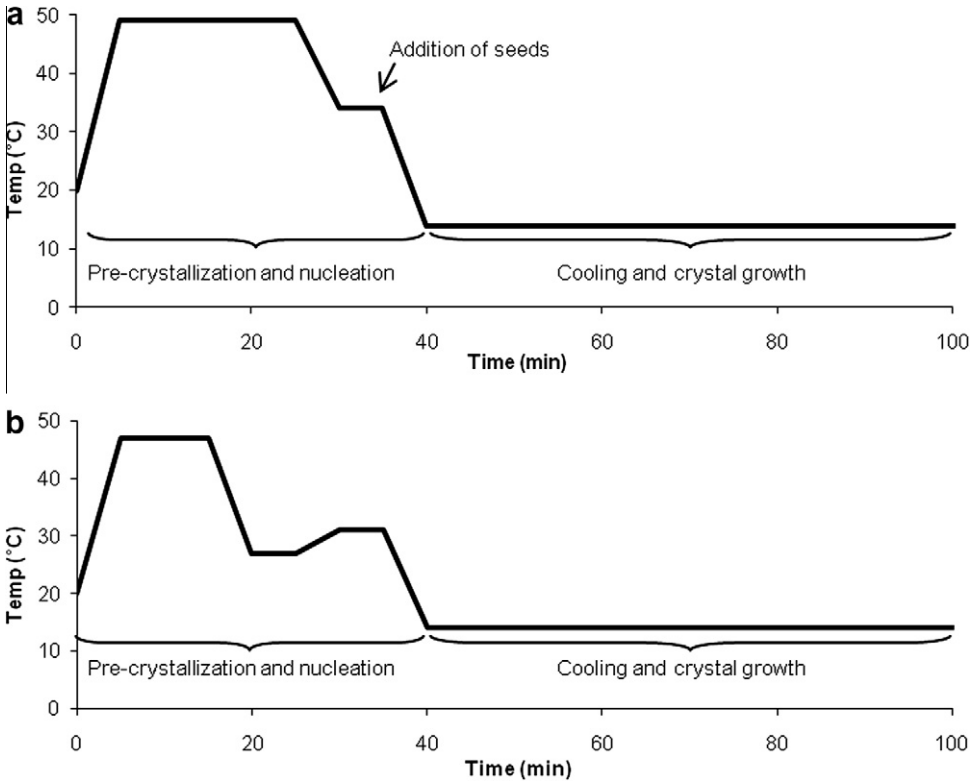

Fig. 1. Schematic image of the pre-crystallisation and cooling initiating nucleation and crystal growth for (a) seeded and (b) non-seeded samples.

Table 1
Preparation and pre-crystallisation of chocolate model systems containing cocoa butter (CB), cocoa particles (CP), sugar (Sug) and lecithin (Lec).

Chocolate model system	Total weight [g]	Cocoa butter [wt%]	Sugar [wt%]	Cocoa particles [wt%]	Lecithin [wt%]	Pretreatment	
						Non-seeded	Seeded
1. Pure CB	0.54	100				+	
2. CB + Sug	0.54	66.7	33.3			+	
3. CB + CP	0.54	66.7		33.3		+	
4. CB + Sug + CP	0.54	66.7	16.65	16.65		+	
5. CB + Lec	0.54	99.5			0.5	+	
6. CB + Sug + Lec	0.54	66.2	33.3		0.5	+	
7. CB + CP + Lec	0.54	66.2		33.3	0.5	+	
8. CB + Sug + CP + Lec	0.54	66.2	16.65	16.65	0.5	+	
9. Pure CB	0.54	100					+
10. CB + Sug	0.54	66.7	33.3				+
11. CB + CP	0.54	66.7		33.3			+
12. CB + Sug + CP	0.54	66.7	16.65	16.65			+
13. CB + Lec	0.54	99.5			0.5		+
14. CB + Sug + Lec	0.54	66.2	33.3		0.5		+
15. CB + CP + Lec	0.54	66.2		33.3	0.5		+
16. CB + Sug + CP + Lec	0.54	66.2	16.65	16.65	0.5		+

2.2.2. Cooling and crystal growth

The crystal growth in chocolate model systems during cooling was followed using a Leica TCP SP2 confocal laser scanning microscope, or CLSM, (Mannheim, Germany). Illumination was provided by an argon laser with emission maximum at 488 nm and the fluorescent signal between 494 and 570 nm was recorded. Images at a central point of each sample were taken at three different length scales, computer zoom 1×, 2× or 4×, with 1–2 min interval using an HCX PL AP0 oil objective with 63 times magnification and a numerical aperture of 1.4. All images were recorded at a resolution of 1024 × 1024 pixels.

During the first minute of cooling, a cover glass was placed over the samples and the focal plane was adjusted to penetrate the sample at 10 μm from where the fluorescence was first noted. The collection of CLSM images started after 3–5 min at 14 °C, and followed the microstructure development over the next 55 min.

2.3. Image analysis

CLSM micrographs were collected and analysed digitally using the Analysis Five program (Soft Imaging System GmbH). Each image was converted to a greyscale image and a median and N × N average filter was applied to correct for noise, shadings and inclinations. Segmentation between solid particles (i.e., sugar and cocoa particles, crystalline and liquid cocoa butter) was made through manual thresholding, which shows solid particles as black and

liquid fat as significantly bright, allowing it to be distinguished from the crystals. For each image series, corresponding to one replicate, we analysed 5–7 representative images covering the phase transition from liquid to solid crystals. The areas representing solid particles where subtracted and the percentage of crystals in the fat phase was plotted against the time kept at 14 °C. At time 0 at 14 °C, the amount of crystals was assumed to be negligible and was therefore set to zero in the plots.

2.4. Differential scanning calorimetry

The melting properties of chocolate model systems directly after the termination of pre-crystallisation were determined utilising a Mettler Toledo, DSC821. Approximately 5 mg of the sample was placed in hermetically-sealed aluminium pans with an empty pan as a reference. Samples were placed in the DSC immediately after pre-crystallisation, at a temperature set to 3 °C below cooling temperature, i.e., 11 °C. This was done to ensure that the sample temperature did not exceed 14 °C prior to surveying the melting in the DSC. The temperature was then raised by 4 °C/min to 50 °C. All DSC measurements were performed in duplicate.

Melting curves were analysed utilising MatLab 7.4 (The Mathworks Inc., Massachusetts, USA), and each curve was divided into four parts based on previous reports on melting points of cocoa butter polymorphs (Dimick and Davis, 1986), and integrated separately:

(i) 20–23 °C
(ii) 23–28 °C
(iii) 28–31 °C
(iv) 31–34 °C

Since the exact amount of fat was known for each sample, and sugar and cocoa particles were not considered to go through any phase change at the investigated temperatures, this allowed an estimation of the amount of less stable crystals (i–iii) and stable crystals (vi) present in the fat phase.

3. Results and discussion

Chocolate model systems were produced and investigated with CLSM in combination with image analysis in order to evaluate the effects of pre-crystallisation techniques, both seeded and non-seeded, and chocolate components (sugar, cocoa particles and lecithin) on the crystallisation process in cocoa butter. Both the pre-crystallisation techniques and the ingredients proved to have a significant impact on the kinetics and morphology of cocoa butter crystallisation, and substantially different microstructures were observed. Complementary DSC measurements were performed to estimate the amount of stable versus non-stable crystals present in the samples. These calculations also displayed large variations between seeded and non-seeded samples, as well as between the additions of ingredients, especially lecithin.

3.1. Pure cocoa butter

CLSM micrographs illustrating the time-dependent structure evolution of seeded and non-seeded pure cocoa butter (samples 1 and 9 in Table 1) are presented in Fig. 2. The staining dye (BODIPY) exhibited significantly higher affinity to the liquid cocoa butter compared to the crystals, thereby enabling the two phases to be distinguished by negative contrast. Thus, in all micrographs, liquid cocoa butter is represented by bright yellow/orange areas and crystals are illustrated by distinct darker areas.

The structure formed after 60 min differed substantially between the two pre-crystallisation techniques. All seeded samples formed multiple nucleation sites, which induced rapid growth of crystals within the first 10 min of cooling, as illustrated in Fig. 2(a) and supported by image analysis presented in Fig. 3(a). Seeds were easily dispersed throughout the samples, resulting in a homogenous microstructure, with small proportions of liquid fat trapped within the fat crystal network.

A more heterogeneous microstructure was observed in the non-seeded samples. In some cases, large spherical crystals evolved after an initiation time of approximately 20 min (Fig. 2(b)), while others remained in a semi-liquid state throughout the entire cooling step (Fig. 2(c)). In the latter case, small compact crystals formed in the central point of the sample, after which no further crystal growth occurred during the 60 min of cooling. However, closer to the sample edge, a heterogeneous microstructure was observed with large inclusions of liquid fat, connected by large irregular elongated domains structures penetrating the fat crystals (Fig. 2(d)). The substantial variation in microstructure for the non-seeded samples was also indicated by large standard deviations in the image analysis presented in Fig. 3(a).

According to the image analysis procedure presented in Section 2.3, it was possible to estimate the percentage of crystals in the fat phase in the observed area during the 60 min of cooling (Fig. 3). Fig. 3(a) clearly shows that in the seeded cocoa butter samples crystal growth was occurring during the first 20 min of cooling while the non-seeded samples showed much larger variations and crystal growth was significantly retarded.

The large variation in microstructure within the non-seeded samples was further supported by the DSC analysis. An estimation of the amount of crystals, represented by "energy required to melt per gram fat" for the temperature intervals is presented in Fig. 4(a). Non-seeded cocoa butter proved to have significantly higher amounts of unstable crystals in the interval 23–28 °C, while stable crystals, represented by interval 31–34 °C, were present in explicitly small quantities. The seeded samples had a higher total amount of crystals and the stable crystals were present in larger numbers. To further elucidate the results from the DSC measurement and the large variations between seeded and non-seeded cocoa butter representative melting curves for the two different model systems are presented in Fig. 4(b).

Although based on a "conventional" tempering procedure, the non-seeded samples were not produced in an optimal way and must therefore represent improper pre-crystallisation. The heterogeneous structure in these samples coincides with previous reports, where the microstructure of poorly tempered chocolate has been investigated with various microscopy techniques (Afoakwa et al., 2008; Bowser, 2006; Kinta and Hartel, 2010).

The non-seeded samples demanded an accurate temperature treatment where the required temperature in each step was to be reached in all parts of the samples. Chocolate has relatively low thermal conductivity and therefore the transport of heat is slow (Beckett, 2000). Thus, it may be easier to disperse seeds than to obtain an even temperature distribution in chocolate. The results found in the present study support this theory, as non-seeded samples had few nucleation sites and slow crystal growth compared to the seeded samples.

3.2. Effect of sugar and cocoa particles

3.2.1. Sugar

The microstructure of both seeded and non-seeded samples with cocoa butter and sugar exhibited the same properties as the corresponding samples made with pure cocoa butter. Thus, seeded samples had multiple nucleation sites from which the fat crystals grew, while the non-seeded samples showed large spherical

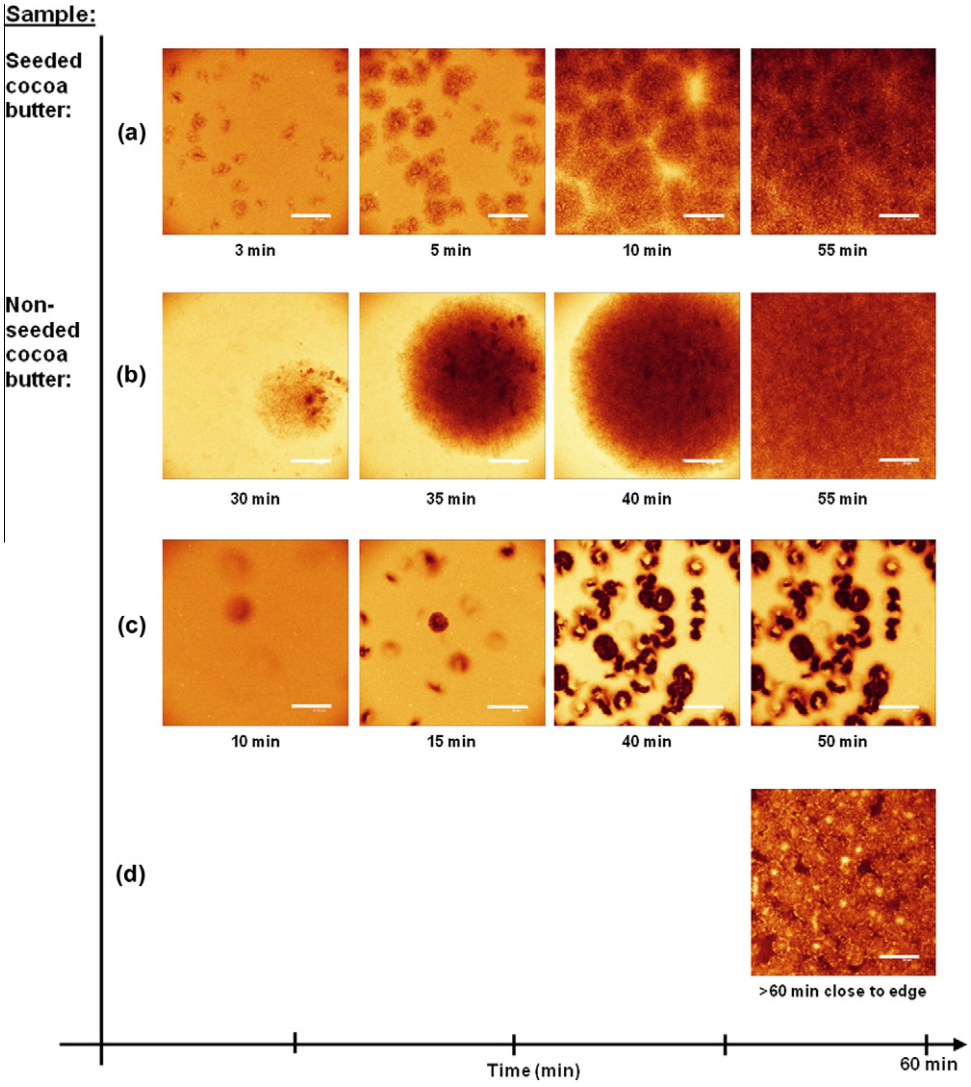

Fig. 2. CLSM micrographs showing the time-dependant crystal growth in (a) seeded and (b–d) non-seeded cocoa butter during the initial 55 min of cooling. The yellow/orange background represents liquid fat, while the crystals are identified by the darker areas. In the non-seeded samples, large spherical (b) or small compact crystals (c) were formed. In the latter case, no connected crystal network evolved in the central point during 60 min of cooling. However, close to the edge of the sample container, a heterogeneous microstructure was observed with large inclusions of liquid fat (d). Scale bars represent 50 μm in all images and the time indicated below each image correlates to minutes kept at 14 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

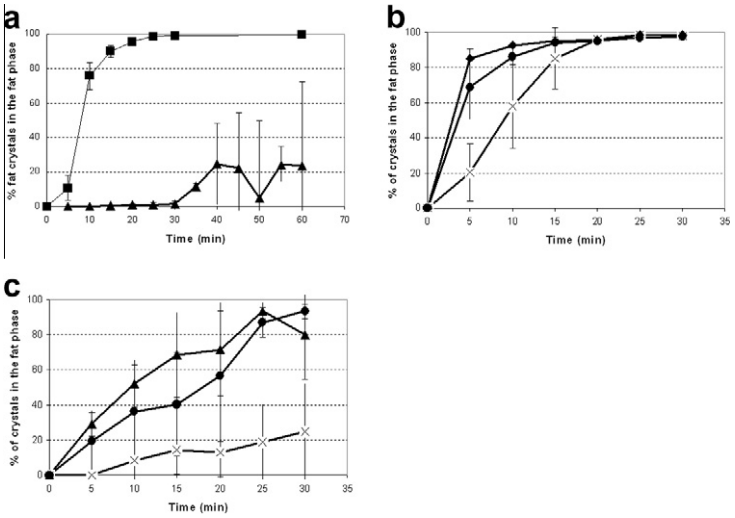

Fig. 3. Percentage of crystals in the fat phase versus time kept at 14 °C, obtained from image analysis for (a) seeded [–■–] versus non-seeded [–♦–] cocoa butter, (b) seeded samples with CB + Sug [–×–], CB + CP [–▲–], CB + Sug + CP [–●–] and (c) non-seeded samples with with CB + Sug [–×–], CB + CP [–▲–], CB + Sug + CP [–●–].

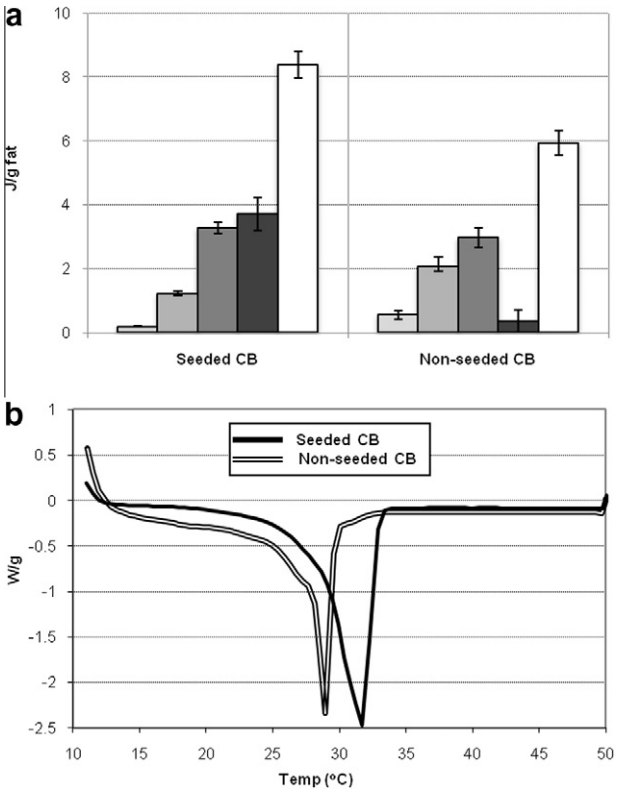

Fig. 4. (a) Estimated amount of crystals calculated from integrated DSC curves for seeded and non-seeded cocoa butter at 20–23 °C [□], 23–28 °C [▒], 28–31 °C [▓] and 31–34 °C [■]. Right column represents estimated total amount of crystals [□]. (b) Representative melting curves for seeded [▬] and [═] non-seeded cocoa butter.

crystals or small compact ones with severe heterogeneous microstructures as a result. Representative CLSM micrographs of the microstructure in seeded and non-seeded samples after 60 min of cooling are presented in Fig. 5. The sugar appeared as black angular shapes in the micrographs and could therefore be distinguished from the fat phase illustrated by yellow (liquid) and orange (crystal) areas. The difference in microstructure between the two pre-crystallisation techniques is clearly shown, since the non-seeded samples exhibited large irregular elongated domains structures identified by connective yellow areas (Fig. 5(b)).

For the seeded samples, the addition of sugar to cocoa butter proved to have little effect on the crystal growth rate compared to corresponding samples with pure cocoa butter (Fig. 3(a and b)). However, in the non-seeded samples the sugar particles showed a tendency to enhance crystal growth (Fig. 3(a and c)). This coincides with a previous report by Dhonsi and Stapley (2006), where a faster shear-induced crystallisation was observed when sugar was added to cocoa butter compared to a corresponding sample solely using fat. The authors suggested that this was due to sugar particles providing sites for *heterogeneous nucleation* of cocoa butter crystallisation. *Heterogeneous nucleation* denotes the phenomena where foreign surfaces acts as nucleation sites for crystallisation (Garside, 1987). Thus, special attention was paid to whether the surface of sugar particles themselves acted as nucleation sites for crystallisation. However, in this study no micrographs showing fat crystals originating from the plain surface of sugar was detected, either in the seeded or non-seeded samples. This is illustrated in Fig. 6, where the crystal network traps the dark and angular sugar particle rather than originates from it.

3.2.2. Cocoa particles

For the non-seeded samples, cocoa particles showed a pronounced impact on both morphology and crystal growth rate. Fig. 7 presents representative CLSM micrographs of the microstructure in seeded and non-seeded samples with cocoa butter and cocoa particles. Cocoa particles appeared as dark, irregularly shaped areas in the micrographs and could therefore be distinguished from both liquid and crystalline cocoa butter. The non-seeded

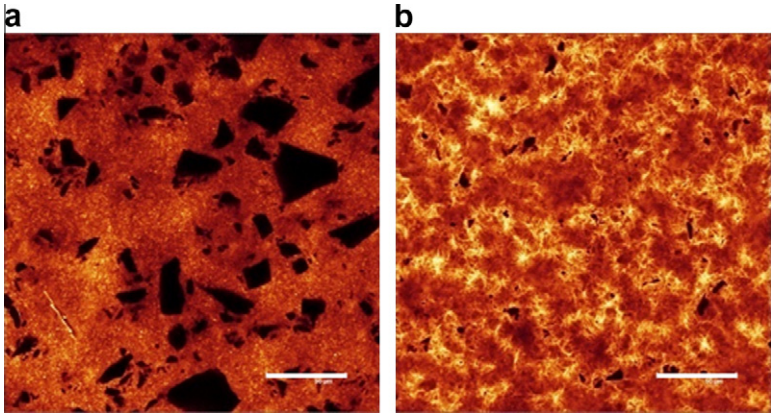

Fig. 5. CLSM micographs of (a) seeded and (b) non-seeded cocoa butter and sugar, after being cooled at 14 °C for 60 min. Scale bars represent 50 μm.

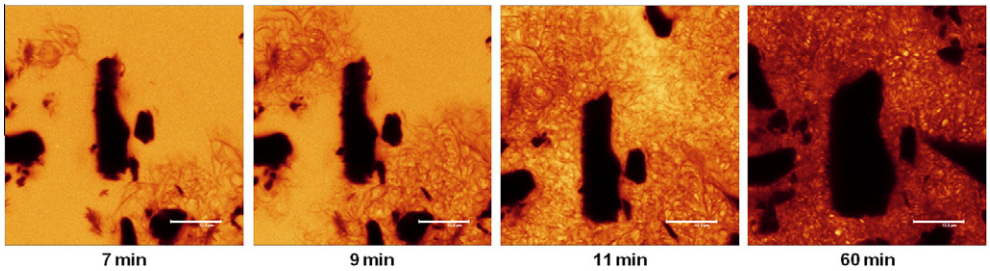

Fig. 6. CLSM micrographs of seeded cocoa butter and sugar. Scale bars represent 12.5 μm in all images and the time indicated below each image correlates to minutes kept at 14 °C.

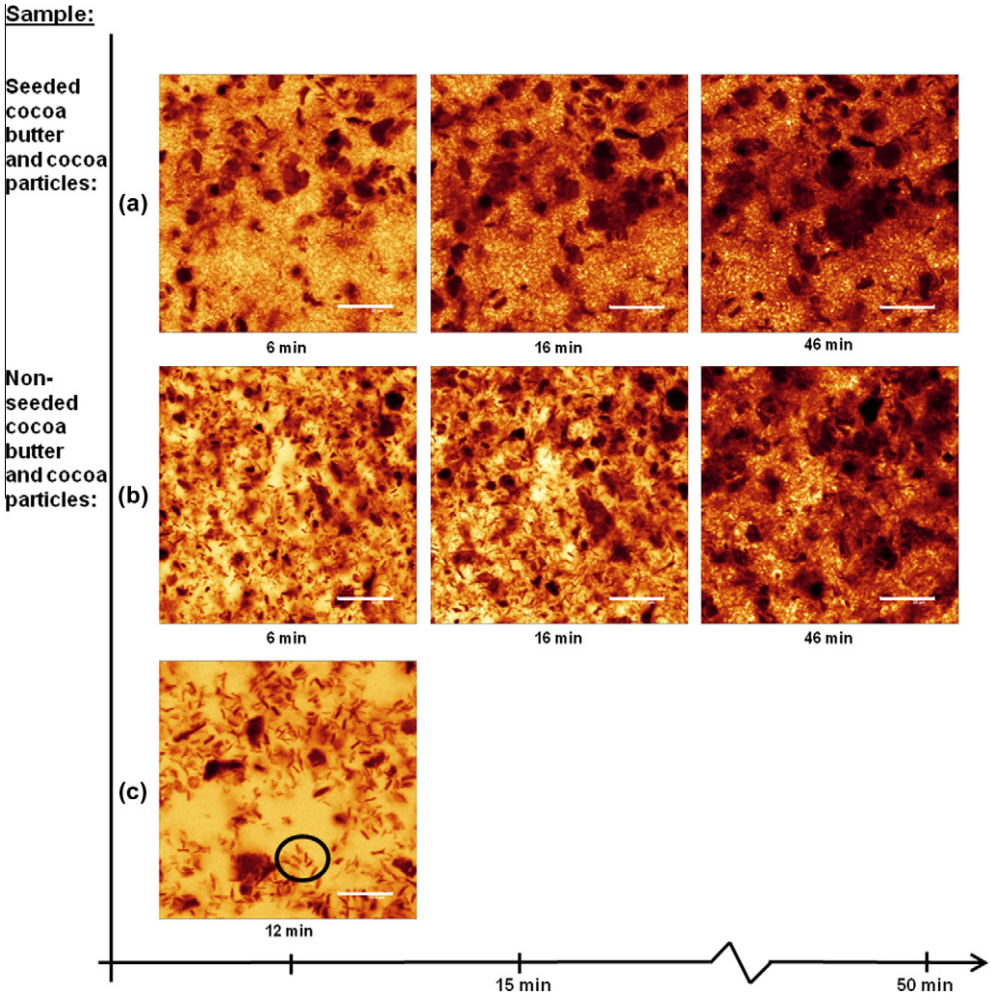

Fig. 7. Representative CLSM micrographs of (a) seeded and (b) non-seeded cocoa butter and cocoa particles mixtures. In the seeded samples (a) a homogenous fat crystal network evolved, whereas in the non-seeded samples (b) small rod-shaped crystals were formed. A 4× zoom-in micrograph of the rod-shaped crystals is presented in (c), and representative crystals are indicated with a circle. Scale bars represent 25 μm in (a and b) and 12.5 μm in (c). The time indicated below each image correlates to minutes kept at 14 °C.

samples formed small rod-shaped crystals, indicated by a circle in Fig. 7(c), which differed significantly from the homogenous microstructure formed in the seeded samples where no such crystal morphology was detected (Fig. 7(a)).

In order to determine whether these crystals originated from fat or other by-products such as cellular membranes, polysaccharides or proteins from the cocoa particles, additional non-seeded samples containing cocoa butter and cocoa particles were produced. The additional samples were placed under the CLSM as early as the final part of non-seeded pre-crystallisation (i.e., at 31 °C), in order to investigate whether rods were present before quenching the samples to 14 °C. Furthermore, after inducing crystal growth at 14 °C for 60 min during which rods were readily detected, the samples were re-melted at 50 °C. No rods were observed, either at 31 °C prior to crystallisation, or during re-melting at 50 °C—indicating that they are fat-based and not originating from other cocoa particle components. This result is not entirely unexpected since the cocoa particles contained 10–12% fat. One could therefore, hypothesise that the seeds that were inducing the formation of rod-shaped crystals are dissolved fatty acids, di- or triacylglycerides originating from cocoa particles. For the non-seeded samples, no other seeding material was present; initiation and growth of rod-shaped crystals was therefore not hindered, resulting in a heterogeneous microstructure with a large inclusion of liquid fat.

The results presented in Fig. 3(b and c) indicate that within both pre-crystallisation techniques, samples containing cocoa butter and cocoa particles had a faster crystal growth rate compared to corresponding samples with cocoa butter and sugar. This correlates with previous results observed by Bowser (2006), where defatted cocoa particles showed a pronounced impact on the rate of crystal growth in insufficient pre-crystallised cocoa butter. Results obtained from the DSC measurements showed that amount of stable crystals within the temperature interval 31–34 °C were significantly higher for samples containing cocoa butter and cocoa particles compared to corresponding samples with cocoa butter and sugar (Fig. 8(a)). It is unlikely that cocoa particles in itself nucleates stable polymorphic forms however, by inducing a more rapid nucleation and crystal growth during the cooling step it provides more time for less stable polymorphs (e.g., α-form) to transform into higher stable forms. To further elucidate the difference between sugar and cocoa particles, representative melting curves for the seeded model systems are presented in Fig. 8(b).

Heterogeneous nucleation, or the initiation of crystallisation on foreign surfaces, is predominantly dependant on three interfacial free energies: liquid–crystal, liquid-surface and crystal-surface (Garside, 1987). Thus, one possible explanation for the enhanced rate of crystal growth could be that the surface of cocoa particles is more hydrophobic thereby offering the possibility for

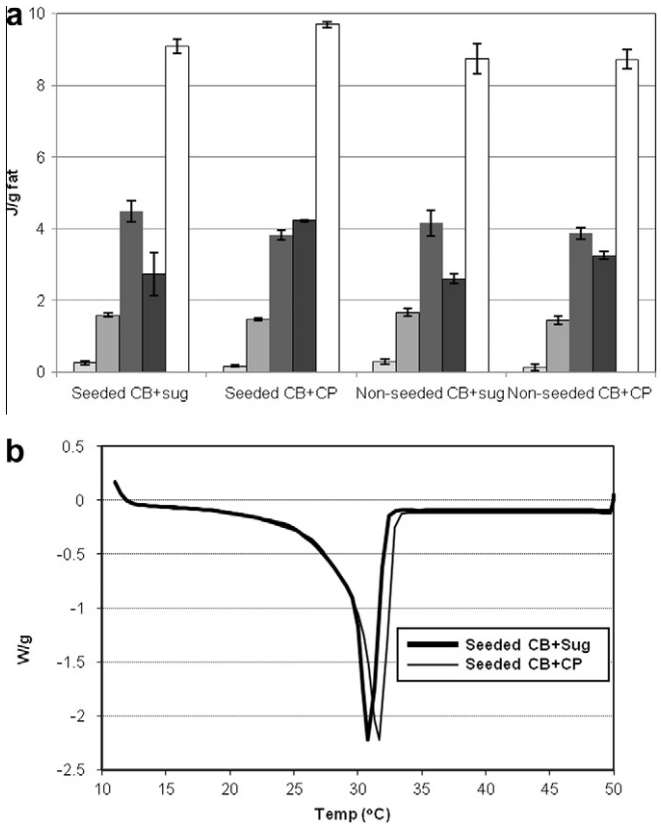

Fig. 8. Estimated amount of crystals calculated by the integration of DSC melting curves for seeded versus non-seeded cocoa butter and sugar or cocoa particles at 20–23 °C [□], 23–28 °C [□], 28–31 °C [□] and 31–34 °C [■]. The right column represents the estimated total amount of crystals [□]. (b) Representative melting curves for seeded CB + Sug [▬] and seeded CB + CP [—].

heterogeneous nucleation from their surface. However, in this study no micrographs showing fat crystals originating from the surface of cocoa particles was detected, in either the seeded or non-seeded samples.

3.2.3. Sugar and cocoa particles

Representative micrographs of the microstructure of samples containing cocoa butter, sugar and cocoa particles from both pre-crystallisation techniques are presented in Fig. 9. Small proportions of the staining dye dissolved within the internal structure of the cocoa particles, making them slightly brighter and allowing them to be distinguished from the angular and completely black sugar particles. However, discrimination between fat crystals and cocoa particles was still possible since the cocoa particles were significantly darker. It is worth nothing that the rod-shaped crystals appeared in the non-seeded samples, (Fig. 9(b)).

Although not significant, the results from the image analysis indicated that the rate of crystal growth for samples containing both sugar and cocoa particles was slower compared to samples containing solely cocoa butter and cocoa particles, for both the seeded and non-seeded samples (Fig. 3(b and c). Based on the results presented in Fig. 3(b and c) and Fig. 8(a), this is not unexpected since the sugar and cocoa butter samples tended to have a slower rate of crystal growth.

3.3. Effect of lecithin

In the investigated chocolate model systems, lecithin showed a pronounced impact on the kinetics of cocoa butter crystallisation for the non-seeded samples, as well as for the seeded samples containing cocoa butter and sugar. In the non-seeded samples with sugar and lecithin, it was possible to distinguish a heterogeneous nucleation where fat crystals evolved from the sugar particle surface, as indicated by a circle in Fig. 10(a). For the same samples, results obtained from the image analysis also showed a significant increase in crystal growth rate compared to corresponding samples without lecithin (Fig. 11(a)). Lecithin's primary location is at the interface between fat and sugar particles, with their hydrophobic tail facing the fat phase (Vernier, 1997). Based on the theory of

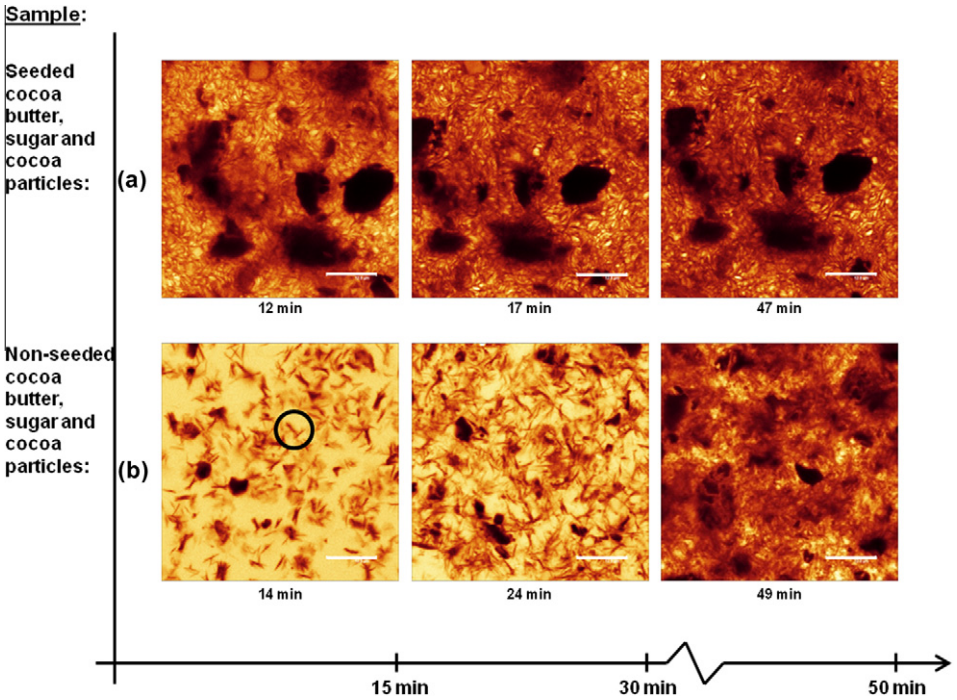

Fig. 9. CLSM micrographs of seeded (a) and non-seeded (b) samples containing cocoa butter, sugar and cocoa particles. The characteristic rod-shaped crystals, highlighted by a circle in (b) and connected to non-seeded samples containing cocoa particles are explicitly detected, scale bars represent 12.5 μm and the time indicated below each image correlates to minutes kept at 14 °C.

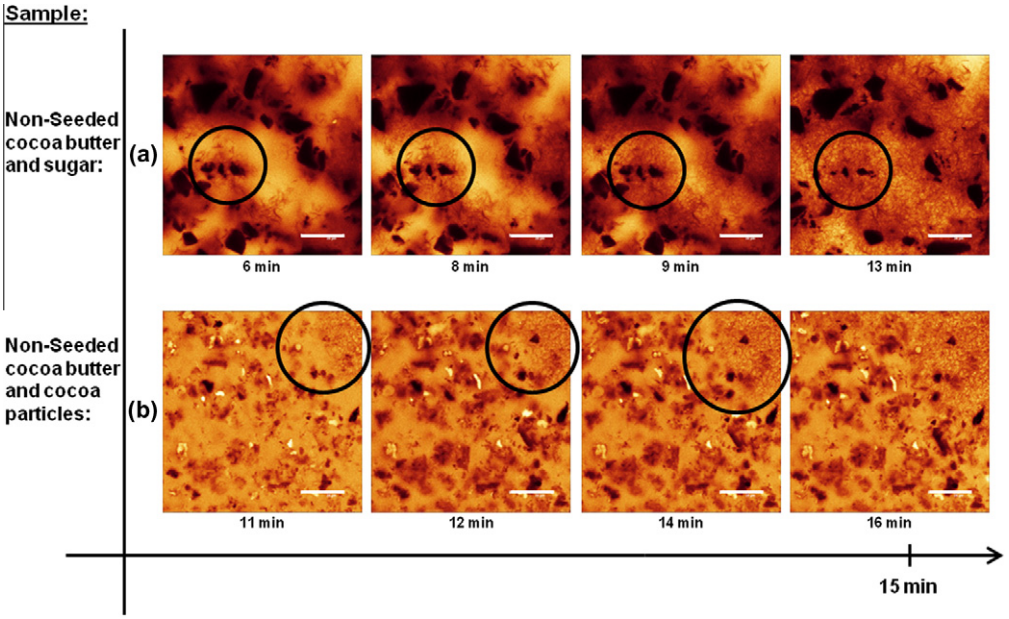

Fig. 10. Representative micrographs of the microstructure of non-seeded (a) cocoa butter, sugar and lecithin, and (b) non-seeded cocoa butter, cocoa particles and lecithin. Scale bars represent 25 μm and the time indicated below each image correlates to minutes kept at 14 °C.

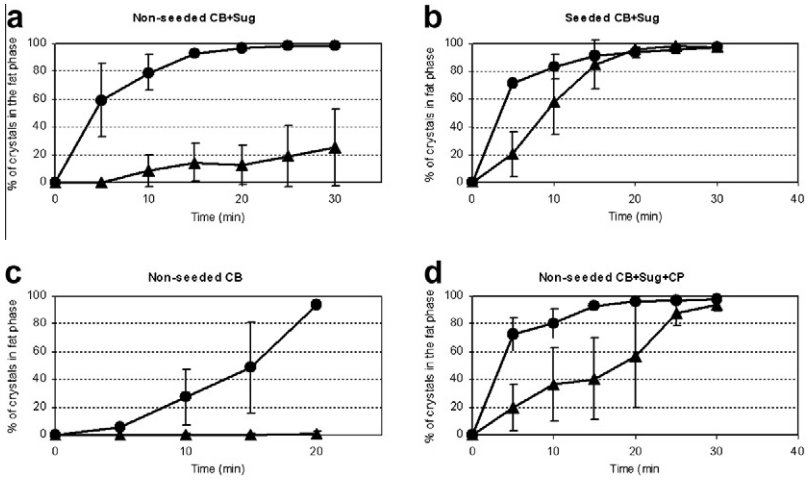

Fig. 11. Percentage of crystals in the fat phase obtained from image analysis for (a) non-seeded CB + Sug with [–●–] and without [–▲–] lecithin, (b) seeded CB + Sug with [–●–] and without [–▲–] lecithin, (c) non-seeded CB with [–●–] and without [–▲–] lecithin and (d) non-seeded CB + Sug + CP with [–●–] and without [–▲–] lecithin.

heterogeneous nucleation, this creates a more favourable environment for fat crystals to evolve from, as compared to the hydrophilic sugar surface. However, lecithin also proved to have an effect on the microstructure in samples containing solely cocoa particles. As can be observed in Fig. 10(b), the non-seeded samples with cocoa particles and lecithin initially formed rod-shaped crystals. However, after approximately 15 min at 14 °C a homogenous fat network evolved, which can be observed in the top right portion of the micrographs, illuminated by a circle (Fig. 10(b)).

To further understand the effect of lecithin on the kinetics of crystallisation, additional samples were produced, during which lecithin was added to pure cocoa butter and subjected to each pre-crystallisation. In the non-seeded pure cocoa butter, lecithin itself acted as the seeding material. This is illustrated in Fig. 12(a) as small irregular spots from which the fat crystals developed. In comparing this with the micrographs shown in Fig. 2(b–d) and the image analysis in Fig. 11(c), the impact of lecithin is remarkable with respect to both morphology and crystal growth rate. After the addition of lecithin, the non-seeded cocoa butter tended to resemble the seeded cocoa butter, with rapid crystal growth and numerous nucleation sites. In seeded pure cocoa butter it was not possible to distinguish any significant effect from the addition of lecithin, as they already contained seeding materials (Fig. 12(b)).

Previous reports have identified the seeding effect of phosphatidylcholine (PC), which is a phospholipid constituting 15% (wt) of the total fat content in lecithin. Due to its tendency to form micelles in the H_{II} mesophase (i.e., with polar heads facing the centre and non-polar parts oriented towards the exterior region), it provides an appropriate foundation for the nucleation of fat crystals (Garti and Sato, 2001; Savage and Dimick, 1995). This could indicate that the seeds inducing crystal growth in the non-seeded samples with cocoa butter and lecithin (Fig. 12(a)) are micelle structures of PC. However, other reports contradict these findings,

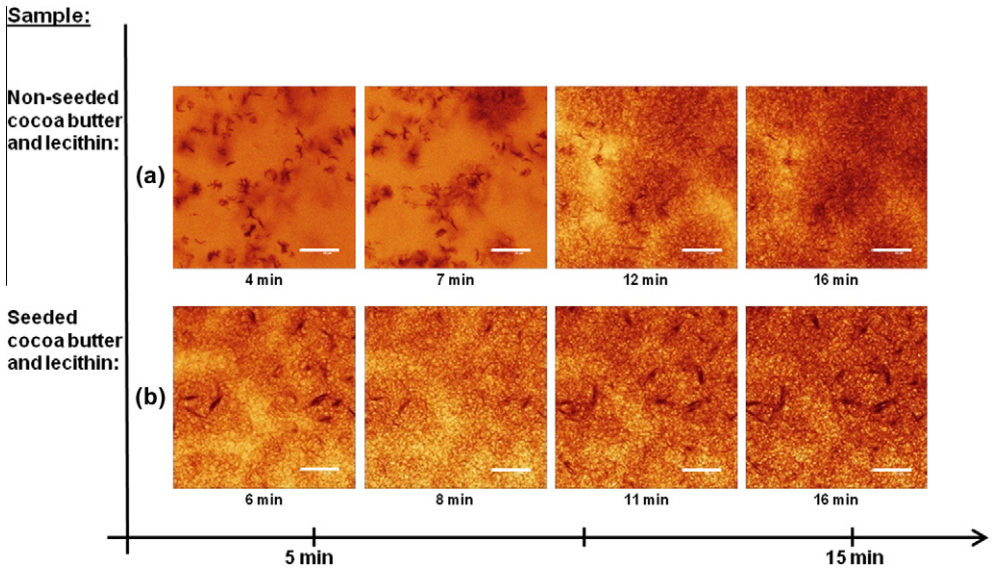

Fig. 12. Representative CLSM micrographs of (a) non-seeded cocoa butter and lecithin, and (b) seeded cocoa butter and lecithin. Scale bars represent 25 μm and the time indicated below each image correlates to minutes kept at 14 °C.

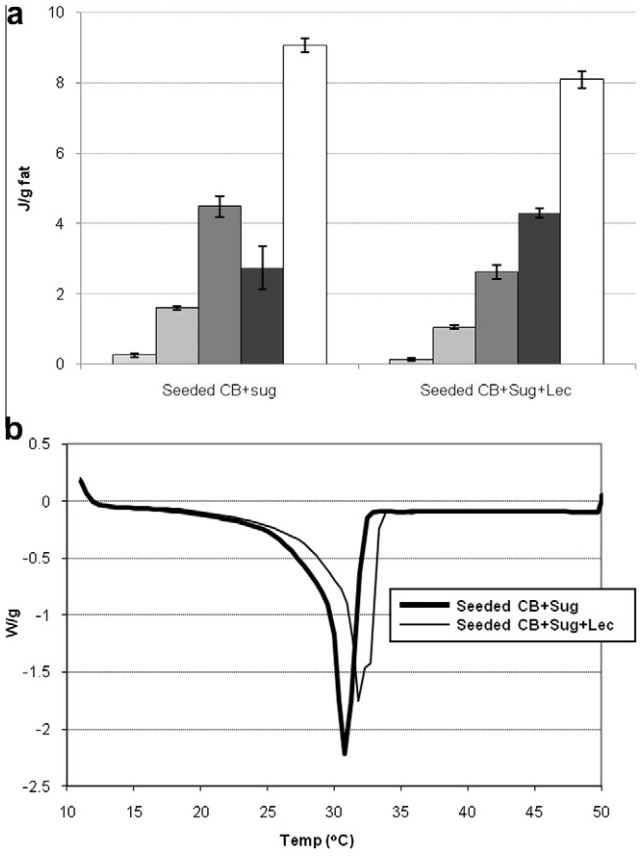

Fig. 13. Estimated amount of crystals calculated from integrated DSC melting curves for seeded cocoa butter and sugar in the absence (left sample) or the presence (right sample) of lecithin at 20–23 °C [□], 23–28 °C [▨], 28–31 °C [▩] and 31–34 °C [■]. The right column represents the estimated total amount of crystals [□]. (b) Representative melting curves for seeded CB + Sug [▬] and seeded CB + Sug + Lec [—].

claiming instead that lecithin delays cocoa butter crystallisation (Dhonsi and Stapley, 2006).

The results from image analysis (i.e., the percentage of crystals in the fat phase during the cooling step for samples containing lecithin) compared to the corresponding sample without lecithin are presented in Fig. 11. The crystal growth rate was significantly increased in non-seeded samples containing sugar and lecithin, as presented in Fig. 11(a) and corresponding seeded samples showed a similar tendency (Fig. 11(b)). As mentioned, previous reports state that lecithin primarily attaches itself to the surface of sugar particles (Vernier, 1997). It was thus not unexpected that crystal growth was significantly influenced in both seeded and non-seeded cocoa butter and sugar samples. Lecithin also significantly enhanced the crystal growth rate in non-seeded pure cocoa butter, although the variation in these samples was quite substantial, as indicated by the large standard deviation (Fig. 11(c)). Furthermore, when cocoa particles were added to the non-seeded cocoa butter and sugar, lecithin continued to have a significant impact on the crystal growth rate (Fig. 11(d)). For the non-seeded samples with cocoa butter and cocoa particles, it was not possible to distinguish any significant difference as the samples without lecithin also showed a fast rate of crystal growth within the first 20 min of cooling, as presented in Fig. 3(c). The enhanced crystal growth rate due to the addition of lecithin observed in the present study are both supported (Bowser, 2006) and contradicted (Dhonsi and Stapley, 2006) in previous literature.

Results obtained from the DSC measurements showed that the addition of lecithin to seeded samples with cocoa butter and sugar enhanced the amount of crystals present in the 31–34 °C interval (Fig. 13(a and b)).

For the investigated chocolate model systems in this study it can be concluded that lecithin, at a fraction of 0.5% (which is typical in chocolate recipes), had a significant impact on crystal growth rate. Whereas from the CLSM/image analysis we can derive a general increase in fat crystal nucleation and growth for all non-seeded model systems as well as for seeded cocoa butter and sugar samples, the respective DSC measurements for the sugar and cocoa butter samples give an additional indication of the crystal polymorphs generated (Fig. 13(a)). From this one can see the impact of added lecithin on the generation of higher stable β-polymorphs. Before DSC measurements were carried out, the model system was cooled down to 14 °C for 60 min and stabilised at 11 °C, before heating up at 4 °C/min within the DSC pan containing 5 mg of the sample mass. It is unlikely that lecithin preferably nucleates higher stable fat crystal polymorph forms, although a more

intensive nucleation and growth of fat crystals during cooling at 14 °C will provide more time for lower-melting polymorphs (e.g., in α-form) to transform into a higher stable form.

Subsequent investigations currently being planned will monitor the fat crystal content and polymorph characteristics using the so-called direct DSC method during a cooling cycle that compares to the temperature–time history chocolates are subjected to in industrial cooling tunnels (Breitschuh and Windhab, 1996). This direct DSC method measures only the fat crystals generated until sampling from the cooling melt. In the event of good reproducibility, this method will then be applied to real chocolate systems in order to prove whether similar crystallisation enhancement effects through the addition of lecithin can be detected. Furthermore, increasing lecithin concentration may provide further insight into the mechanism of crystallisation enhancement caused by the lecithin.

4. Conclusions

CLSM, DSC and image analysis were found to be a good combination of techniques for evaluating the kinetics and morphology of cocoa butter crystallisation in chocolate model systems. Both pre-crystallisation techniques and ingredients proved to have a pronounced impact on the kinetics of crystallisation and final microstructure in all model systems. Seeded samples formed multiple nucleation sites, which induced a rapid growth of crystals and resulted in a more homogenous microstructure, since seeds were easily dispersed throughout the samples. This indicated that proper pre-crystallisation adjust for inconsistencies in microstructure caused by cocoa particles. The non-seeded samples showed a more random structure, with some areas developing large spherical crystals while other parts gained a more heterogeneous microstructure with large inclusions of liquid fat and small compact crystals.

Non-seeded samples containing cocoa particles induced the formation of rod-shaped crystals resulting in a heterogeneous microstructure. We also concluded that the seeds inducing the formation of rod-shaped crystals are fat-based substances deviating from the cocoa particles. For both pre-crystallisation techniques, the addition of sugar prolonged the nucleation and growth of cocoa butter crystals, compared to corresponding samples with cocoa particles. On the other hand, lecithin significantly enhanced the crystal growth rate in seeded cocoa butter and sugar samples, as well as for all non-seeded samples. In the absence of homogenous nucleation sites, whether they were added as seeds or created by correct tempering, lecithin showed an equivalent behaviour and induced crystal growth. However, further studies are required to establish whether the effect of lecithin remains in commercial chocolate systems.

Overall, a deeper knowledge of how to obtain a fast and controlled crystallisation process shows great potential for developing novel techniques for the production of high quality chocolate with homogeneous microstructure.

Acknowledgements

This work was financially supported by the European Commission through the Seventh Framework Programme on *Research for the benefit of specific groups (in particular SMEs)*, within the project "ProPraline: Structure and processing for high quality pralines".

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