Research Article

Modified soybean lecithins as inducers of the acceleration of cocoa butter crystallization

Eriksen Koji Miyasaki[1], Valdecir Luccas[2] and Theo Guenter Kieckbusch[1]

[1] School of Chemical Engineering, University of Campinas, Campinas, SP, Brazil
[2] Cereal Chocotec – Food Technology Institute, Campinas, SP, Brazil

Modified soy lecithins are introduced in chocolate processing as an alternative to standard lecithin to improve rheological characteristics and the fat crystallization process. The influence of the type of these additives (hydroxylated, enzymatically hydrolyzed, acetylated, and defatted lecithins) as well as the amount added (0.2, 0.5, 0.8% [w/w]) to cocoa butter was evaluated. The results were compared to samples containing standard lecithin and polyglycerol polyricinoleate (PGPR). The influence of emulsifier addition was investigated measuring the melting and crystallization properties by differential scanning calorimetry (DSC) and the crystallization kinetics using a Nuclear Magnetic Resonance (NMR) spectrometer. The addition of modified lecithins to cocoa butter increased fat crystallization rates, mainly at the lowest concentration evaluated. The isothermal crystallization of the emulsifiers suggests that lecithins may act as co-crystallizers in cocoa butter crystallization. Thus, the acceleration of the crystallization events can be attributed to presence of the high melting point phospholipids and also to solids present in lecithins. Pure PGPR did not crystallize and did not influence the cocoa butter crystallization. The use of modified lecithins in cocoa butter decreased the maximum temperature of melting (T_{max}) and increased the crystallization (ΔH_c) and melting (ΔH_m) enthalpies.

Practical application: The modified soy lecithins may be used as alternatives to expand the range of applications of phospholipids containing emulsifiers like standard soybean lecithin. These new commercial emulsifiers may act as crystallization agent, anti-bloom agent, to control polymorphism and as improver of rheological properties of chocolate or the like. Our results showed that the effects of modified lecithins were more prominent than standard lecithin on crystallization process, mainly in low concentration, so that their use can significantly reduce costs in industrial chocolate processing and produce more appealing products.

Keywords: Cocoa butter / Crystallization / Melting / Modified lecithins / Polymorphism

Received: February 18, 2015 / Revised: October 8, 2015 / Accepted: November 3, 2015

DOI: 10.1002/ejlt.201500093

Supporting information available online http://dx.doi.org/10.1002/ejlt.201500093

1 Introduction

Cocoa butter represents the continuous phase in chocolates, serving as matrix for the dispersion of the solid particles of cocoa, sugar, and milk. Cocoa butter is also responsible for several quality attributes in the final product, such as hardness, melting at mouth temperature, surface gloss, and volume contraction during the demolding step [1]. The main components of cocoa butter are triacylglycerols (TAG) that represent between 97 and 98% of total lipids. The remainder consists of diacylglycerols (DAG), monoacylglycerols (MAG), and free fatty acids (FA). Two saturated fatty acids are predominant, palmitic acid (27%), and stearic acid (34%), with oleic acid as the main monounsaturated fatty acid (34%) [2]. Cocoa butter crystallizes in six polymorphs: I, II, III, IV, V, and VI. Due to this complex polymorphism, chocolates require tempering before the step of molding.

Correspondence: Prof. Theo Guenter Kieckbusch, School of Chemical Engineering, University of Campinas, 13083-852 Campinas, Brazil
E-mail: theo@feq.unicamp.br

Abbreviations: CB, cocoa butter; **DAG,** diacylglycerol; **DSC,** differential scanning calorimetry; **FA,** fatty acids; **MAG,** monoacylglycerol; **O,** oleic acid; **P,** palmitic acid; **PGPR,** polyglycerol polyricinoleate; **S_t,** stearic acid; ***S_3*,** tri-saturated; ***S_2U*,** di-saturated or monounsaturated; **SFC,** solid fat content.; **TAG,** triacylglycerol; ***U_2S*,** di-unsaturated; ***U_3*,** tri-unsaturated

Among emulsifiers applied to fatty products, lecithin is widely used. The term lecithin is a commercial designation of emulsifiers composed by a mixture of molecules called phospholipids. When the phosphoric group in phosphoric acid molecules is esterified with choline, ethanolamine, serine, inositol, glycerol, or diacylglycerol, produces derivatives called phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), respectively. Lecithin is a mixture of these derivatives. The composition of standard lecithin presents 19–21, 8–20, and 20–21% of PC, PE, and PI, respectively [3–6]. Other components like soybean oil, sterols, free carbohydrates, water, and other phosphatides correspond approximately to 33–35, 2–5, 5, 1, and 5–11%, respectively [3]. The fatty acid composition of lecithin is different from soybean oil. In lecithins, the major fatty acids content are around 16, 4, 17, 55, and 7% of palmitic, stearic, oleic, linoleic, and linolenic acid, respectively [7].

The most commonly used emulsifiers in chocolate industries are standard soy lecithin and polyglycerol polyricinoleate (PGPR), which are added to the chocolate mass mainly for rheological adequacy. These two additives have complementary effects on viscosity and yield stress. Lecithin is reported to have greater effect on plastic viscosity and PGPR reduces the yield stress. Small amounts added suffice to adequate the rheological behavior of molten chocolates. This is due to the ability of emulsifier molecules to cover the surface of polar particulates (sugar and cocoa powder), turning them lipophilic so to reduce frictions between particles dispersed in the fatty phase. As a consequence, molten chocolate shows better flow properties [2, 8–10]. Besides functioning as emulsifiers, they are also said to affect the polymorphic transition, the crystal morphology, and the fat bloom development [5]. According to Miskandar et al. [11], standard soy lecithins can act as crystallization promoters or inhibitors, depending on the concentration added to the fat phase. Bowser [12] showed that the addition of PGPR or standard soy lecithin to cocoa butter produces numerous crystals of small sizes or few crystals of larger size, respectively. In general, the effect of emulsifiers on fat is associated to the development of different crystalline organizations and/or imperfections [13]. Two different mechanisms have been reported in order to interpret the influence of emulsifiers on fats crystallization. The first one refers to the activity of these additives as hetero-nuclei, promoting the acceleration of the crystallization rate by a direct catalytic action. During crystal growth, emulsifiers would be adsorbed on the surface of the crystals and therefore, the rate of incorporation of TAGs and the crystals morphology might be modified. The second mechanism, accepted by most authors, states that the emulsifiers and triacylglycerols could co-crystallize due to the similarity of their chemical structures. On the other hand, structural dissimilarities should cause delays in nucleation, inhibiting crystal growth [5, 14, 15].

There are several types of lecithins and modified soy lecithins emerging as an alternative to expand their range of applications. Changes carried out in the molecular structure of the phospholipids may promote different effects on a matrix [16], and consequently each modified lecithin can improve specific sensorial properties of products in the confectionery, dairy, and bakery areas. Modifications are made by physical, enzymatic, and chemical processes. The first one occurs when the modification is by physical fractionation of the components of crude lecithin, using solvents like acetone, alcohol, or others. Thus, the final product will have a high concentration of specific phospholipids or will be highly defatted. The enzymatic process subjects the phospholipid molecule to a partial hydrolysis using phospholipase. The chemical modification is based on partial hydroxylation of unsaturated fatty acids in the phospholipid molecules or by acetylation of the amino group of phosphatidylethanolamine molecule (PE) [3, 17]. These modifications produce defatted lecithin (or deoiled lecithin), PC-enriched lecithin, PI-enriched lecithin, enzymatically hydrolysed lecithin, hydroxylated lecithin, and acetylated lecithin [17].

The changes affect the balance between hydrophilic and lipophilic groups (HLB) of emulsifiers molecules. Different HLB values classify emulsifiers according to their application in emulsified foods. The modified lecithins generally have higher HLB values than those of standard lecithin, which are generally reported to be around 4. According to the literature, PE-enriched lecithin, PC-enriched lecithin, acetylated lecithin, enzymatically hydrolyzed lecithin, and hydroxilated lecithin have HLB values of 2–3, 5, 6–7, 7, and 9, respectively [17].

In the field of fat crystallization, studies of modified lecithins involving fats and the correlated crystallization phenomena have not been disclosed, yet. Thus, the objective of this study was to evaluate the effect of added modified lecithins on the melting and crystallization properties of cocoa butter. Results of applications of modified lecithins to chocolate mass will be presented in a complementary paper.

2 Materials and methods

2.1 Materials

Refined cocoa butter was supplied by Barry Callebaut Brasil S/A (Ilhéus-BA, Brazil). The fat was stored under controlled temperature (23 ± 0.5°C) and relative humidity until use.

The lecithins were provided by Solae Company (Pillar-RS, Brazil) and their Hydrophilic Lipophilic Balance (HLB) values are: acetylated lecithin(6), enzymatically hydrolyzed lecithin (8), hydroxylated lecithin(9-10), standard lecithin(4), defatted (or deoilded lecithin)(7). PGPR Grinsted 90 was supplied by Danisco (Cotia-RS/Brazil).

2.2 Methods

2.2.1 Fatty acid composition

The fatty acid composition was determined by capillary gas chromatography (CGC Agilent 6850 Series GC System [Santa Clara, USA]) after esterification, according to the method Hartman and Lago [18]. The separation of the fatty acids methyl esters was carried out according to the procedure AOCS Ce 1f-96 [19] using a capillary Agilent DB-23 (50% cyanopropyl – methylpolysiloxane) column, dimensions: 60 m long × 0.25 mm internal diameter and 0.25 μm film thickness. The operating conditions were: helium at a flow rate of 1.0 mL/min; linear velocity: 24 cm/s; FID detector temperature: 280°C; injector temperature: 250°C; oven temperature: 110–215°C (5°C/min), 215°C/24 min; injection volume: 1.0 μL. The results are expressed as the mean of two determinations.

2.2.2 Triacylglycerol composition

The TAG composition was obtained according to method AOCS Ce 5–86 [14] by capillary gas chromatography (CGC Agilent 6850 Series GC System) with a capillary column DB-17HT, Agilent Catalog no. 122-1811 (50% phenyl – methylpolysiloxane), 10 m long, 0.25 mm internal diameter, and 0.15 μm film thickness. The operating conditions were: helium at a flow rate of 1.0 mL/min; linear velocity: 40 cm/s; FID detector temperature: 375°C; injector temperature: 360°C; oven temperature: 250–350°C (5°C/min), 350°C/20 min; injected volume: 1.0 μL; split injection: 1:100; sample concentration without prior preparation: 10 mg/mL of tetrahydrofuran. The results were expressed as the mean of two determinations.

2.2.3 Emulsifiers addition to cocoa butter

Five different lecithins were tested (acetylated, enzymatically hydrolyzed, hydroxylated, standard, defatted [or deoiled lecithin]). Samples containing the emulsifiers (0.2, 0.5, and 0.8%, w/w) were prepared by melting 200 g of cocoa butter, at 60°C for 30 min, adding the emulsifier followed by homogenization for 10 min using a magnetic stirrer (150 rpm) (IKA Instruments [Staufen im Breisgau, Germany] RH basic1).

Samples containing PGPR (0.2, 0.5, and 0.8%) were also prepared and tested for comparison. Pure cocoa butter (without any emulsifiers) was also subject to the same treatment and served as reference.

2.2.4 Isothermal crystallization using NMR

The samples were melted (70°C/10min) and kept in a high precision dry bath (TCON 2000, Duratech, Carmel, USA) at 60°C for 1 h aiming to destruct their crystalline history. The increase in SFC due to crystallization was monitored by a NMR spectrometer Bruker pc120 Minispec (Rheinstetten, Germany), with the reading compartment stabilized at 15°C. The data acquisition was automatic, with measurements taken every minute, during 110 min for cocoa butter with and without emulsifiers, and 40 min for the emulsifiers. The analysis ended when the difference between SFC values were lower than 1% in a time interval of 10 min. All SFC data were used to fit to Avrami model (Eq. 1) using nonlinear regression.

$$\frac{\mathrm{SFC}(t)}{\mathrm{SFC}_{\max}} = 1 - e^{-kt^n} \tag{1}$$

The parameter n is the Avrami exponent and k is the Avrami constant (min^{-n}), $\mathrm{SFC}(t)$ is the solid fat content at any specific time, and $\mathrm{SFC}_{\max}$ corresponds to the maximum Solid Fat Content [20].

The experimental data were used to obtain complementary parameters: (a) the induction time (ζ), which reflects the time required for a stable critical nucleus to be formed in the liquid phase [20, 21]; the values of this parameter were confirmed visually and also verified with data obtained from NMR measurements and (b) the half life of crystallization $t_{1/2}$ (Eq. 2), which is defined as the moment at which the degree of crystallization is 50% completed. The measurements were carried out in triplicate and the results are shown as the mean ± standard deviation.

$$t_{1/2} = \left(\frac{ln2}{k}\right)^{\frac{1}{n}} \tag{2}$$

2.2.5 Solid fat content (SFC)

Samples were placed in a dry thermostatic bath with the temperature controlled by a Peltier system (Duratech TCON, USA), and then submitted to the tempering procedures for non-stabilizing fats, according to the direct method recommended in AOCS Method Cd 16b-93 [22]. The measurements of the SFC were performed at temperatures of 10, 15, 20, 25, 30, 35, and 40°C, using the NMR spectrometer. Samples were analyzed in duplicate and the values are reported as the mean of two measurements.

2.2.6 Melting and crystallization properties using DSC

Melting and crystallization events of samples of cocoa butter and cocoa butter with 0.2% of emulsifier were determined according to AOCS method 1-94 cj [19] with some adjustments using a differential scanning calorimeter (DSC), model Q2000 TA-plus TX (Hamilton, USA), connected to an auto sampler. The cooling was performed using liquid nitrogen. Approximately 10 mg of samples were weighed and hermetically sealed in aluminum pans. For the

determination of the crystallization events, a cooling rate of 2°C/min was used in the temperature range of 80°C to −40°C. The samples were stabilized for 30 min at this temperature and subsequently heated to 80°C at a heating rate of 10°C/min.

An empty sealed aluminum pan was used as reference and the equipment was previously calibrated using indium. The results are presented as heat flow (W/g) normalized per gram of sample.

The onset or initial temperature (T_{onset}) and maximum peak temperature (T_{max}) of melting and of crystallization, all in degrees Celsius, the melting (ΔH_m) and the crystallization (ΔH_c) enthalpy in J/g, and the peak intensity in W/g, were obtained from the curves. Samples were analyzed in triplicate and the values are reported as the mean ± standard deviation.

2.2.7 Statistical analysis

The values of the parameters obtained were analyzed by descriptive statistics, the analysis of variance (ANOVA), and Tukey test was used to determine significant differences of means at a probability level of 5% ($p < 0.05$).

3 Results and discussion

3.1 Fatty acid and triacylglycerol composition of cocoa butter

The fatty acid composition of the cocoa butter samples is in agreement with literature data on Brazilian cocoa butter [18–20]. The major fatty acids were palmitic (C16:0), stearic (C18:0), and oleic acid (C18:1), representing 93.9% of total fatty acids. The sum of the unsaturated fatty acids content in the cocoa butter samples was higher than values found by Ribeiro et al. [23], and slightly lower than data disclosed by Lipp and Anklan [24] and Spangenberg and Dionisi [25], both for Brazilian cocoa butter. Compared to Malaysia and Ivory Coast cocoa butters, the samples used in this research presented about 2% less stearic acid and about 2% more oleic acid contents.

The triacylglycerols composition of cocoa butter presented as major components POP (19.9%), POS_t (39.7%), S_tOS_t (21.6%), summing up to 81.2% of the total triacylglycerols content. The content of trisatured (S_3) TAG was lower and monounsaturated (S_2U) was similar or slightly lower than the majority of cocoa butter composition disclosed in the literature [23, 25, 26]. The di- (U_2S) and tri-unsaturated (U_3) TAG had higher values than those presented by Ribeiro et al. [23] and Foubert et al. [26]. Thus, based to this compositional profile, one can anticipate that the cocoa butter used in this study has a lower melting point, a slower crystallization kinetic and lower SFC at high temperatures than those reported in the literature.

3.1.1 Solid fat content

The SFC or solids profile refers to the melting behavior of the fat. The solids content data obtained for the cocoa butter plus emulsifiers and for the reference (pure cocoa butter) samples showed very similar values and overall behavior. Due to this reason, and for the sake of illustration, Fig. 1 shows only the SFC values of pure cocoa butter and of those containing standard lecithin. At temperatures higher than 25°C, cocoa butter showed a prominent reduction of SFC, and melted below 35°C. Cocoa butter with SFC around 51% at 25°C and melting temperature around mouth temperature are desirable for uses in the production of chocolates [9]. The shape of all curves is similar to the one found by Ribeiro et al. [23], confirming that the sample used is representative of the behavior of Brazilian cocoa butter.

3.1.2 Isothermal crystallization at 15°C

The curves obtained are drawn in Figs. 2 and 3 show the typical sigmoidal shape of crystallization isotherms of cocoa butter. The addition of modified lecithins to cocoa butter alters the kinetic of crystallization. Cocoa butter with lower concentrations of lecithin showed the highest rates of crystallization.

Additions of emulsifiers to cocoa butter caused different effects on crystallization patterns depending on each type of lecithin. The crystallization rates of cocoa butter samples with enzymatically hydrolyzed (Fig. A – Supporting Information) and hydroxylated lecithin (Fig. B – Supporting Information) at concentrations of 0.2 and 0.5%, showed similar changes. The same behavior was found with the samples containing standard (Fig. 2) and defatted lecithin (Fig. C – Supporting Information). Variation in the concentration of PGPR hardly affected the crystallization curve (Fig. 3). Compared to the 0.5% curve, most samples with 0.8% of emulsifier presented a drift of the crystallization behavior toward the curve of pure cocoa butter, with the sample containing 0.8% acetylated lecithin transposing it (Fig. D – Supporting Information).

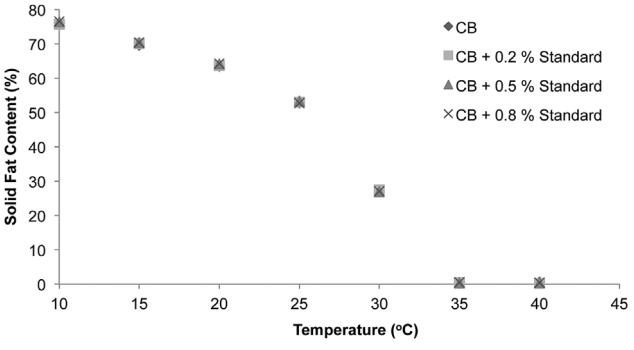

Figure 1. Solid Fat Content (SFC) of pure cocoa butter and cocoa butter with standard lecithin at concentrations of 0.2, 0.5, and 0.8% at different temperatures.

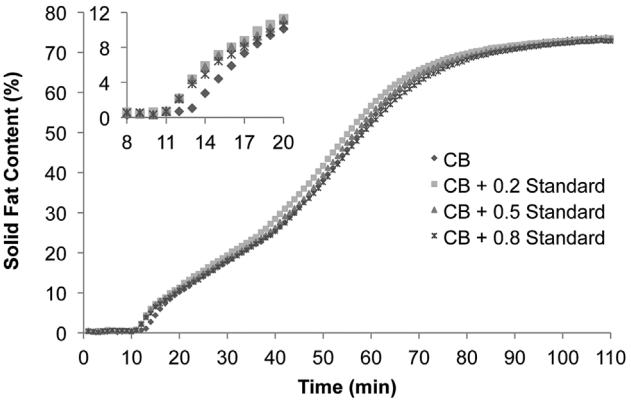

Figure 2. Crystallization isotherms of cocoa butter with standard lecithin, at 15°C.

In all determinations, the SFC_{max} value reached 73% in approximately 110 min. The curves can be divided in two regions with distinct effects of the emulsifiers. The first region refers to the period between 10 and 14 min where the nucleation and crystal growth start, and the second one refers to the period of rapid crystal growth between 35 and 80 min. In the last region, it is possible to observe differences of SFC values between samples with emulsifier and pure cocoa butter. The literature reports that this region includes the period in which the polymorphic transition from Form II to Form IV takes place [27]. The addition of emulsifier increased the crystallization rate, which means that the evolution of polymorphic transitions may also have been accelerated. The parameters induction time, half life of crystallization and maximum SFC proved to be well suited for a quantitative description of these regions and were able to establish differences between cocoa butter samples with and without emulsifiers.

According to Nieuwenhuyzen and Tomás [7], each type of emulsifier acts differently on a matrix, and the specific effects are strongly associated to the presence of different chemical structures. Lecithins, however, are two phased mixtures of several components, so that the amount added to the lipid matrix as well as the soluble fraction (oil, carbohydrates, and others) and the insoluble fraction (phosphatidylcholine [PC], phosphatidylinositol [PI], phosphatidylethanolamine [PE], phosphatidic acid [PA], phosphatidylglycerol [PG], phosphatidylserine [PS], lysophosphatidylcholine [LPC]) can also affect the crystallization behavior. For example, defatted lecithins, which are oil free, have higher phospholipid concentration than standard lecithins. Thus, for the same amount of total lecithin added to the fat system, defatted lecithin will have a higher content of phospholipids. However, the results indicated that the effect of defatted lecithin on cocoa butter crystallization was similar to that of the sample containing standard lecithin. On the other end, enzymatically hydrolyzed lecithin and hydroxylated lecithin have small differences in phospholipids content (PC, PI, PE, PG, PS, and PA), since the chemical changes (hydrolysis or hydroxylation) are made at the *sn*-2 position of the phospholipids molecules [7]. These structural changes are reported to increase the polar fraction of the molecules of phospholipids, and consequently, increase the HLB values. The structural changes could account for the pronounced effects on accelerating the cocoa butter crystallization accomplished by these two modified lecithins.

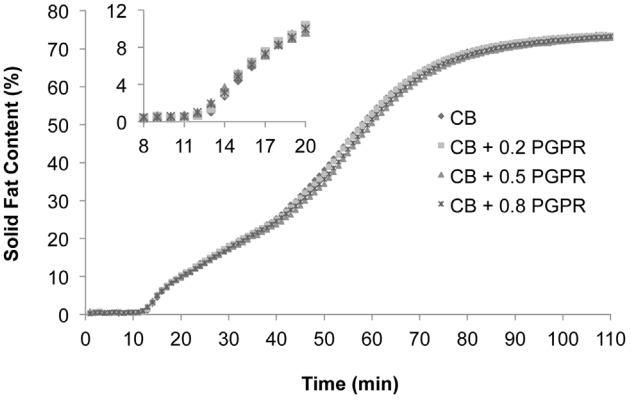

Figure 3. Crystallization isotherms of cocoa butter with PGPR, at 15°C.

The quantification of the crystallization kinetics was performed through the Avrami parameters n and k which were obtained by nonlinear regression of the experimental data. The range of SFC values used in the regression comprised all data, from the beginning until the time when equilibrium was reached. The parameters obtained are shown in Fig. 4. The table also contains the parameters induction time (ζ), the half life of crystallization $(t_{1/2})$ and the maximum solid fat content (SFC_{max}). The Avrami model fitted well to the experimental data with coefficients of determination (R^2) values higher than 0.99 for all samples.

The Avrami parameter k has been used to evaluate the effect of additives in crystallization acceleration [20]. In the present study, the values of k indicate an influence of emulsifiers on the cocoa butter crystallization, particularly at the lowest concentration of this additive. All samples showed a general decreasing trend of $k$values with increasing emulsifier concentration, except those containing PGPR. Samples that reached the highest values of k were those containing enzymatically hydrolyzed and hydroxylated lecithin, at concentrations of 0.2 and 0.5%, with no significant differences among them. Compared to pure cocoa butter, samples with acetylated lecithin at a concentration of 0.8% (w/w), and all samples containing PGPR showed no statistical differences at 5% level of significance ($p < 0.05$).

In isothermal crystallizations, the Avrami parameter n describes the type and size of the formed fat crystals. The values found for the exponent n were between 2.2 and 2.5,

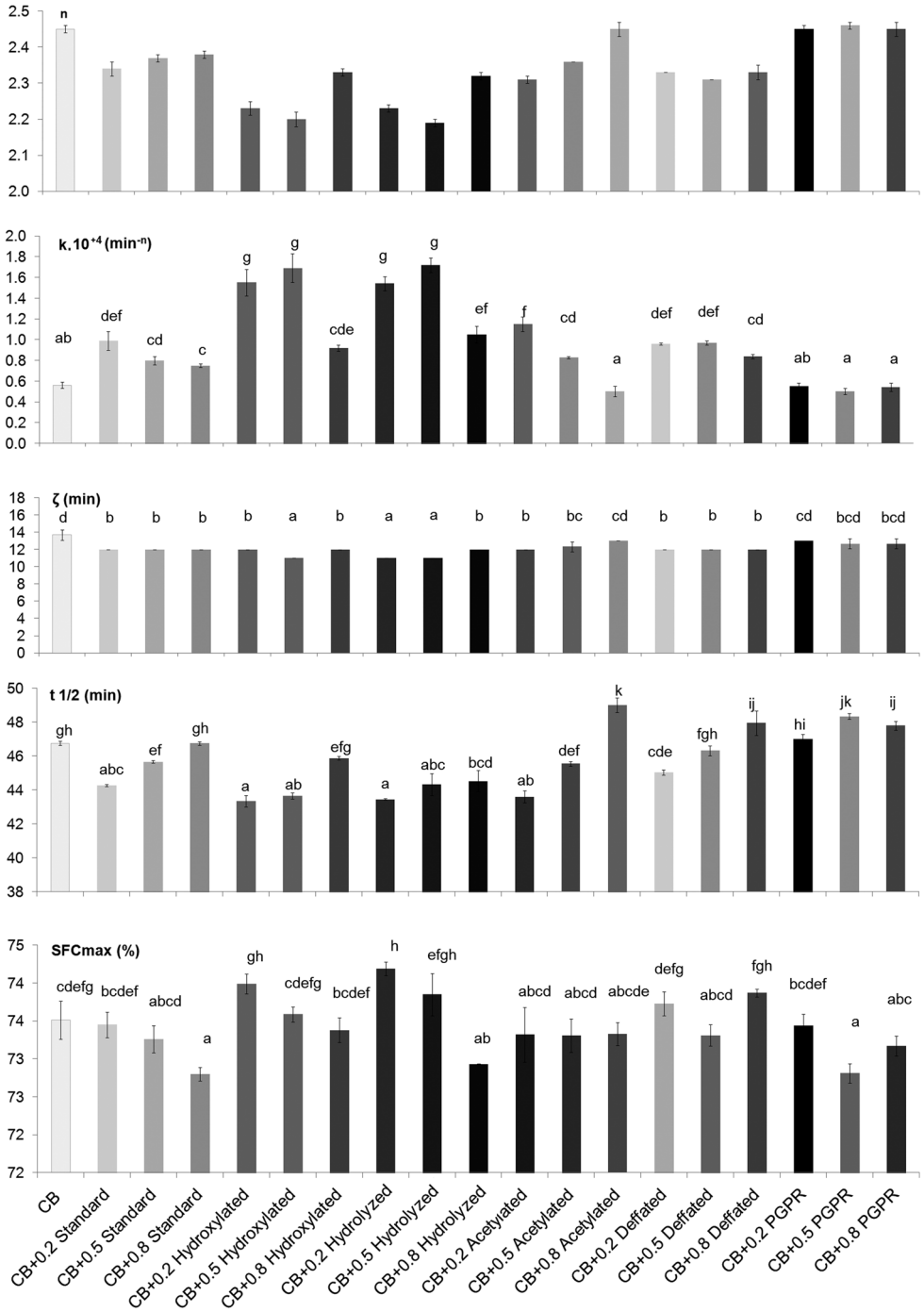

Figure 4. Avrami parameters obtained from isothermal crystallization, at 15°C, of pure cocoa butter and cocoa butter with emulsifier.

suggesting the formation of mixed crystals, either in samples of pure cocoa butter or in cocoa butter combined with emulsifiers. According to Avrami [28], non-integer values of n between 2 and 3 indicate up to three dimensional crystal growth forming needle, disc or spherulite structures, with instantaneous or sporadic nucleation. Thus, the process of crystallization follows a well-defined trend, indicating that static and isothermal crystallizations may induce the formation of mixed crystals.

The majority of the emulsifiers added to cocoa butter decreased the induction time (ζ), signalizing that emulsifiers affected the crystallization rate of pure cocoa butter. The

induction time of samples containing lecithin varied between 11 and 12 min while for pure cocoa butter sample the average value was 13.7 min. For most blends, an increase in emulsifier concentrations did not affect the induction time. Samples with 0.5% hydroxylated lecithin and with 0.2 and 0.5% hydrolyzed enzymatically lecithin had the lowest value of this parameter. All samples containing PGPR did not present statistically significant differences in relation to pure cocoa butter. Moreover, samples containing PGPR showed values of SFC_{max}, n, and k close to pure cocoa butter. This behavior suggests that the addition of PGPR does not modify the crystallization behavior of cocoa butter, at the studied concentration levels.

Samples with the highest concentrations of emulsifier showed the highest values of half life of crystallization, $t_{1/2}$. Among these, cocoa butter with defatted and acetylated lecithin, both at a concentration of 0.8%, presented the highest values of $t_{1/2}$, as well as to all blends containing PGPR. In general, as expected, a good correlation between Avrami parameters k induction time (ζ), and $t_{1/2}$ was obtained. The samples containing hydroxylated and enzymatically hydrolyzed lecithin for instance, presented the shortest values of induction time, the lower values of half life of crystallization and the largest value of the parameter k. All SFC_{max} values varied within a narrow range. Cocoa butter with standard, hydroxylated and hydrolyzed lecithin had a statistically well-defined trend of reducing SFC with the increase in additive concentration.

The most widely accepted mechanism to explain the positive influence of some additives in accelerating lipid crystallization is based on similarity of chemical structures.

It is reported in the literature that phospholipids can co-crystallize with the TAGs due to structural similarity [15] and act as hetero-nuclei, promoting an increase of the crystallization rate by the direct catalytic action as an impurity [5]. By the same point of view, small solid particles and impurities present in the lecithin product can be considered as surface active agents and can act like emulsifiers [29, 30]. According to Williams and Chapman [31], the melting point of the phospholipids is high, between 67 and 240°C. Jeffrey [29] suggests that the phospholipids PC, PG, PA, PI, PE, and PA accelerate crystallization because they are able to nucleate prior to the triacylglycerol molecules nucleation, serving as initial site to start the packing of triacylglycerol molecules.

There are also a few mechanisms that could be considered in order to explain the influence of emulsifier additions at different concentration on the crystallization kinetics of cocoa butter. The reduction of fat crystallization rate with increasing concentration of additives could be attributed to different micelles formations, direct or reverse. Micelles can arrange in normal hexagonal or reverse hexagonal structures. According to Savage and Dimick [32], cocoa butter induction periods and subsequent crystal-growth rates are speculated to depend upon the proportion between the normal hexagonal phase (H_I) forming phospholipids (lysophosphatidylcholine [LPC] and phosphatidylinositol [PI]), and the phosphatidylcholine (PC), which typically forms reverse hexagonal phase (H_{II}). They also stated that the composition of phospholipids among the rapid-nucleating cocoa butter was generally made up by high proportions of PC and low proportions of LPC and PI. The slow-nucleating cocoa butters exhibited opposite trends. As each lecithin contains in its composition a mixture of different phospholipids as previously mentioned, different micelle formation can potentially be formed in the lipid system. In the normal hexagonal phase (H_I), the nonpolar part (fatty acids) of the micelles' molecular structure is oriented toward the fat phase and the polar part directed outwards the micelle [33, 34]. As a consequence, micelles remain isolated from others by repulsive forces between their polar groups. An increase of emulsifier concentration could increase the electrostatic repulsion between the micelles [33, 34], and consequently, the crystallization rate decreases. In addition, excess emulsifier could also hamper a proper packing of the TAGs in the crystal lattice due to steric hindrance [12, 34].

To support these hypotheses the crystallization isotherms of emulsifiers were obtained using the same temperature of 15°C and NMR equipment. Figure 5 shows these isotherms and the presence of solids can be identified at the very beginning of the measurements. The initial solids may consist of particulate matter, impurities, or some phospholipids already crystallized due to their high melting points. These solids could act as a catalyst surface, leading to heterogeneous nucleation [31, 34]. A careful comparison between the cocoa samples and the pure additives isotherms, however, leads to another possible mechanism. The curves in Fig. 5 (except for PGPR) show a steady increase in solids content and the crystallization rate levels off after 13–14 min. Coincidentally, the nucleation induction times of the cocoa butter samples containing lecithin (Fig. 2A–C) are in the range of 11 min or higher. This finding may indicate that the lecithin components that are in the process of crystallization could be active

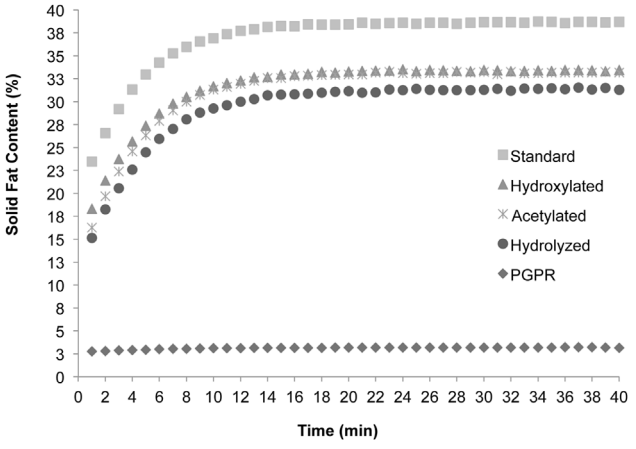

Figure 5. Crystallization isotherms of the emulsifiers, at 15°C.

and available to co-crystallize with the cocoa butter lipids. Despite the small amounts of lecithins added to cocoa butter, they could maintain their activity as minor components and crystallize. Thus, these crystallization isotherms may indicate that lecithin components could act as co-crystallizers and surface-active agents. PGPR did not crystallize, and it might be the key explanation for its poor performance when added to cocoa butter.

The discussion could not be carried out based solely on the phospholipids composition of lecithin (PC, PE, PA, PI, and PG). It is noteworthy to consider, however, that modified lecithins have higher values of HLB than standard lecithin (Item 2.1). According to the literature [10, 17], higher HLB values of emulsifiers indicate that they are suitable to be used in O/W emulsion (hydrophilic medium). However, addition of low concentration of modified lecithin to cocoa butter produced effects at least equivalent to the standard lecithin.

Standard lecithin has a HLB value considered rheologically more appropriate for oily products (HLB = 4). Moreover, hydroxylated and enzymatically hydrolyzed lecithins have higher HLB values, 9–10 and 8, respectively, and exerted a more prominent effect on cocoa butter crystallization than the addition of standard lecithin. This suggests that the stronger the polar fraction in the phospholipids molecules, the greater may be its effect on cocoa butter crystallization. These conclusions are limited to pure cocoa butter and therefore, should be extrapolated with great care to chocolate masses crystallization since in these systems other polar components, such as sugar and cocoa solids can interfere.

3.2 Thermal analysis

Thermal analysis determinations were limited to samples with 0.2% of emulsifiers, since they showed the most pronounced effect on crystallization kinetics. Figure 6 shows the crystallization thermograms obtained by DSC, and one can observe that the shape of the curve for all samples was very similar. However, the pure CB and CB containing acetylated, standard, defatted, and hydroxylated lecithins

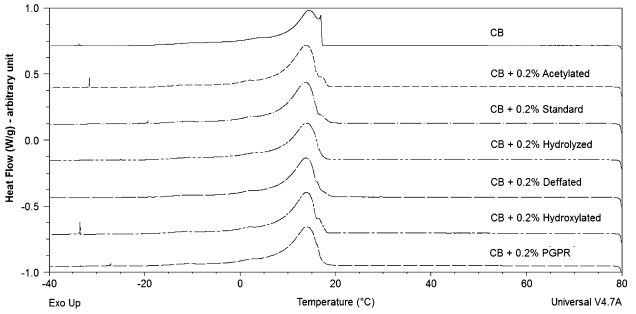

Figure 6. DSC crystallization thermograms of pure cocoa butter and cocoa butter with 0.2% of emulsifiers at a cooling rate of 2°C/min.

presented double peaks. Explanations for such behavior are not clear, yet. For a quantitative evaluation of the crystallization properties, the following parameters obtained from the thermograms were used: initial or onset temperature of crystallization, T_{onset} (°C), the maximum temperature of crystallization, T_{max} (°C), peak intensity (W/g), and the enthalpy of crystallization, ΔH_c (J/g), determined by integration of the area under the curve, between T_{onset} and T_{endset}. The values found are shown in Fig. 8.

The values in Fig. 8 indicate that the addition of emulsifier changed the crystallization behavior of butter. With respect to the onset temperature of crystallization (T_{onset}), only pure cocoa butter was significantly different, with its crystallization starting at a temperature lower than the others samples.

As for the crystallization parameter T_{max}, pure cocoa butter presented significantly higher T_{max} value than other samples. Among the prepared samples significant differences were only found between cocoa butter with standard lecithin and with PGPR. Since crystallization is a process that releases heat, a larger T_{max} value is related to the point where crystallization or heat release is maximum. The addition of emulsifiers to cocoa butter immediately started the nucleation, and these samples showed lower T_{max} values than those obtained for pure cocoa butter. The low T_{max} values of the samples containing emulsifiers may be related to the ability of the emulsifiers to act as catalysts [5, 14], and thus, a smaller amount of energy is involved to accelerate crystals growth [35].

The enthalpy of crystallization refers to the total amount of energy released during the crystallization. The enthalpy values found are in the range 70–85 J/g, with pure cocoa butter as the lowest value. According to the literature, high enthalpy values are associated to the formation of the predominant homogeneous crystals and/or high-density crystals in the crystal lattice [36–39]. The peak intensity of pure cocoa butter was the lowest of all samples, but no defined trend was found.

Pure cocoa butter showed the most distinct behavior. It began to crystallize at a lower temperature, showed the highest value of T_{max} and lower values of enthalpy and peak intensity. As can be concluded by comparing values in Figs. 2, 3 and 6, the DSC crystallization outputs were unable to confirm the effect of modified lecithin obtained by NMR determinations. The samples containing hydrolyzed and hydroxylated lecithin, at concentration of 0.2% (higher k values), were not outstandingly different from each other. This suggests that the use of different cooling rates between both procedures (DSC and NMR) may have promoted variations in the interactions.

Figure 7 shows the melting curves obtained by DSC in the temperature range −40 to 80°C at a heating rate of 10°C/min. The parameters for the evaluation of melting properties, T_{onset}, T_{max}, melting enthalpy, and peak intensity were obtained from the melting curves and are shown in Fig. 9.

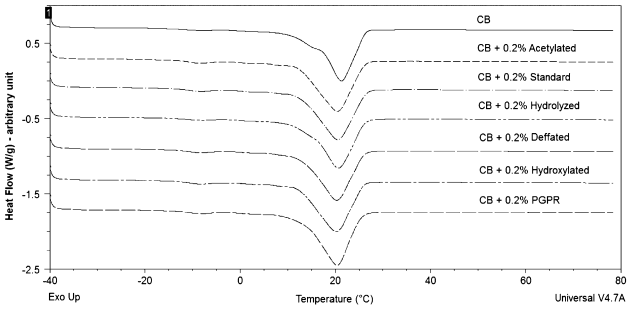

Figure 7. DSC melting thermograms of pure cocoa butter and cocoa butter with 0.2% of emulsifiers at a heating rate of 10°C/min.

The values of melting onset temperatures (T_{onset}) aligned around −11.3°C and showed no significant differences among all samples. Pure cocoa butter presented a T_{max} value of melting significantly superior than others samples. This behavior may be related to the high crystallization rate of pure cocoa butter, so that it reached the maximum crystallization peak before the other samples (Fig. 6). This situation may have accelerated the polymorphic transitions, since the more intensively the crystallization process takes place, the faster the transition to a more stable polymorphic form [10, 38]. The presence of more stable polymorphs may justify the highest T_{max} melting value of pure cocoa butter. Emulsifiers shorten the onset of cocoa butter crystallization, even so the presence of emulsifiers promoted less intense crystal growth than that of pure cocoa butter. Due to this behavior, lower T_{max} melting values were obtained. Among the samples with emulsifier, the hydrolyzed lecithin showed T_{max} melting values significantly different from the others.

Interestingly, the standard deviation of the melting enthalpy is higher than that of other melting parameters. The literature reported that depending on the crystallization technique a larger distribution of different polymorphs in the fat matrix may occur resulting in less dense structures and heterogeneous crystalline fat. Thus, the diversified polymorphs formation of heterogeneous crystals are reflected in higher values of standard deviation than for the other melting parameters [39].

An examination of the crystallization enthalpy change (Fig. 8) and the melting enthalpy change (Figure 9) reveals that cocoa butter with 0.2% of standard lecithin and cocoa butter with 0.2% of acetylated lecithin have the highest values of both parameters. The results may indicate that the fat crystal network of these samples is formed by more homogeneous crystals and more stable polymorphs [39].

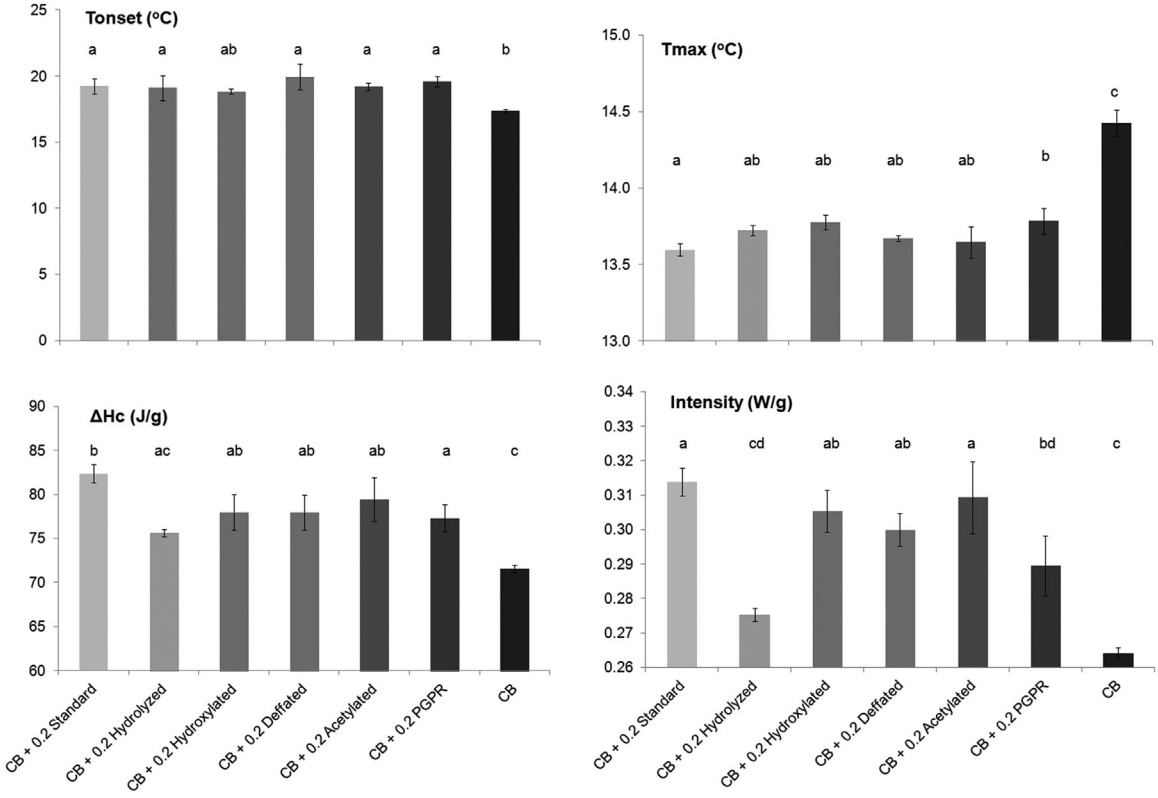

Figure 8. Onset (T_{onset}) and maximum (T_{max}) temperatures during the crystallization process, crystallization enthalpy (ΔH_c) and peak intensity of cocoa butter with and without emulsifiers subjected to cooling rates of 2°C/min in a DSC.

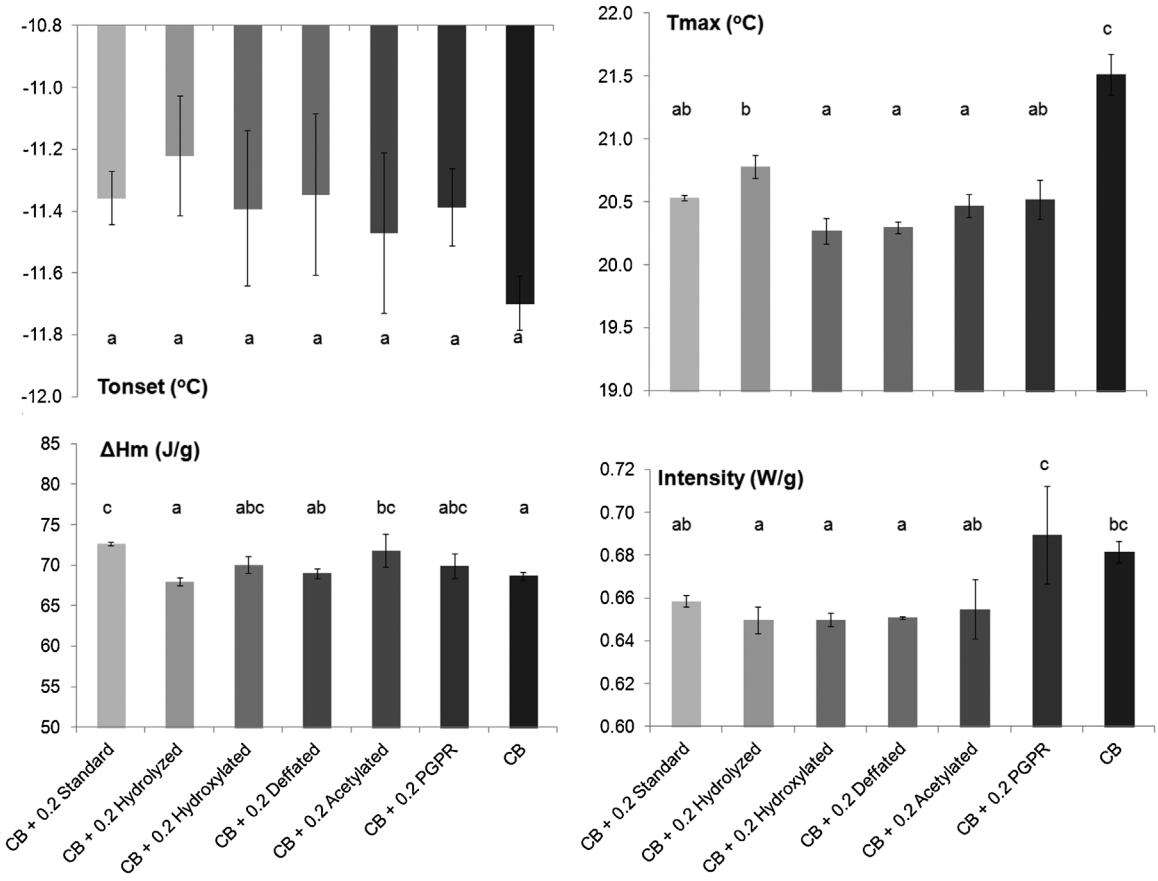

Figure 9. The onset (T_{onset}) and maximum (T_{max}) temperatures during the melting process, melting enthalpy (ΔH_m) and the peak intensity of cocoa butter with and without emulsifiers subjected to heating rates of 10°C/min in a DSC.

The values of peak intensity of all samples containing lecithin showed values around 0.65 W/g. The peak intensity of cocoa butter alone and containing PGPR were statistically higher.

4 Conclusions

Among the five modified lecithins tested, enzymatically hydrolyzed and hydroxylated lecithins showed the most pronounced effect on cocoa butter crystallization. This was confirmed mainly by the lowest induction time and the remarkable crystallization rate at the central region of the isotherms. According to Avrami theory, the general tendency was to form different crystal types and dimensions and the highest fat crystallization rate was found at the lowest emulsifier concentration (0.2%) added to cocoa butter. Crystallization isotherms of the commercial lecithins used confirmed that phospholipids with a high melting point and/or other kind of solids present in these products are prone to accelerate cocoa butter crystallization. The results suggest that these components may also be acting as co-crystallizers. PGPR did not crystallize and did not influence the cocoa butter crystallization

The addition of emulsifiers to cocoa butter increased the onset temperature of crystallization, confirming their effect on accelerating the nucleation. Blends of cocoa butter and emulsifiers having the largest values of crystallization enthalpy also presented the highest values of melting enthalpy. Thus, emulsifiers may act forming more cohesive fat network, denser and with the presence of more homogeneous polymorphs.

The results showed that enzymatically hydrolyzed and hydroxylated lecithins change the crystallization rate of cocoa butter so that they may be used as an alternative to standard lecithin with reassuring and promising potential applications in chocolate manufacture.

The authors acknowledge the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – Proc. 2009/53006-0) for financial support.

The authors have declared no conflicts of interest.

References

[1] Timms, R. E., in: Timms, R. E. (Ed.), *Confectionery Fats Handbook: Properties, Production and Applications*, The Oily Press, Bridgwater, UK 2003, pp. 63–104.

[2] Afoakwa, E. O., Paterson, A., Fowler, M, Factor influencing rheological and textural qualities in chocolate – A review. *Trends Food. Sci. Tech. 18*, 290–298.

[3] Tanno, H., *Lecithin-Ullmann's Encyclopedia Of Industrial Chemistry*, Wiley-VCH Verlag GmbH & Co, Weinheim, Germany 2012.

[4] Pokorný, J., in: Gunstone, F. D. (Ed.), *Modifying Lipids for Use in Food*, CRC Press, Boca Raton, USA 2006, pp. 369–390.

[5] Garti, N., in: Marangoni, A. G., Narine, S. (Eds.), *Physical Properties of Lipids*. Marcel Dekker, New York, USA 2002, pp. 265–386.

[6] Gunstone, F. D. in: Gunstone, F. D. (Ed.), *Structured and Modified Lipids*, 1st Ed., Marcel Dekker, New York, USA 2001, pp. 241–250.

[7] Nieuwenhuyzen, W. V., Tomás, M. C., Update on vegetable lecithin and phospholipid technologies. *Eur. J. Lipid Tech.* 2008, *110*, 472–486.

[8] Nebesny, E., Zyzelewicz, D., Effect of lecithin concentration on properties of sucrose-free chocolate masses sweetened with isomalt. *Eur. Food Res. Technol.* 2005, *220*, 131–135.

[9] Beckett, S. T., *Industrial Chocolate Manufacture and Use*, Blackwell Science, Oxford, UK 1994.

[10] Hasenhuettl, G. L., in: Hasenhuettl, G. L., Hartel, R. W. (Eds.), *Food Emulsifiers and their Applications*, Springer, New York, USA 2008, p. 183.

[11] Miskandar, M. S., Che Man, Y. B., Rahman, R. A., Aini, I. N., Yusoff, M. S. A., Effects of emulsifiers on crystallization properties of low-melting blends of palm oil and olein. *J. Food Lipids* 2006, *13*, 57–72.

[12] Bowser, A., Crystallization of cocoa butter. *Manuf. Confect.* 2006, *86*, 115–118.

[13] Aronhime, J. S., Sarig, S., Garti, N., Mechanistic considerations of polymorphic transformations of tristearin in the presence of emulsifiers. *J. Am. Oil Chem. Soc.* 1987, *64*, 529–533.

[14] Cerdeira, M., Martini, S., Hartel, R. W., Herrera, M. L., Effect of sucrose ester addition on nucleation and growth behavior of milk fat-sunflower oil blends. *J. Agr. Food. Chem.* 2003, *51*, 6550–6557.

[15] Ribeiro, A. P. B., Masuchi, M. H., Miyasaki, E. K., Domingues, M. A. F., et al., Crystallization modifiers in lipid systems. *J. Food Sci. Technol.* 2014, *2014*, 3925–3946.

[16] Szuhaj, B. F., in: Shahidi, F. (Ed.), *Bailey's Industrial Oil and Fat Products*, John Wiley & Sons, New York 2005, vol. *3*, pp. 361–456.

[17] Nieuwenhuyzen, W. V., in: Kjellin, M., Johansson, I. (Eds.), *Surfactants from Renewable Resources*, John Wiley & Sons, Chichester, United Kingdom 2010, pp. 191–212.

[18] Hartman, L., Lago, R. C., A rapid preparation of fatty acid methyl esters from lipids. *Lab. Pract* 1973, *22*, 475–476.

[19] AOCS, *American Oil Chemists' Society. Official Methods and Recommended Practices of the American Oil Chemists' Society*, 6th Edn., American Oil Society, Champaign 2009.

[20] Aguilera, J. M., Lillford, P. J., *Food Materials Science: Principles and Practice*, Springer, New York, USA 2008.

[21] Himawan, C., Starov, V. M., Stapley, A. G. F., Thermodynamic and kinetic aspects of fat crystallization. *Adv. Colloid Interface Sci.* 2006, *122*, 3–33.

[22] AOCS, *American Oil Chemists' Society. Official Methods and Recommended Practices of the American Oil Chemists' Society*, 5th Ed., American Oil Society, Champaign 2004.

[23] Ribeiro, A. P. B., Silva, R. C., Gioielli, L. A., Gonçalves, M. I. A., et al., Physico-chemical properties of Brazilian cocoa butter and industrial blends. Part I – Chemical composition, solid fat content and consistency. *Grasas Aceites* 2012, *63*, 79–88.

[24] Lipp, M., Anklam, E., Review of cocoa butter and alternative fats for use in chocolate – Part A. Compositional data. *Food Chem.* 1998, *62*, 73–97.

[25] Spangenberg, J. E., Dionisi, F., Characterization of cocoa butter and cocoa butter equivalents by bulk and molecular carbon isotope analyses: Implications for vegetable fat quantification in chocolate. *J. Agric. Food Chem.* 2001, *49*, 4271–4277.

[26] Foubert, I., Vanrolleghem, P. A., Thas, O., Dewettinck, K., Influence of chemical composition on the isothermal cocoa butter crystallization. *J. Food Sci.* 2004, *69*, 478–487.

[27] Marangoni, A. G., Mcgauley, S. E., Relationship between crystallization behavior and structure in cocoa butter. *Cryst. Growth Des.* 2002, *3*, 95–108.

[28] Avrami, M., Kinetics of phase change I: General theory. *J. Chem. Phys.* 1939, *7*, 1103–1112.

[29] Jeffrey, M. S., The effect of cocoa butter origin, milk fat and lecithin levels on the temperability of cocoa butter systems. *Manuf. Confect.* 1991, *71*, 76–82.

[30] Mcclements, D. J., in: Akoh, C. C., Min, D. B. (Eds.), *Food Lipids: Chemistry, Nutrition and Biotechnology*, CRC Press, Boca Raton, USA 2008, pp. 63–97.

[31] Williams, R. M., Chapman, D., in: Holmes, R. T. H. (Ed.), *Progress in the Chemistry fats and other Lipids*, Academic Press, London, UK 1970, pp. 1–79.

[32] Savage, C. M., Dimick, P. S., Influence of phospholipids during crystallization of hard and soft cocoa butters. *Manuf. Confect* 1995, *75*, 127–132.

[33] Hasenhuettl, G. L., Hartel, R. W., *Food Emulsifiers and their Applications*, Springer, New York, USA 2008, p. 426.

[34] Cordiez, J. P., Grange, G., Mutaftschiev, B., Solidification of stearic acid-water emulsion. effect of the droplet-medium interface on nucleation kinetics. *Stud. Surf. Sci. Catal.* 1982, *10*, 103–106.

[35] Kloek, W., Walstra, P., Vliet, T. V., Nucleation kinetics of emulsified triglyceride mixtures. *J. Am. Oil Chem. Soc.* 2000, *77*, 643–652.

[36] Arishima, T., Sagi, N., Mori, H., Sato, K., Density measurement of the polymorphic forms of POP, POS and SOS. *J. Jpn. Oil Chem. Soc.* 1995, *44*, 431–437.

[37] Seguine, E. S., Tempering the inside story. *Manuf. Confect.* 1991, *71*, 117–125.

[38] Macmillan, S. D., Roberts, K. J., Rossi, A., Wells, M. A., et al., In situ small angle X-ray scattering (SAXS) studies of polymorphism with the associated crystallization of cocoa butter fat using shearing conditions. *Cryst. Growth Des.* 2002, *2*, 221–226.

[39] Svanberg, L., Ahrné, L., Lorén, N., Windhab, E., Impact of pre-crystallization process on structure and product properties in dark chocolate. *J. Food Eng.* 2013, *114*, 90–98.

