

CRYSTALLIZATION MECHANISMS IN CAMEL MILK CREAM DURING PHYSICAL RIPENING: EFFECT OF TEMPERATURE AND RIPENING DURATION

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Abstract

The effects of temperature (5 °C, 12 °C, and 21 °C) and time (0 h, 1 h, 3 h, 5 h, 24 h) on the camel cream crystallization mechanisms during ripening were investigated. Particles size, solid fat content (SFC), melting behavior and polymorphism were evaluated. Significant increase of the number of larger droplets was observed for samples ripened at 21 °C as a function of ripening time. Analysis of the solid fat content and the total enthalpy of melting curves indicated that, regardless of the temperature, the crystallization of camel milk cream occurs especially during cooling step and during the first hour of ripening. The results revealed that ripening of camel milk cream at 5 °C and 12 °C promoted a transition from α to β' 2 and appearance of β . However, β' 1 and β crystals were found in camel cream throughout the ripening at 21 °C. The present study enables a better understanding of the crystallization mechanisms of camel milk cream during ripening, which is needful during the production of butter camel milk.

1. Introduction

During butter manufacturing, cream ripening is one of the most crucial steps that enable the formation of a continuous fat network during churning. The ripening step mainly depends on the fatty acids composition of the cream (Frede and Buchheim, 1994). Differences in physicochemical composition, especially regarding the fatty acids profile may change the crystallization mechanisms during ripening of the cream. The overall composition of camel milk is similar to that of cow milk (El-Agamy, 2006).

However, several studies have reported some differences between camel and cow milk in relation to the fat composition (Abu-Lehia, 1989; Attia et al., 2000; Gorban and Izzeldin, 2001). Camel milk fat contains a smaller amount of short-chain fatty acids, a higher concentration of long-chain fatty acids, and a higher content of unsaturated fatty acids (Attia et al., 2000). Moreover, compared to cow milk fat microstructure, the camel milk presents two major dissimilarities: on the one hand, camel milk fat has a higher frequency of smaller globules which negatively influenced the butter manufacturing process (Mehaia, 1993; Attia et al., 2000; Karray et al., 2005a,b). On the other hand, camel fat globules are characterized by a thicker membrane with a higher amount of phospholipids allowing better stability of camel milk emulsion (Mehaia, 1993; Attia et al., 2000). Hence, due to these particular features (fatty acids composition and microstructure), the camel milk butter cannot be obtained by following the same technology of bovine milk.

In general, during butter making, the phase inversion requires the presence of partially crystallized fat globules which are necessary to partial coalescence phenomenon. The crystallization mechanisms of cow milk cream have been extensively investigated (Ronholt et al., 2012; Buldo et al., 2013; Lee and Martini, 2018). Cow milk fat crystallization during ripening is an extremely complex phenomenon as it is controlled by processing conditions, such as ripening time and temperature, cooling rate, and agitation rate. These factors influence mainly the solid fat content (SFC), the droplet size distribution, the melting behavior, and the polymorphism of cream. Lee and Martini, (2018) reported that the SFC increased faster at 5 °C than 10 °C and 15 °C during ripening. They also stated that agitating cream at 40 rpm increased the droplet size particles regardless of ripening temperature. In addition, longer ripening time leads to the formation of stable polymorphic forms, especially β form (Ronholt et al., 2012; Buldo et al., 2013). Moreover, by increasing the ripening time from 0 h to 1 h, the melting point of medium melting fraction also increased (Buldo et al., 2013). Besides, the fat crystallization in emulsions can also be affected by the emulsion droplet size and the triacylglycerols (TAGs) composition (Coupland, 2002).

Previous studies reported the possibility of producing butter from camel milk with some technological difficulties (Farah et al., 1989; Yagil et al., 1994; Berhe et al., 2013). Only a few studies have investigated the crystallization behavior of camel milk fat in bulk (Karray et al., 2004, 2005a,b) and camel milk cream (Lopez et al., 2005). In bulk, Karray et al. (2004) found the presence of the unstable α_{-2L} and the stable β'_{-2L} during cooling at 1 °C min⁻¹ and 5 °C min⁻¹. However, a single species corresponding to the most stable β'_{-2L} form was generated during cooling at 0.1 °C min⁻¹. Lopez et al. (2005) showed that similar crystalline structures (α'_{-2L} and β'_{-2L}) were formed in camel milk cream and in anhydrous camel milk fat during cooling at 1 °C min⁻¹. However, the same author indicated that different TAGs crystallization kinetics was observed in the dispersed state as compared to bulk state.

In these previous studies, the focus was mainly on elucidating the thermal and structural behavior of milk fat crystals. To the best of the author's knowledge, no research has assessed the effect of the thermal treatment of camel milk cream on the crystallization mechanisms during physical ripening. Understanding of the crystallization mechanisms during ripening will contribute to the optimization of the camel butter production process. Hence, this study aimed to evaluate the effect of the ripening time and temperature on the crystallization mechanisms. The droplet size distribution, the solid fat content (SFC) (using pulsed nuclear magnetic resonance (NMR)), the melting behavior (using Differential Scanning Calorimetry (DSC)) and the polymorphism (using X-Ray diffraction (XRD))

determinations were performed at predefined ripening time (0 h, 1 h, 3 h, 5 h, and 24 h) for each ripening temperature (5, 12, and 21 °C).

2. Materials and methods

2.1. SAMPLES PREPARATION

2.1.1. MILK, CREAM AND ANHYDROUS MILK FAT SAMPLES

Fresh raw camel milk (*Camelus dromedarius*) containing 27gL⁻¹ of fat was collected from 12 healthy milking females located in local cattle in the south of Tunisia; while cow milk was collected from a local farm (Gembloux). Both cow and camel milk were transported to the laboratory at 4 °C within 2 hours from milking, and then a pH measurement was performed. Afterward, camel milk cream was obtained by skimming operation at 3000g for 20 min at 4 °C using a Gyrozen 1580MGR Centrifuge (Gangnam-gu, Seoul, Korea). The fat content was determined by Gerber method (ISO 11870:2009) and was equal to 43% and 42% for camel and cow milk cream, respectively. Anhydrous camel milk fat and anhydrous cow milk fat were isolated as described by Karray et al. (2004). After churning of the cream at 21 °C, the obtained butter was melted at 60 °C and centrifuged at 3000g for 2 min. The anhydrous milk fat was obtained after vacuum filtration of the upper phase in the presence of anhydrous sodium sulfate (Na₂SO₄).

2.1.2. CREAM HEAT TREATMENT AND RIPENING

A laboratory-scale device was developed to control the heat treatment of the cream. Prior to the ripening step, camel milk cream (with 43% of fat) was heated at 65 °C for 15 min to erase crystal memory (Ronholt et al., 2012). The cream was then cooled (using a cooling rate of about 4.5 °Cmin⁻¹) to the ripening temperature and held at that temperature for 24 hours to allow crystallization. Three ripening temperatures (5 °C, 12 °C, and 21 °C) were considered. The 5 °C and 12 °C temperatures were within normal ripening temperature of bovine milk (Chandan et al., 2015); whereas 21 °C was chosen based on the melting curves of camel compared to that of bovine milk. A low agitation (30 rpm) was used during ripening to produce homogeneous crystallization.

2.2. CHEMICAL CHARACTERIZATION: FATTY ACID COMPOSITION BY GAS CHROMATOGRAPHY

The fatty acid composition was carried out by using a Trace GC Ultra gas chromatograph (GC) (Thermo Fisher Scientific, Belgium) fitted with a flame ionization detector (FID). The fatty acid methyl ester was prepared according to AOCS Ce 2-66 method (Walker, 1990), and were analyzed in GC-FID using a Stabliwax DA column (Restek Corporation, USA) of 30 m × 0.25 μm × 0.25 μm (length × thickness × diameter)). The injection was performed in splitless mode (splitless time: 0.85 min) at 250 °C. The temperature program was set as follow: 50 °C (hold of 1 min) to 150 °C at 30 °C/min and to 240 °C (hold for 10 min) at 5 °C/min. FID was set at 250 °C. The identification of fatty acid methyl ester was performed by comparison of their retention times with those of pure reference.

2.3. PHYSICAL CHARACTERIZATION

2.3.1. FAT DROPLET SIZE DISTRIBUTION

To track the aggregation of fat globules, their size distribution was quantified at different stages of ripening by laser scattering analyzer (Mastersizer 2000, Malvern Instruments, Ltd, UK). The refractive indexes used for water and globules were 1.33 and 1.46, respectively. Cream samples were diluted in distilled water until reaching an obscuration rate of 20%. The size distribution was determined by the Malvern Mastersizer software (Ver. 5.61) using the Mie theory (Anihouvi et al., 2013).

2.3.2. PULSED-NMR ANALYSIS

The solid fat content (SFC) in the cream was measured by a pulsed NMR spectrometer (Minispec-mq20, BRUKER, Germany). Automatic calibration was made daily using 3 standards (supplied by Bruker, Germany) containing respectively 0.0, 31.3 and 74.8% of solids. Approximately 2 ml of cream was transferred to a nuclear magnetic resonance tube. Raw data (displayed on the screen) was used without any further transformation.

2.3.3. DSC ANALYSIS

The melting properties of each cream sample and anhydrous milk fat were studied during ripening using a differential scanning calorimeter (Q1000, TA Instruments, New Castle, USA) coupled with a refrigerated cooling system. The melting profile of anhydrous camel and cow milk fat was carried out according to the method described by Danthine(2012) with slight modifications. Indeed, 3 to 4 mg of anhydrous milk fat was loaded to an SFI (Solid Fat Index, TA Instruments, New Castle, USA) aluminum pan (an empty SFI pan was used as a reference). The samples were heated to 80 °C and held at that temperature for 5 min. Then, they were frozen at -60 °C with a cooling rate of 10 °Cmin⁻¹ and kept for 10 min at -60 °C. Melting curves of anhydrous camel and cow milk fat were recorded from -60 °C to 70 °C at a heating rate of 5 °C min⁻¹. For camel cream, 3 to 8 mg of each sample was loaded in a T0 (Tzero hermetic cell, TA Instruments, New Castle, USA) hermetically sealed aluminum pan. An empty sealed pan was used as a reference. DSC measurements were performed from the ripening temperature (5 °C, 12 °C, 21 °C) to 65 °C with a heating rate of 5 °C min⁻¹. Universal Analysis software (version, 4.5A) was used to determine the melting behavior by identifying the peak melting temperature (°C) and change in enthalpy (J/g) associated with the melting process.

2.3.4. XRD ANALYSIS

The polymorphism of the fat crystals in camel milk cream was evaluated during ripening by XRD spectroscopy using a D8 Advance Diffractometer (Bruker, Germany) (λ Cu = 1.54178 Å, 40 kV, 30 mA) equipped with a LynxEye (Bruker, Germany) detector, a TTK450 low-temperature chamber and a temperature control unit (TCU 110 system, Anton Paar, Austria) connected to a circulating water bath. Diffraction patterns were recorded in short-spacing (15°–27° 2 θ , 0.02° 2 θ s⁻¹) and long-spacing (1–13° 2 θ , 0.02° 2 θ s⁻¹) ranges. Measurements were performed at the ripening temperature of cream samples (5 °C, 12 °C, and 21 °C).

2.4. Statistical analysis

Measurements were performed in triplicate and the results are presented as average value along with their standard deviation. The statistical differences were examined using SPSS 19 (IBM SPSS statistics, Version 19, USA) according to the one-way ANOVA followed by the Tukey post-hoc test.

Table 1 – Fatty acid composition of camel milk fat and cow milk fat (mol%).

| Fatty acids | Camel milk fat | Cow milk fat |
|-------------------------|----------------|--------------|
| C6:0 | Traces | 3.31 ± 0.11 |
| C8:0 | 0.17 ± 0.01 | 2.12 ± 0.05 |
| C9:0 | – | 0.06 ± 0.01 |
| C10:0 | 0.20 ± 0.02 | 4.94 ± 0.19 |
| C10:1n6 | – | 0.46 ± 0.01 |
| C12:0 | 1.25 ± 0.03 | 5.56 ± 0.21 |
| C12:1n1 | 0.06 ± 0.00 | 0.31 ± 0.01 |
| C14:0 iso | 0.22 ± 0.00 | 0.15 ± 0.01 |
| C14:0 | 11.56 ± 0.16 | 13.58 ± 0.50 |
| C14:1n5 | 0.73 ± 0.04 | 1.05 ± 0.01 |
| C15:0 anteiso | 0.86 ± 0.03 | 0.47 ± 0.00 |
| C15:0 | 1.13 ± 0.05 | 1.31 ± 0.02 |
| C16:0 iso | 0.25 ± 0.01 | 0.30 ± 0.00 |
| C16:0 | 27.94 ± 0.47 | 32.50 ± 0.99 |
| C16:1 | 0.71 ± 0.21 | 0.13 ± 0.00 |
| C16:1n9 | 7.21 ± 0.24 | 1.32 ± 0.04 |
| C16:1n7 | 0.31 ± 0.03 | 0.25 ± 0.04 |
| C17:0 iso | 0.62 ± 0.04 | 0.36 ± 0.01 |
| C17:0 | 0.60 ± 0.03 | 0.53 ± 0.01 |
| C17:1n7 | 0.46 ± 0.06 | 0.21 ± 0.02 |
| C18:0 | 12.53 ± 0.11 | 9.73 ± 0.23 |
| C18:1n9 | 24.30 ± 0.5 | 14.00 ± 0.04 |
| C18:1n7 | 2.72 ± 0.11 | 2.15 ± 0.08 |
| C18:1n3 | 0.33 ± 0.00 | 0.35 ± 0.04 |
| C18:2n6 | 2.57 ± 0.10 | 2.05 ± 0.05 |
| C18:3n3 | 0.64 ± 0.03 | 0.82 ± 0.01 |
| C18:2n7(c9,t11) | 0.83 ± 0.01 | 0.48 ± 0.48 |
| C20:0 | 0.34 ± 0.04 | 0.13 ± 0.01 |
| C20:1n11 | 0.16 ± 0.01 | 0.23 ± 0.05 |
| C22:0 | 0.13 ± 0.01 | 0.04 ± 0.01 |
| C4-C12 | 1.68 | 16.74 |
| C13-C22 | 97.15 | 82.14 |
| Saturated fatty acids | 57.80 | 75.06 |
| Unsaturated fatty acids | 41.03 | 23.82 |

3. Results and discussion

3.1. FATTY ACIDS COMPOSITION

The fatty acids composition of camel milk fat and cow milk fat is shown in **Table 1**. Camel milk fat was characterized by a very small amount of short-chain fatty acid (C4-C12) and a high proportion of long-chain fatty acids (C13-C22) (1.68% and 97.15%, respectively). This finding is in agreement with results previously obtained (Attia et al., 2000; Konuspayeva et al., 2008; Berhe et al., 2013). The amount of short-chain fatty acids was notably lower than that of cow milk fat (16.74%). This difference might be explained by the fact that camel tissues rapidly metabolize these fatty acids before being extracted in milk (Karray et al., 2005a,b).

The most abundant saturated fatty acids observed in this study in camel milk fat were C14:0, C16:0, C18:0 with proportions equal to $11.56 \pm 0.16\%$, $27.94 \pm 0.47\%$, $12.53 \pm 0.11\%$, respectively. Our results are in accordance with the findings of Haddad et al. (2010) (14.9%, 28.5%, 10.52%, respectively). These fatty acids were also the main saturated fatty acids found in cow milk fat. The amount of C14:0 in camel milk fat was relatively similar to those for cow milk fat ($13.58 \pm 0.50\%$). However, the amount of C16:0 in camel milk fat was lower than that of cow milk fat ($32.50 \pm 0.99\%$). The content of stearic acid (C18:0) observed in this study was higher than that found for cow milk fat ($9.73 \pm 0.23\%$). These findings are in agreement with those reported by Attia et al. (2000).

Furthermore, our data revealed a higher amount of unsaturated fatty acids in camel milk fat than in cow milk fat (41.03%, 23.82%, respectively). The major unsaturated fatty acids observed in this study were the C16:1n9 and C18:1n9. The content of C16:1n9 and C18:1n9 found in camel milk fat ($7.21 \pm 0.24\%$, $24.3 \pm 0.5\%$, respectively) was higher than that of cow milk fat ($1.32 \pm 0.04\%$, $14.00 \pm 0.04\%$, respectively). Those results are consistent with data reported by Farah et al. (1989) and Attia et al. (2000).

The difference in fatty acid composition and thus, TAGs composition were highlighted by different thermal behaviors of camel and cow milk fat (**Fig. 1**). The DSC melting profiles of anhydrous camel milk fat and anhydrous cow milk fat are presented in **Fig. 1**.

The melting curves of cow milk fat showed three melting peaks, representing the low-, medium-, high-melting fraction (Danthine (2012)). However, the melting curve of camel milk fat shows several weak peaks and a major peak at 40 °C, the latter represents the high melting fraction. Melting of the camel milk fat starts around -26 °C and is complete at about 44 °C. The final melting temperature is higher in the camel milk fat than in cow milk fat (44 °C, 39 °C, respectively). These results are similar to that previously reported by Rüegg and Farah, (1991), Smiddy et al. (2012) and Bakry et al. (2020). The higher melting point of camel milk fat could be explained by the low fraction of short-chain fatty acids and to the higher content of long-chain fatty acids (**Table 1**), resulting in a higher content of TAGs with the higher melting point. Indeed, Smiddy et al. (2012), by using the principal component analysis, have shown that the higher molecular weight TAGs predominantly determined the melting profiles. In addition, Haddad et al. (2011) and Bakry et al. (2020) reported a high content of TAGs with long-chain fatty acids in camel milk fat.

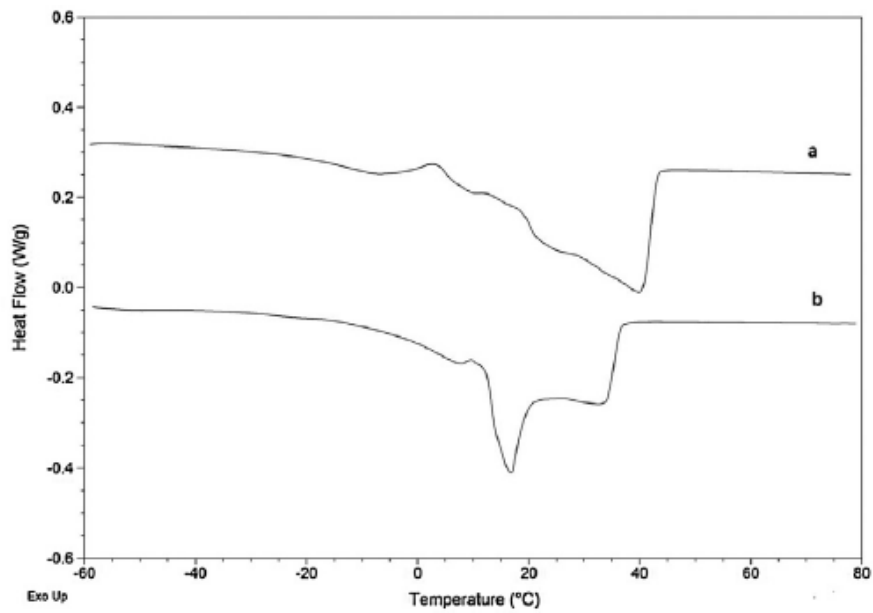


Fig. 1 – DSC melting curves of camel milk fat (a) and cow milk fat (b), heating rate = 5°C/min.

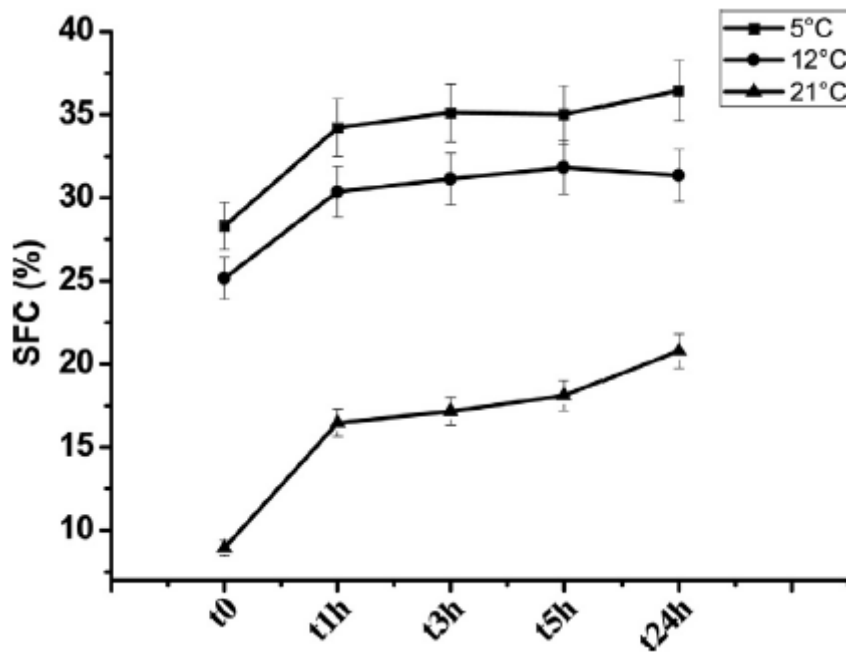


Fig. 2 – Evolution of the solid fat content (SFC) as a function of the ripening time (0 h, 1 h, 3 h, 5 h, 24 h) at 5 °C, 12 °C and 21 °C.

3.2. EVOLUTION OF SOLID FAT CONTENT (SFC)

Fig. 2 shows the evolution of the SFC values in camel milk cream during ripening conducted at 5 °C, 12 °C, and 21 °C for 24 hours.

For all tested temperatures, a significant increase in the SFC value of camel milk cream was highlighted during the first hour of ripening. No further significant changes were observed after this first hour for cream ripening (at 5 °C and 12 °C). This trend allowed the SFC of camel milk cream to reach a plateau at these ripening temperature (at 5 °C and 12 °C). The observed results can be explained by the fact that an increase of the SFC level leads to an increase of the viscosity. When viscosity increases, the nucleation rate decreases (Wright and Marangoni, 2006), and thus the SFC level reaches a plateau during ripening. Different crystallization behavior was reported for cow milk fat by Buldo et al. (2013) during ripening at 10 °C for 17 h. These authors observed that the SFC level of cow cream still remarkably increased even after the first hour of ripening. This difference in crystallization behavior between two kinds of creams can be related to the higher viscosity of camel milk cream reported by Attia et al. (2000). This characteristic may promote that the camel milk cream reaches more rapidly a plateau during ripening, than cow milk cream. Furthermore, considering the difference in fatty acid composition and necessarily in TAGs between the two types of milk, the different crystallization behavior observed is not at all surprising.

However, our results showed that the SFC level slightly increased after the first hour of ripening at 21 °C. Furthermore, it is important to note that even at high crystallization temperature (21 °C) there was sufficient supercooling, which promoted nucleation mechanism in camel milk cream. This behavior can be related to the low proportion of low melting TAGs (BuPO and BuOO) and the high proportion of high melting TAGs in camel milk fat (PPO: and PPP) (Timms, 1980) (Bu:C14:0, P: C16:0, O: C18:1). Indeed, Haddad et al. (2011) and Bakry et al. (2020) have reported a high content of PPP and PPO in camel milk fat.

Therefore, it may be concluded that the crystallization of camel milk cream takes place mainly during the first hour of ripening step regardless of the ripening temperature. In addition, it is worth noticing that only at 21 °C, the crystallization process continues even after the first hour with a low crystallization rate.

Furthermore, our results showed that, as expected, the highest SFC values were found at 5 °C followed by 12 °C and then 21 °C. The SFC values obtained after 24 h of ripening at 5 °C, 12 °C, and 21 °C were equal to $36.46 \pm 1.82\%$, $31.34 \pm 1.56\%$, $20.8 \pm 1.04\%$, respectively. These values were higher than those reported by Lee and Martini, (2018) for cow milk cream aged at 5 °C, 10 °C and 15 °C during 90 min of ripening. This difference could be attributed to the differences in fatty acid composition resulting in differences in TAGs. Indeed, the higher level of SFC of camel milk cream could be explained by the higher melting point of camel milk cream as it is composed of a high content of TAG with a high melting point (**Fig. 1**). In fact, a higher melting point gives a lower solubility of the crystallized phase in the liquid phase, leading to higher SFC values (Vereecken et al., 2009).

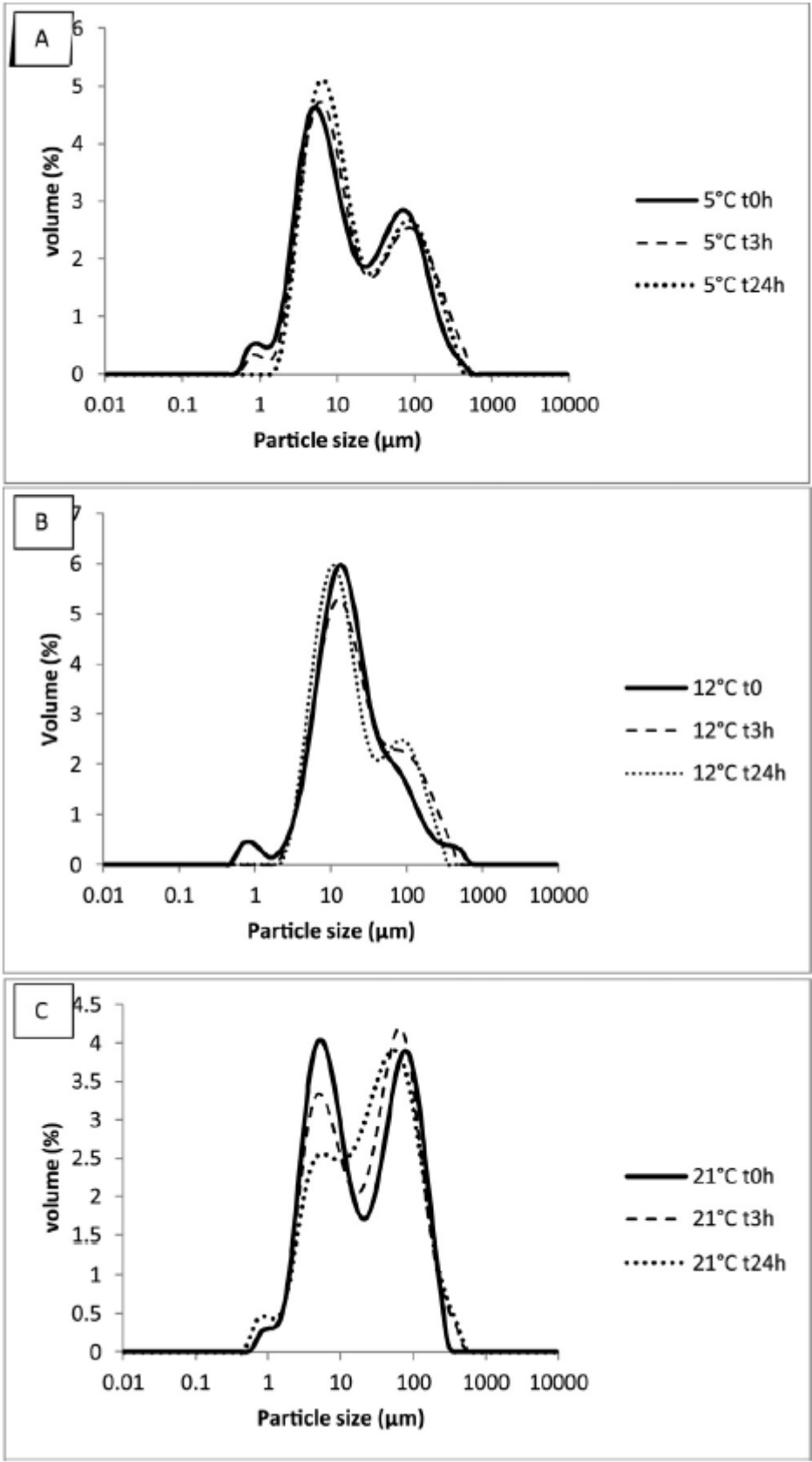


Fig. 3 – Droplet size distribution for camel cream during ripening (0 h, 1 h, 3 h, 5 h, 24 h) at 5 °C (A) 12 °C (B) 21 °C (C).

3.3. DROPLET SIZE DISTRIBUTION

By following the droplet size distribution, it is possible to track the aggregation of fat globules and thus the partial coalescence during ripening. The droplet size distribution of camel milk cream as a function of ripening conditions is presented in **Fig. 3A** (5 °C), **3B** (12 °C), **3C** (21 °C); in order to obtain clear graphs, only curves measured at 0 h, 3 h, and 24 h are presented.

The droplet size distribution of cream samples ripened at 5 °C and 12 °C was initially characterized by the presence of three distinct populations of aggregates ($t = 0$ h). During ripening at 5 °C and 12 °C, the smallest population disappeared, which led to a bimodal distribution. However, cream samples ripened at 21 °C showed three populations of aggregates regardless of the ripening time.

Furthermore, for all tested temperatures, an increase in the number of larger particles was observed during ripening step. These results could be explained by the occurrence of the partial coalescence phenomenon caused by the increase of the SFC level during the ripening step. Indeed, Boode and Walstra, (1993) stated that more partial coalescence is prone to occur in emulsions at higher SFC level. Moreover, the increase in the number of larger droplets was more significant for samples ripened at 21 °C than for samples ripened at 5 °C or 12 °C. Thus, it can be concluded that the higher aggregation rate was observed for camel milk cream ripened at 21 °C. Fredrick et al. (2010) reported that partial coalescence phenomenon could only be achieved when solid particles are present in the dispersed phase and the remaining liquid oil is not entirely immobilized by crystal network. Thus, we suggest that the high SFC value level found for camel milk cream ripened at 5 °C and 12 °C may limit the aggregation of fat globules due to the lack of oil to hold the globules together.

Furthermore, it is important to notice that after 24 h of ripening, the highest number of the largest population was observed for camel cream ripened at 21 °C. Hence, we can suggest that the SFC level obtained at 21 °C after 24 h ($20.8 \pm 1.04\%$) promoted a higher aggregation level of fat globules. Our results highlighted that the aggregation of camel milk fat globules during ripening was mainly influenced by the crystallization temperature and thus by the SFC level of the emulsion.

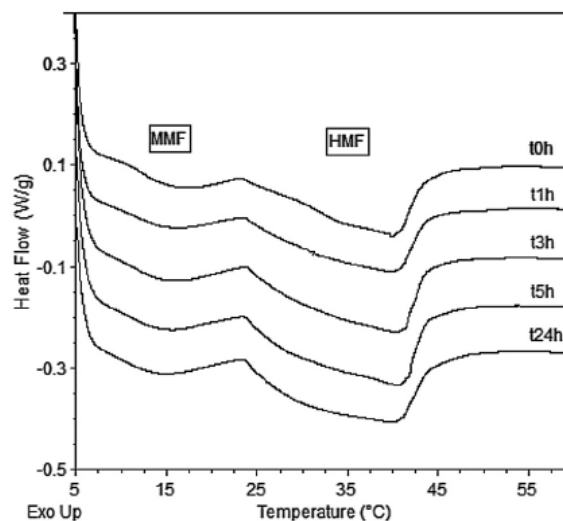


Fig. 4 – Evolution of the DSC melting curves of camel milk cream samples collected at different ripening time: 0 h, 1 h, 3 h, 5 h, 24 h; ripening performed at 5°C. (HMF: high melting fraction, MMF: medium melting fraction).

Table 2 – Melting behavior of camel milk cream during ripening at 5 °C, 12 °C, and 21 °C.

| Temperature | Time | ΔH_{total} (J/g) | ΔH_{MMF} (J/g) | ΔH_{HMF} (J/g) | T_{PMMF} (°C) | T_{PHMF} (°C) |
|-------------|------|---------------------------------|-------------------------------|-------------------------------|-----------------------------|-----------------------------|
| T = 5 °C | 0 h | 36.33 ± 1.70 ^{a,*} | 10.3 ± 0.53 ^{a,*} | 26.64 ± 1.30 ^{a,*} | 15.63 ± 0.78 ^{a,*} | 39.93 ± 1.99 ^{a,*} |
| | 1 h | 41.77 ± 2.10 ^{b,*} | 12.07 ± 0.55 ^{b,*} | 29.6 ± 1.50 ^{b,*} | 15.53 ± 0.77 ^{a,*} | 39.74 ± 1.79 ^{a,*} |
| | 3 h | 41.57 ± 2.30 ^{b,*} | 12.08 ± 0.60 ^{b,*} | 29.98 ± 1.48 ^{b,*} | 15.58 ± 0.79 ^{a,*} | 40.06 ± 2.1 ^{a,*} |
| | 5 h | 42.87 ± 1.50 ^{b,*} | 11.08 ± 0.55 ^{a,b,*} | 31.17 ± 1.56 ^{b,*} | 15.37 ± 0.75 ^{a,*} | 40.47 ± 2.03 ^{a,*} |
| | 24 h | 40.23 ± 2.10 ^{b,*} | 10.47 ± 0.52 ^{a,*} | 29.75 ± 1.47 ^{b,*} | 15.2 ± 0.76 ^{a,*} | 39.78 ± 1.98 ^{a,*} |
| T = 12 °C | 0 h | 22.27 ± 1.12 ^{a,§} | 1.87 ± 0.20 ^{a,§} | 21.47 ± 1.07 ^{a,§} | 17.78 ± 0.90 ^{a,§} | 40.31 ± 2.01 ^{a,*} |
| | 1 h | 25.17 ± 1.26 ^{b,§} | 1.95 ± 0.12 ^{a,§} | 23.42 ± 1.17 ^{a,b,§} | 17.65 ± 0.88 ^{a,§} | 40.68 ± 2.02 ^{a,*} |
| | 3 h | 25.51 ± 1.28 ^{b,§} | 1.66 ± 0.23 ^{a,§} | 23.97 ± 1.20 ^{b,§} | 17.49 ± 0.79 ^{a,§} | 40.55 ± 2.03 ^{a,*} |
| | 5 h | 25.06 ± 1.25 ^{b,§} | 1.78 ± 0.28 ^{a,§} | 23.27 ± 1.16 ^{b,§} | 17.8 ± 0.90 ^{a,§} | 40.56 ± 2.04 ^{a,*} |
| | 24 h | 24.93 ± 1.25 ^{b,§} | 1.57 ± 0.35 ^{a,§} | 23.22 ± 1.15 ^{b,§} | 17.31 ± 0.87 ^{a,§} | 40.30 ± 2.01 ^{a,*} |
| T = 21 °C | 0 h | 18.47 ± 0.92 ^{a,§} | ND | 18.47 ± 0.92 ^{a,§} | ND | 40.59 ± 2.03 ^{a,*} |
| | 1 h | 20.89 ± 1.01 ^{b,§} | ND | 20.89 ± 1.01 ^{b,§} | ND | 40.36 ± 2.02 ^{a,*} |
| | 3 h | 21.17 ± 1.05 ^{b,§} | ND | 21.17 ± 1.05 ^{b,§} | ND | 40.34 ± 2.04 ^{a,*} |
| | 5 h | 21.29 ± 1.02 ^{b,§} | ND | 21.29 ± 1.02 ^{b,§} | ND | 40.74 ± 2.04 ^{a,*} |
| | 24 h | 21.91 ± 1.07 ^{b,§} | ND | 21.91 ± 1.07 ^{b,§} | ND | 40.44 ± 2.02 ^{a,*} |

Same letter (for each temperature) or symbol (for each time) represent the statistical data significance ($p > 0.05$). ND indicates that data were not detected. ΔH_{total} : total melting enthalpy associated with the entire melting process; ΔH_{MMF} : Enthalpy associated with the melting of the medium melting fraction; ΔH_{HMF} : Enthalpy associated with the melting of the high melting fraction; T_{PMMF} : melting temperature of the medium melting fraction; T_{PHMF} : melting temperature of the high melting fraction.

3.4. MELTING BEHAVIOR

The melting properties of camel milk cream as a function of ripening conditions are summarized in **Table 2**. Melting curves of sample ripened at 5 °C are presented in **Fig. 4**, as the same tendency was observed for all tested temperatures.

The final melting point of camel milk cream was around 44 °C (**Fig. 1, Fig. 4**). This value is higher than that observed for bovine milk fat (39 °C, **Fig. 1**). As previously discussed, this tendency may be explained by the higher amounts of long-chain fatty acid presents in camel milk fat (**Table 1**) and the high proportion of large TAGs (C48-C52) in camel milk (Haddad et al., 2010; Smiddy et al., 2012). Furthermore, the fat melting thermograms of camel cream samples ripened at 5 °C and 12 °C displayed two melting peaks. The first weak peak was found close to 15 °C, which corresponds to the medium melting fraction (MMF). The second one was observed at 40 °C, which corresponds to the higher melting fraction (HMF). However, only one melting peak was observed in camel cream ripened at 21 °C. This peak represents the melting of the higher melting fraction (**Fig. 4, Table 2**) as at this temperature (21 °C) the medium melting fraction is already at liquid state.

Moreover, our results showed that the ripening temperature and time had a significant effect on the enthalpy values. In fact, the highest total enthalpy values were logically obtained at 5 °C followed by 12 °C and 21 °C (40.23 ± 2.1 J/g, 24.93 ± 1.2 J/g, and 21.16 ± 1.06 J/g, respectively). In addition, for all tested temperatures, a significant increase in the total melting enthalpy and the enthalpy of HMF was found during the first hour of ripening. No statistical changes in the total enthalpy and HMF enthalpy were observed after one hour of ripening (**Table 2**). Hence, these findings confirm that for all experimental conditions, camel milk crystallization occurs mainly during the cooling step and the first hour of ripening, especially the crystallization of high melting TAGs. In this study, it is important to notice that the enthalpy and the SFC values showed the same increase tendency for cream ripened at 5 °C and 12 °C (**Fig. 2**). However, although there was a slight increase of the SFC values for cream ripened at 21 °C, no significant changes were observed by DSC after one hour of ripening at this

temperature even if a small increase tendency was observed. Furthermore, the fat melting thermograms of all tested temperatures show some “shoulders”, which become increasingly apparent during ripening (Fig. 4). This may be due to polymorphism transition phenomena.

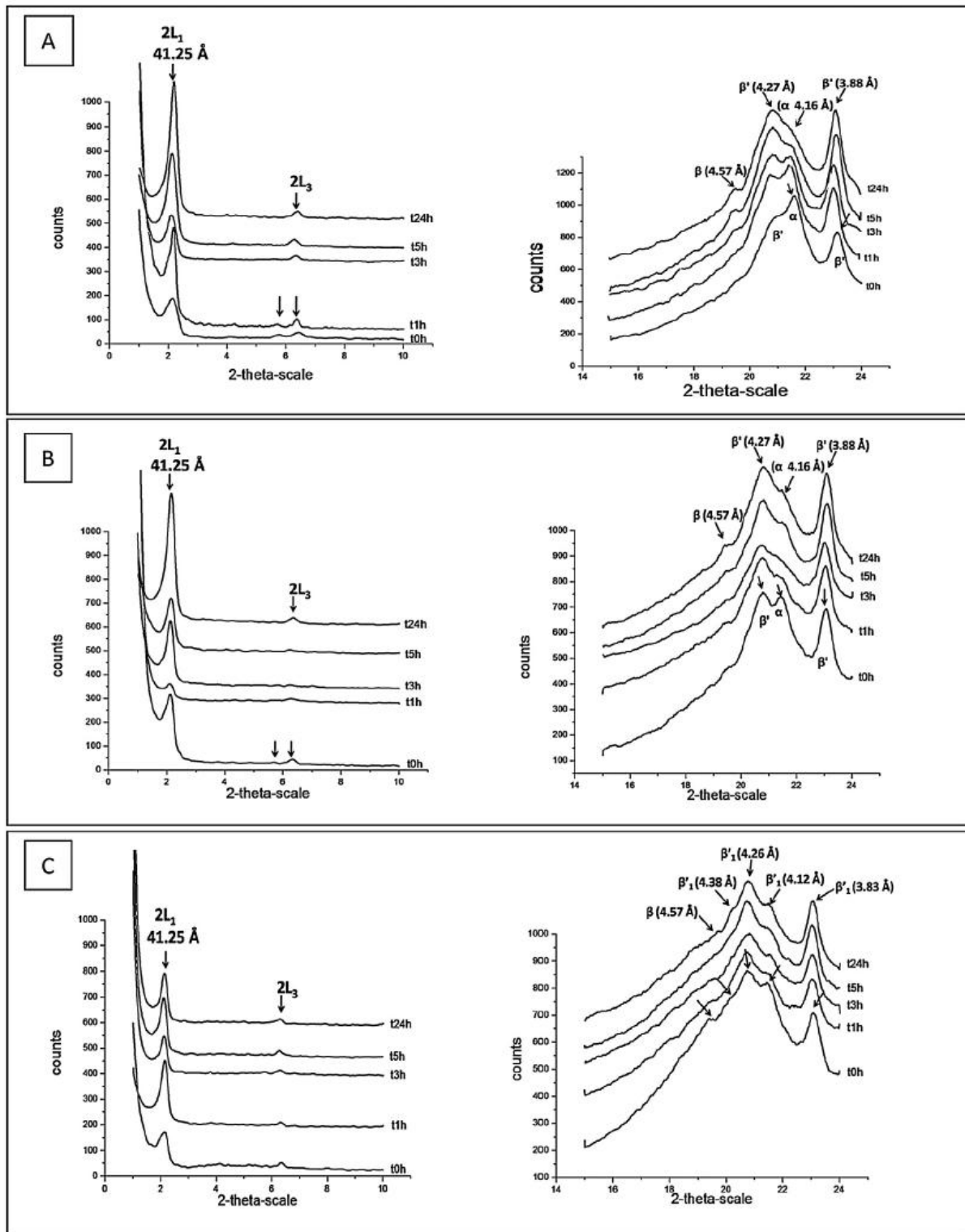


Fig. 5 – XRD diffraction pattern recorded during ripening (0 h, 1 h, 3 h, 5 h, 24 h) at 5 °C (A), 12 °C (B), and 21 °C (C).

3.5. POLYMORPHISM

In order to further understand the obtained DSC results, the polymorphic behavior of camel milk cream during ripening was studied by using X-ray diffraction analysis (**Fig. 5**).

The short spacings diffraction patterns of cream samples without ripening ($t = 0$ h) at 5 °C and 12 °C showed three peaks corresponding to a mixture of the unstable form (4.16 Å) and β' 2 form of TAGs (3.8 Å and 4.2 Å). During the first hour of ripening at 5 °C and 12 °C, the intensity of α form peak decreased whereas the intensity of β' 2 form peak increased remarkably. After three hours of ripening, a weak peak (4.6 Å) started to emerge, indicating the occurrence of the triclinic sub-cell of the form TAGs. For the rest of the ripening, coexistence of these three crystal forms was observed. Hence, the ripening of camel milk cream at 5 °C and 12 °C caused a transition from to β' 2 form and the appearance of β form.

The observed α to β' transition in camel milk cream was also reported for bovine cream ripened at 10 °C and 5 °C (Ronholt et al., 2012; Buldo et al., 2013).

On the other hand, the short spacing diffraction spectra recorded at 21 °C showed directly the presence of five main peaks corresponding to a mixture β' 1 (3.83 Å, 4.12 Å, 4.26 Å, 4.38 Å) and a small amount of β (4.57 Å) forms; this was moreover observed regardless of the ripening time. In addition, the peak intensities increased during ripening time. The presence of β' 1 and forms in the non-ripened sample cooled to 21 °C could be explained by the occurrence of polymorphic transition (α to β' to β) during cooling of the cream to the ripening temperature 21 °C.

The same conclusion can be highlighted from the diffraction pattern recorded in long spacing at 5 °C and 12 °C and 21 °C. Indeed, samples without ripening at 5 °C and 12 °C, showed an α 2L (45 Å) and a β' 2L (42 Å) structures. Upon ripening at 5 °C and 12 °C, the 2L packing (45 Å) decreased in intensity and the 2L packing (42 Å) increased, indicating a crystal rearrangement from α to β' . In addition, it is also important to note that the camel milk cream did not show 3L stacking, which was observed for bovine cream (Lopez et al., 2005). The absence of this structure can be explained by the lower amounts of short-chain fatty acid observed in camel milk (**Table 1**). In fact, the 3L stacking may correspond to the crystallization of TAGs with fatty acid chains of different lengths, like BuPP (Lopez, 2018).

Our results highlight that camel milk fat crystallized into three forms: α , β' and β . Moreover, the polymorphic transition occurs not only during the first hour of ripening, but it could be observed all over the ripening process.

4. Conclusion

In this study, the effects of temperature (5, 12, and 21 °C) and duration (0, 1, 3, 5, and 24 h) of ripening on the crystallization mechanisms of camel cream milk were studied. Our results showed that during ripening the largest particles are formed mainly at 21 °C. For all tested temperatures, monitoring of the SFC and the total enthalpy of melting curves over time showed that crystallization takes place mainly during the cooling step and the first hour of ripening. The study of crystal polymorphism during

ripening showed that the ripening of the cream at 5 °C and 12 °C caused a transition from α to β' 2 form and appearance of form. On the other hand, β' 1 and forms were detected in creams ripened at 21 °C regardless of ripening time. The present study gives a general overview of the crystallization process which occurs before churning of camel milk cream.

Declaration of interest

None.

Declaration of Competing Interest

The authors report no declarations of interest.

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