Insights into the mechanism of the formation of the most stable crystal polymorph of milk fat in model protein matrices

P. R. Ramel and A. G. Marangoni¹

Department of Food Science, University of Guelph, Guelph, ON, N1G 2W1, Canada

ABSTRACT

The effect of incorporation and presence of various ingredients in a model sodium caseinate-based imitation cheese matrix on the polymorphism of milk fat was comprehensively described using powder x-ray diffraction, differential scanning calorimetry, and microscopy. With anhydrous milk fat (AMF) in bulk used as control, the embedding of AMF as droplets in a protein matrix was found to result in a greater extent of formation of the β polymorph than AMF alone and AMF homogenized with water and salts solution. The use of other protein matrices such as soy and whey protein isolate gels revealed that the nature of the protein and other factors associated with it (i.e., hydrophobicity and molecular structure) do not seem to play a role in the formation of the β polymorph. These results indicated that the most important factor in the formation of the β polymorph is the physical constraints imposed by a solid protein matrix, which forces the triacylglycerols in milk fat to arrange themselves in the most stable crystal polymorph. Characterization of the crystal structure of milk fat or fats in general within a food matrix could provide insights into the complex thermal and rheological behavior of foods with added fats.

Key words: milk fat, triacylglycerol, polymorphism, protein matrix, processed cheese product

INTRODUCTION

Milk fat (MF) is one of the most widely studied fats because of its importance in the production of various food products such as butter, ice cream, cheese, and chocolate (Wright et al., 2001; Lopez et al., 2006; Gliguém et al., 2009, 2011; Sonwai and Rousseau, 2010; Méndez-Velasco and Goff, 2012). The crystal network that MF forms at different length scales has been extensively characterized and was shown to affect the various physical, mechanical, and rheological proper-

ties of MF (Narine and Marangoni, 1999; Wright et al., 2001; Campos et al., 2002; Ramel and Marangoni, 2016; Ramel et al., 2016). Polymorphism refers to the subcell packing of triacylglycerols (TAG) in a crystalline lattice. It is one of the most commonly used structural characterization parameters of MF crystal networks, and it was found to be well correlated with the melting properties of fats (Timms, 1984; deMan, 1992; Grotenhuis et al., 1999; Sato, 2001). Milk fat has been shown to crystallize into 3 major polymorphs: α , β' , and β . The α polymorph of MF is metastable and readily transforms into a more stable form, usually the β' polymorph, while the β form is seldom found (Woodrow and deMan, 1968; Lopez et al., 2001a,b,c; Wright and Marangoni, 2002). Because of the presence of unsaturated fatty acids (FA) that have “kinks” (*cis*-double bonds) in their structure and the abundance of short-chain FA in MF, the packing of TAG in the most compact form (triclinic system) is generally inhibited (van Aken et al., 1999; Sato, 2001; Jensen, 2002). In previous studies, the β polymorph was shown to be formed when a sufficient amount of liquid fat is present along with high amounts of long-chain saturated FA (Wright et al., 2000; Mazzanti et al., 2004; Tzompasosa et al., 2016). Furthermore, the β polymorph was observed in native cream and nanoemulsions of milk lipids (Lopez et al., 2002a; Bugeat et al., 2011; Rønholt et al., 2012; Truong et al., 2014). Various processing conditions such as crystallization temperature, cooling rate, application of shear, and addition of emulsifiers have also been used to study the formation of different crystal polymorphs in MF (van Aken and Visser, 2000; Lopez et al., 2001a,b; Martini et al., 2001; Wiking et al., 2009; Kaufmann et al., 2012). The polymorphism of MF has been extensively studied using x-ray diffraction (XRD) technique. Coupled with Bragg’s law, characteristic *d*-spacings related to the geometry that TAG molecules arrange or conform into, are determined with the technique. The α , β' , and β polymorphs are characterized by *d*-spacings in the wide angle region of ~ 4.1 , ~ 4.2 and 3.8 , and ~ 4.6 Å, respectively (Woodrow and deMan, 1968; D’Souza et al., 1990; deMan, 1992).

Most of these studies have been carried out using anhydrous milk fat (AMF) and cream alone or as they

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¹Corresponding author: amarango@uoguelph.ca

are. However, MF is also usually incorporated in food products, and conditions that have been previously studied may not fully explain the crystallization behavior of MF within the matrix of a solid food product (e.g., cheese) that contains other various ingredients such as protein. Results of our previous experiments on commercial processed cheese products showed that MF in processed cheese has higher ratios of β to β' polymorph than MF in bulk (Ramel and Marangoni, 2017). We proposed that the embedding of the fat globules in the protein matrix, the presence of other ingredients, or both could have forced the TAG in MF to arrange into the most stable crystal form.

Therefore, the main objective of this study was to determine specific factors in processed cheese that allow or force the formation of the β polymorph in MF, using a model sodium caseinate-based imitation cheese product. With AMF in bulk used as the control, a reductionist approach was also employed by mixing AMF with various individual ingredients of the model cheese and then determining polymorphism. Lastly, to investigate the effect of the nature of the protein matrix on the polymorphism of MF, soy and whey proteins were also used as the main protein components.

MATERIALS AND METHODS

Materials

Anhydrous milk fat was kindly provided by The Kraft Heinz Company (Waukegan, IL). For cheese-making, sodium caseinate (90.8% protein, Fonterra Cooperative Group, Auckland, New Zealand), sodium citrate, citric acid, and potassium sorbate (Hela Spice, Uxbridge, ON, Canada), disodium phosphate (Fisher Scientific, Pittsburgh, PA), table salt and Cheddar cheese flavoring were used. Soy protein (Hela Spice) and whey protein isolate (BiPro, Agropur Inc., Le Sueur, MN) were also used as main protein components. Furthermore, as a negative control, canola oil (from the supermarket) was also used as the fat component in cheese.

Sample Preparation

Samples were prepared according to a modified formulation and laboratory-scale processing used in the literature (Mounsey and O'Riordan, 2008; Sołowiej et al., 2014). First, sodium caseinate (21%) was dissolved in water (48.8%) at room temperature ($\sim 21^\circ\text{C}$) using a counter-top mixer (Hobart, North York, ON, Canada) at speed setting 1. Liquefied fat (e.g., 100°C for 2–5 min, AMF or canola at 26%) was then added to the caseinate suspension and mixed for 2 min. Emulsifying salts [2.18% (1.08% (tri)sodium citrate, 0.62% citric acid, 0.48% disodium phosphate)], salt (1.50%), cheddar flavoring (0.42%), and potassium sorbate (0.1%) were then added and mixed for 1 min. A water bath set at 80°C was then attached to the mixer, and the mixture was homogenized at $\sim 10,000$ rpm for 10 min using an Ultra Turrax homogenizer (IKA, Staufen, Germany). Samples were placed in a plastic container with aluminum foil, kept at room temperature for 30 min, and then stored at 4 to 7°C before analyses. For the breakdown approach, AMF was mixed with individual ingredients (at amounts similar to those used in making cheese) and then processed at similar conditions. Three batches per sample were produced and are summarized in Table 1.

Cryogenic-Scanning Electron Microscopy

To ensure that MF is emulsified in the protein matrix, cryogenic-scanning electron microscopy (**cryo-SEM**) was performed. Thin slices of the cheese sample were cut and inserted into a copper sample holder. Using a Tissue-Tek embedding medium, the samples were kept in place. The sample holder was then immersed in a liquid nitrogen slush (approximately -210°C) using an Emitech K1250x cryo-preparation unit (Ashford, UK). Fracturing of the samples was then performed to expose the inside structure of the cheese. Sublimation at -80°C for 60 min was then done to remove excess water, and samples were sputter-coated with ~ 30 nm gold.

Table 1. Summary of the samples prepared and their corresponding compositions

Sample name	Composition
1. AMF ¹ cheese	AMF, water, sodium caseinate, emulsifying salts, salt, flavoring
2. Canola cheese	Canola oil, water, sodium caseinate, emulsifying salts, salt, flavoring
3. AMF	AMF
4. AMF H ₂ O	AMF, water
5. AMF caseinate	AMF, water, sodium caseinate
6. AMF salts	AMF, water, emulsifying salts, salt, flavoring
7. AMF soy	AMF, water, soy protein
8. AMF whey	AMF, water, whey protein

¹AMF = anhydrous milk fat.

to increase conductivity. Samples were then transferred onto the scanning electron microscopy unit, which was kept at a temperature below -140°C (Hitachi S-5700, Japan). The scanning electron microscopy unit was operated at 10-kV accelerating voltage of the electron beam, and images were captured digitally using Quartz PCI imaging package (Quartz Imaging Corp., Vancouver, BC).

Powder X-Ray Diffraction

For wide-angle x-ray diffraction (**WAXD**), samples were prepared by filling the well of a glass sample holder until the well was level with the surrounding slide. All samples were prepared inside a walk-in refrigerator to keep conditions cold. Wide-angle x-ray scans ($12^{\circ} < 2\theta < 35^{\circ}$ at $1^{\circ}/\text{min}$) were obtained using a Rigaku Multiflex x-ray diffractometer ($\text{Cu-K } \alpha$, $\lambda = 1.5418 \text{ \AA}$; Rigaku Corporation, Tokyo, Japan). Measurements were performed at 4°C by putting the glass slide on top of a peltier that is attached to a temperature-controlled water bath. Characteristic d -spacings were determined using Bragg's equation, $d = n\lambda/2\sin\theta$, where d is the spacing between atoms ($\text{\AA}$), n is an integer related to the order of reflection, usually equal to 1, and θ is the angle of diffraction. Samples were measured after 1 and 3 wk of storage at 4 to 7°C . To confirm that the diffraction peaks came from AMF crystals, samples were melted by putting the glass slides in a 45°C oven for 30 min. The WAXD measurements were then performed at 45°C . These measurements were done in duplicate per batch. Peak analysis and quantification of the relative proportion of the β polymorph to the β' form was performed by taking the ratio of the % heights of the corresponding peaks using MDI Jade 9 (Materials Data Inc., Livermore, CA).

Differential Scanning Calorimetry

Differential scanning calorimetry (**DSC**) measurements were performed using a Mettler-Toledo DSC-1 Analyzer (STAR^c-System; Mettler-Toledo International Inc., Greifensee, Switzerland). Samples (5–10 mg for AMF and AMF mixtures, and 15–20 mg for cheeses and AMF caseinate) were weighed into an aluminum pan. The DSC procedure was programmed to stabilize samples at 0°C for 10 min before melting to 60°C at $5^{\circ}\text{C}/\text{min}$. This measurement was done every week for 3 wk and was performed in duplicate.

Graph Plotting, Statistical and Image Analysis

Graph plotting and statistical analysis were performed using GraphPad Prism v.6 (GraphPad Soft-

ware, San Diego, CA). Statistical differences in Table 2 were determined by performing an ordinary one-way ANOVA, and choosing Tukey's multiple comparison test as a post test at 95% CI. Image analysis of scanning electron micrographs was done using Image J software 1.42q (National Institutes of Health, Bethesda, MD). The distance covered by drawing a line in pixels was calibrated or converted into micrometers, using the scale bar in the images. Circles were drawn around distinguishable fat droplets in the micrographs, and diameters were calculated from the measured area of these circles. Four micrographs were analyzed, and 42 measurements were made.

RESULTS AND DISCUSSION

Microstructure of Model Cheese

Microstructural characterization of the model imitation cheese in Figure 1 shows that AMF was emulsified as droplets in the protein matrix. This emulsification was achieved by homogenization and cooking. This microstructure is similar to that of commercial or previously studied processed cheese products (Mounsey and O'Riordan, 1999; Tamime, 2011; Solowiej et al., 2014), indicating that the conditions present in the current investigation were relevant or applicable to our previous study on commercial processed cheese products (i.e., embedding of fat globules in the protein matrix; Ramel and Marangoni, 2017). Fat droplet size estimations by image analysis are shown in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2017-12758>). Most AMF droplets were approximately 6 to 8 μm .

Melting Behavior of AMF, AMF Mixtures, and Model Cheeses

Differential scanning calorimetry analysis of AMF, AMF homogenized with various ingredients, and cheeses made with AMF and canola oil (Figure 2) differed in melting behavior upon heating from 0 to 60°C . The lack of peaks in canola oil cheese indicates that no crystals were present in this sample, and DSC melting peaks observed in cheeses with AMF arose from the melting AMF crystals only and not from protein and other various ingredients in the model cheese. Furthermore, the temperatures (0 – 60°C) that were investigated are not expected to cause proteins to exhibit thermal transitions (Gliguem et al., 2011).

The AMF embedded in the protein matrix (AMF caseinate and AMF cheese) showed 2 distinct melting peaks corresponding to the melting of the low-melting and middle-melting fractions (**LMF** and **MMF**) at 0 to 22°C and to the high melting fraction (**HMF**) at

Figure 1. Cryogenic-scanning electron micrograph (cryo-SEM) of a model sodium caseinate-based imitation cheese with anhydrous milk fat (AMF) as the main fat component.

about 24 to 42°C. These melting curves are similar to those found in ripened cream (Buldo et al., 2013) and our previous findings in commercial processed cheese products. On the other hand, AMF alone and AMF with water and salts showed overlapping melting peaks, which are commonly found when MF in bulk is melted due to co-melting of 3 fractions (i.e., LMF, MMF, and HMF) (Timms, 1980; Marangoni and Lencki, 1998; Grotenhuis et al., 1999). These results indicate that when AMF is dispersed as cream or throughout the protein matrix, the melting of the LMF and MMF can be distinguishable from the melting of HMF. Furthermore, this finding supports our observations with the scanning electron micrographs that AMF was homogenized or well dispersed in the protein matrix.

Powder X-Ray Diffraction Spectra of AMF, AMF Mixtures, and Model Cheeses After Storage

Wide-angle x-ray diffraction patterns of AMF mixed with various ingredients but the same processing conditions show differences in the peak related to a *d*-spacing of ~ 4.6 Å (Figure 3a). The absence of any characteristic peaks in canola cheese indicates that the diffraction peaks observed in samples made with AMF are from MF crystals only because at 4 to 7°C, canola oil is not expected to crystallize, which is also shown

in the DSC results (Figure 2). The WAXD patterns of AMF cheese and AMF caseinate samples, in which AMF was emulsified or embedded as droplets in the protein matrix, show a very visible 4.6-Å peak, indicating a greater degree of β polymorph formation in these samples. This result supports our previous findings that the physical constraints imposed by the dispersion of fat in a solid matrix induces TAG in MF to arrange themselves into the most stable crystal form (Ramel and Marangoni, 2017). The effect of dispersion on the crystallization kinetics and polymorphism of MF has been studied extensively (Kloek et al., 2000; Lopez et al., 2002b; Bugeat et al., 2011; Fredrick et al., 2011; Rønholt et al., 2012; Buldo et al., 2013; Truong et al., 2014). The formation of the β polymorph co-existing along with the β' form in native cream is due to the inherent slow cooling caused by low concentrations of catalytic impurities or nucleation sites when MF is dispersed (Walstra and van Beresteyn, 1975; Lopez et al., 2002a,b; Rønholt et al., 2012; Buldo et al., 2013). Furthermore, a greater degree of β polymorph formation was observed in nanoemulsions because, although compositions were different, a higher supersaturation is

Figure 2. Differential scanning calorimetry (DSC) melting curves (0–60°C) of anhydrous milk fat (AMF); AMF homogenized with water (AMF H₂O) and emulsifying salts solution (AMF salts); AMF incorporated in sodium caseinate gel (AMF caseinate) and model cheese (AMF cheese); and model cheese made with canola oil (Canola cheese) after 1 wk of storage at 4 to 7°C. Exo = exothermic.

Table 2. Relative proportion of the β polymorph to the β' form by taking the ratio of the percent height of the peaks corresponding to 4.6 Å (β polymorph) and 4.2 Å (β' polymorph)

Sample	Average height ratio (β/β') ²
AMF ¹	0.100 ± 0.018 ^a
AMF H ₂ O	0.187 ± 0.051 ^a
AMF salts	0.247 ± 0.035 ^a
AMF caseinate	0.765 ± 0.072 ^b
AMF cheese	0.552 ± 0.043 ^c

^{a-c}Values with the same letters indicate no significant difference at $\alpha = 0.05$.

¹AMF = anhydrous milk fat.

²Values shown are from the average of 6 measurements ± SEM.

required for TAG in smaller-sized droplets to start crystallizing (Bugeat et al., 2011; Truong et al., 2014). Our system was different from cream and the nanoemulsions previously studied because AMF does not have a membrane around fat droplets, which is naturally present in native cream, and therefore TAG in AMF were directly in contact with various heterogeneous materials in the matrix during crystallization, especially salt crystals that could well enhance nucleation and crystal growth. Furthermore, our results show that when fat is homogenized with emulsifying salts solution (AMF salts), the crystals formed are mostly in the β' polymorph (Figure 3a). In terms of droplet size, the cryo-SEM micrograph in Figure 1 and droplet size frequency distribution in Supplemental Figure S1 (<https://doi.org/10.3168/jds.2017-12758>) show that the fat droplets in AMF

cheese have sizes around the micrometer range. These results therefore suggest that the formation of the β polymorph in AMF cheese and AMF caseinate samples is not caused by low undercooling of a dispersed system (thermodynamic effect), but by the confinement in the solid matrix (physical effect). The AMF H₂O sample behaved in the same way as AMF, even with homogenization, because of the eventual separation between AMF and water during cooling. Therefore, a true dispersion was not achieved, causing AMF H₂O to behave as bulk fat. Paired with our previous findings in commercial processed cheese products (Ramel and Marangoni, 2017), current results indicate that the dispersion or embedding of fat globules in the cheese matrix is the most important factor in the formation of the β polymorph, rather than the templating effect caused by other ingredients (i.e., salts) present in commercial processed cheese products.

Quantification of the relative proportion of the β polymorph to the β' form by taking the ratio of the % height of the peak corresponding to a d -spacing of 4.6 Å (β polymorph) to 4.2 Å (β' polymorph) within the diffraction pattern of each sample revealed that the β polymorph forms to a significantly greater extent in AMF cheese and AMF caseinate (i.e., when fat is dispersed or embedded as droplets within the protein matrix) than in AMF alone or AMF homogenized with water and salts solution (AMF H₂O and AMF salts; Table 2). Furthermore, it can be observed that a greater extent of β polymorph formation occurs in the

Figure 3. Wide-angle x-ray diffraction (WAXD) patterns of anhydrous milk fat (AMF); AMF homogenized with water (AMF H₂O) and emulsifying salts solution (AMF salts); AMF incorporated in sodium caseinate gel (AMF caseinate) and model cheese (AMF cheese); and model cheese made with canola oil (Canola cheese) after (a) 1 wk and (b) 3 wk of storage at 4 to 7°C. Arrows indicate d -spacings corresponding to characteristic polymorphic forms present: 4.6 Å (β) and 4.2 and 3.8 Å (β').

AMF caseinate sample than in the AMF cheese, which could be due to the presence of salts in AMF cheese, which could enhance β' polymorph formation.

The polymorphism of the samples was also determined after 3 wk (Figure 3b) to investigate whether transitions occur over time. The crystals formed after 1 wk were stable because similar WAXD patterns for each of the samples were observed after 3 wk (Figure 3a and 3b). This result is consistent with previous findings that once a stable form is adopted by TAG, further transformation is not observed, especially at a storage temperature of 4 to 7°C. For AMF not embedded in the protein matrix, the most stable form is β' because of its complex and heterogeneous molecular composition.

Powder X-Ray Diffraction Spectra of AMF, AMF in Sodium Caseinate Gel and Model Cheese, and Canola Oil

To be able to correlate DSC and XRD results, we subjected the samples to heating according to the final melting temperature of AMF ($\sim 45^\circ\text{C}$) obtained from DSC and then determined the polymorphic forms present at this temperature. Because of the complete melting of AMF at this temperature, we expected that no more crystalline structures would be present. Results in Figure 4 show that after melting, diffraction peaks were no longer distinguishable in all the samples. Furthermore, the WAXD patterns of AMF cheese and AMF caseinate are similar to that of melted AMF and canola oil, confirming that the characteristic peaks we observed in XRD for all samples were from AMF crystals.

Powder X-Ray Diffraction Spectra of AMF in Soy and Whey Protein Isolate Gels

For determining other factors that could also affect the polymorphism of AMF in a protein matrix such as the surface properties and structure of the protein molecules, we incorporated AMF in soy and whey protein isolate gels, which have amino acid profiles that are different from those of sodium caseinate.

Results in Figure 5 show that the WAXD patterns of AMF incorporated in soy (AMF soy) and whey protein isolate (AMF whey) gels are similar to those of AMF caseinate and AMF cheese. Quantifying the β polymorph to β' form ratio of AMF soy and AMF whey, we were able to get values of 0.454 ± 0.100 and 0.597 ± 0.080 , respectively, which are close to the values obtained for AMF cheese and AMF caseinate. This result therefore indicates that the formation of the β polymorph in AMF is not affected by the nature of the protein (e.g., hydrophobicity or templating effect due

to similar molecular structures), but by the embedding or incorporation of AMF as droplets (physical effect) in the solid protein matrix, which further supports our findings above.

CONCLUSIONS

Using a model sodium caseinate-based imitation cheese product, we have shown that the formation of the most stable crystal polymorph of MF (β form) in processed cheese products is due to the confinement of TAG through embedding of MF droplets in the protein matrix. The physical constraints imposed by this solid matrix could have forced TAG in MF to arrange themselves in the most compact or stable form. Thermodynamic effects (i.e., inherent slow cooling) caused by dispersion of fat into droplets do not seem to play a role in this system because TAG in AMF were in constant interaction with the different ingredients (e.g., salts) in the matrix, which could have enhanced crystallization or nucleation. Correlations between XRD and DSC measurements confirmed that the characteristic peaks we observed were from AMF alone. This work points to the fact that if the quality and functionality of MF-containing food products depend on its melting

Figure 4. Wide-angle x-ray diffraction (WAXD) patterns of anhydrous milk fat (AMF); AMF incorporated in sodium caseinate gel (AMF caseinate) and model cheese (AMF cheese); and model cheese made with canola oil (Canola cheese) after melting at 45°C for 30 min.

Figure 5. Wide-angle x-ray diffraction (WAXD) patterns of anhydrous milk fat incorporated in soy (AMF soy) and whey protein isolate (AMF whey) gels after 1 wk of storage at 4 to 7°C. Arrows indicate *d*-spacings corresponding to characteristic polymorphic forms present: 4.6 Å (β) and 4.2 and 3.8 Å (β').

behavior, then the effects of different food components as well as dispersion state of MF need to be taken into consideration. Results of this study could have a great effect on the understanding of the complex thermal and rheological behavior of foods with MF or fats as one of its main ingredients.

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REFERENCES

- Bugeat, S., V. Briard-Bion, J. Pérez, P. Pradel, B. Martin, S. Lesieur, C. Bourgaux, M. Ollivon, and C. Lopez. 2011. Enrichment in unsaturated fatty acids and emulsion droplet size affect the crystallization behaviour of milk triacylglycerols upon storage at 4°C. *Food Res. Int.* 44:1314–1330. <https://doi.org/10.1016/j.foodres.2011.01.003>.
- Buldo, P., J. J. K. Kirkensgaard, and L. Wiking. 2013. Crystallization mechanisms in cream during ripening and initial butter churning. *J. Dairy Sci.* 96:6782–6791. <https://doi.org/10.3168/jds.2012-6066>.
- Campos, R., S. Narine, and A. G. Marangoni. 2002. Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Res. Int.* 35:971–981. [https://doi.org/10.1016/S0963-9969\(02\)00159-X](https://doi.org/10.1016/S0963-9969(02)00159-X).
- D'Souza, V., J. M. DeMan, and L. DeMan. 1990. Short spacings and polymorphic forms of natural and commercial solid fats: A review. *J. Am. Oil Chem. Soc.* 67:835–843. <https://doi.org/10.1007/BF02540502>.
- deMan, J. M. 1992. X-ray diffraction spectroscopy in the study of fat polymorphism. *Food Res. Int.* 25:471–476. [https://doi.org/10.1016/0963-9969\(92\)90172-2](https://doi.org/10.1016/0963-9969(92)90172-2).
- Fredrick, E., D. Van de Walle, P. Walstra, J. H. Zijtveld, S. Fischer, P. Van der Meeren, and K. Dewettinck. 2011. Isothermal crystallization behaviour of milk fat in bulk and emulsified state. *Int. Dairy J.* 21:685–695. <https://doi.org/10.1016/j.idairyj.2010.11.007>.
- Glignem, H., D. Ghorbel, C. Lopez, C. Michon, M. Ollivon, and P. Lesieur. 2009. Crystallization and polymorphism of triacylglycerols contribute to the rheological properties of processed cheese. *J. Agric. Food Chem.* 57:3195–3203. <https://doi.org/10.1021/jf802956b>.
- Glignem, H., C. Lopez, C. Michon, P. Lesieur, and M. Ollivon. 2011. The viscoelastic properties of processed cheeses depend on their thermal history and fat polymorphism. *J. Agric. Food Chem.* 59:3125–3134. <https://doi.org/10.1021/jf103641f>.
- Grotenhuis, E., G. A. van Aken, K. F. van Malssen, and H. Schenk. 1999. Polymorphism of milk fat studied by differential scanning calorimetry and real-time x-ray powder diffraction. *J. Am. Oil Chem. Soc.* 76:1031–1039. <https://doi.org/10.1007/s11746-999-0201-5>.
- Jensen, R. G. 2002. The composition of bovine milk lipids: January 1995 to December 2000. *J. Dairy Sci.* 85:295–350. [https://doi.org/10.3168/jds.S0022-0302\(02\)74079-4](https://doi.org/10.3168/jds.S0022-0302(02)74079-4).
- Kaufmann, N., V. De Graef, K. Dewettinck, and L. Wiking. 2012. Shear-induced crystal structure formation in milk fat and blends with rapeseed oil. *Food Biophys.* 7:308–316. <https://doi.org/10.1007/s11483-012-9269-9>.
- Kloek, W., P. Walstra, and T. Vliet. 2000. Nucleation kinetics of emulsified triglyceride mixtures. *J. Am. Oil Chem. Soc.* 77:643–652. <https://doi.org/10.1007/s11746-000-0104-7>.
- Lopez, C., C. Bourgaux, P. Lesieur, S. Bernadou, G. Keller, and M. Ollivon. 2002a. Thermal and structural behavior of milk fat. 3. Influence of cooling rate and droplet size on cream crystallization. *J. Colloid Interface Sci.* 254:64–78. <https://doi.org/10.1006/jcis.2002.8548>.
- Lopez, C., C. Bourgaux, P. Lesieur, and M. Ollivon. 2002b. Crystalline structures formed in cream and anhydrous milk fat at 4 °C. *Lait* 82:317–335. <https://doi.org/10.1051/lait>.
- Lopez, C., V. Briard-Bion, B. Camier, and J.-Y. Gassi. 2006. Milk fat thermal properties and solid fat content in Emmental cheese: A differential scanning calorimetry study. *J. Dairy Sci.* 89:2894–2910. [https://doi.org/10.3168/jds.S0022-0302\(06\)72562-0](https://doi.org/10.3168/jds.S0022-0302(06)72562-0).
- Lopez, C., F. Lavigne, P. Lesieur, C. Bourgaux, and M. Ollivon. 2001a. Thermal and structural behavior of milk fat. 1. Unstable species of anhydrous milk fat. *J. Dairy Sci.* 84:756–766. [https://doi.org/10.3168/jds.S0022-0302\(01\)74531-6](https://doi.org/10.3168/jds.S0022-0302(01)74531-6).
- Lopez, C., F. Lavigne, P. Lesieur, G. Keller, and M. Ollivon. 2001b. Thermal and structural behavior of anhydrous milk fat. 2. Crystalline forms obtained by slow cooling. *J. Dairy Sci.* 84:2402–2412. [https://doi.org/10.3168/jds.S0022-0302\(01\)74689-9](https://doi.org/10.3168/jds.S0022-0302(01)74689-9).
- Lopez, C., P. Lesieur, C. Bourgaux, G. Keller, and M. Ollivon. 2001c. Thermal and structural behavior of milk fat. *J. Colloid Interface Sci.* 240:150–161. <https://doi.org/10.1006/jcis.2001.7664>.
- Marangoni, A. G., and R. W. Lencki. 1998. Ternary phase behavior of milk fat fractions. *J. Agric. Food Chem.* 46:3879–3884. <https://doi.org/10.1021/jf9801668>.
- Martini, S., M. L. Herrera, and R. W. Hartel. 2001. Effect of cooling rate on nucleation behavior of milk fat—Sunflower oil blends. *J. Agric. Food Chem.* 49:3223–3229. <https://doi.org/10.1021/jf001101j>.

- Mazzanti, G., S. E. Guthrie, E. B. Sirota, A. G. Marangoni, and S. H. J. Idziak. 2004. Effect of minor components and temperature profiles on polymorphism in milk fat. *Cryst. Growth Des.* 4:1303–1309. <https://doi.org/10.1021/cg0497602>.
- Méndez-Velasco, C., and H. D. Goff. 2012. Fat structure in ice cream: A study on the types of fat interactions. *Food Hydrocoll.* 29:152–159. <https://doi.org/10.1016/j.foodhyd.2012.02.002>.
- Mounsey, J. S., and E. D. O’Riordan. 1999. Empirical and dynamic rheological data correlation to characterize melt characteristics of imitation cheese. *J. Food Sci.* 64:701–703. <https://doi.org/10.1111/j.1365-2621.1999.tb15114.x>.
- Mounsey, J. S., and E. D. O’Riordan. 2008. Characteristics of imitation cheese containing native or modified rice starches. *Food Hydrocoll.* 22:1160–1169. <https://doi.org/10.1016/j.foodhyd.2007.06.014>.
- Narine, S. S., and A. G. Marangoni. 1999. Microscopic and rheological studies of fat crystal networks. *J. Cryst. Growth* 198–199:1315–1319. [https://doi.org/10.1016/S0022-0248\(98\)01016-1](https://doi.org/10.1016/S0022-0248(98)01016-1).
- Ramel, P. R., and A. G. Marangoni. 2016. Engineering the microstructure of milk fat by blending binary and ternary mixtures of its fractions. *RSC Advances* 6:41189–41194. <https://doi.org/10.1039/C6RA07114G>.
- Ramel, P. R., and A. G. Marangoni. 2017. Characterization of the polymorphism of milk fat within processed cheese products. *Food Struct.* 12:15–25. <https://doi.org/10.1016/j.foosr.2017.03.001>.
- Ramel, P. R., F. Peyronel, and A. G. Marangoni. 2016. Characterization of the nanoscale structure of milk fat. *Food Chem.* 203:224–230. <https://doi.org/10.1016/j.foodchem.2016.02.064>.
- Rønholt, S., J. J. K. Kirkensgaard, T. B. Pedersen, K. Mortensen, and J. C. Knudsen. 2012. Polymorphism, microstructure and rheology of butter. Effects of cream heat treatment. *Food Chem.* 135:1730–1739. <https://doi.org/10.1016/j.foodchem.2012.05.087>.
- Sato, K. 2001. Crystallization behaviour of fats and lipids—A review. *Chem. Eng. Sci.* 56:2255–2265. [https://doi.org/10.1016/S0009-2509\(00\)00458-9](https://doi.org/10.1016/S0009-2509(00)00458-9).
- Solowiej, B., I. W. Y. Cheung, and E. C. Y. Li-Chan. 2014. Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. *Int. Dairy J.* 37:87–94. <https://doi.org/10.1016/j.idairyj.2014.03.003>.
- Sonwai, S., and D. Rousseau. 2010. Controlling fat bloom formation in chocolate—Impact of milk fat on microstructure and fat phase crystallisation. *Food Chem.* 119:286–297. <https://doi.org/10.1016/j.foodchem.2009.06.031>.
- Tamime, A. Y. 2011. *Processed Cheese and Analogues: An Overview*. 1st ed. A. Tamime, ed. Blackwell Publishing, Oxford, UK.
- Timms, R.E. 1980. The phase behaviour and polymorphism of milk fat, milk fat fractions and fully hardened milk fat. *Aust. J. Dairy Technol.* 35:47–53.
- Timms, R. E. 1984. Phase behaviour of fats and their mixtures. *Prog. Lipid Res.* 23:1–38. [https://doi.org/10.1016/0163-7827\(84\)90004-3](https://doi.org/10.1016/0163-7827(84)90004-3).
- Truong, T., N. Bansal, R. Sharma, M. Palmer, and B. Bhandari. 2014. Effects of emulsion droplet sizes on the crystallisation of milk fat. *Food Chem.* 145:725–735. <https://doi.org/10.1016/j.foodchem.2013.08.072>.
- Tzompa-Sosa, D. A., P. R. Ramel, H. J. F. Van Valenberg, and G. A. Van Aken. 2016. Formation of β polymorphs in milk fats with large differences in triacylglycerol profiles. *J. Agric. Food Chem.* 64:4152–4157. <https://doi.org/10.1021/acs.jafc.5b05737>.
- van Aken, G. A., E. Grotenhuis, A. J. Langevelde, and H. Schenk. 1999. Composition and crystallization of milk fat fractions. *J. Am. Oil Chem. Soc.* 76:1323–1331. <https://doi.org/10.1007/s11746-999-0146-8>.
- van Aken, G. A., and K. A. Visser. 2000. Firmness and crystallization of milk fat in relation to processing conditions. *J. Dairy Sci.* 83:1919–1932. [https://doi.org/10.3168/jds.S0022-0302\(00\)75067-3](https://doi.org/10.3168/jds.S0022-0302(00)75067-3).
- Walstra, P., and E. C. H. van Beresteyn. 1975. Crystallization of milk fat in the emulsified state. *Neth. Milk Dairy J.* 29:35–65.
- Wiking, L., V. De Graef, M. Rasmussen, and K. Dewettinck. 2009. Relations between crystallisation mechanisms and microstructure of milk fat. *Int. Dairy J.* 19:424–430. <https://doi.org/10.1016/j.idairyj.2009.03.003>.
- Woodrow, I. L., and J. M. deMan. 1968. Polymorphism in milk fat shown by x-ray diffraction and infrared spectroscopy. *J. Dairy Sci.* 51:996–1000. [https://doi.org/10.3168/jds.S0022-0302\(68\)87112-7](https://doi.org/10.3168/jds.S0022-0302(68)87112-7).
- Wright, A. J., and A. G. Marangoni. 2002. Effect of DAG on milk fat TAG crystallization. *J. Am. Oil Chem. Soc.* 79:395–402. <https://doi.org/10.1007/s11746-002-0495-5>.
- Wright, A. J., S. E. McGauley, S. S. Narine, W. M. Willis, R. W. Lencki, and A. G. Marangoni. 2000. Solvent effects on the crystallization behavior of milk fat fractions. *J. Agric. Food Chem.* 48:1033–1040. <https://doi.org/10.1021/jf9908244>.
- Wright, A. J., M. G. Scanlon, R. W. Hartel, and A. G. Marangoni. 2001. Rheological properties of milkfat and butter. *J. Food Sci.* 66:1056–1071.