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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. Marangoni[1]
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; Gliguem 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 properties 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; Tzompa-Sosa 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).

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[1]Corresponding author: amarango@uoguelph.ca

Most of these studies have been carried out using anhydrous milk fat (**AMF**) and cream alone or as they 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 (~21°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 ~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 Kent, 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 to increase conductivity. Samples were then transferred onto the scanning electron microscopy

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_2O	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

[1]AMF = anhydrous milk fat.