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Effects of emulsion droplet sizes on the crystallisation of milk fat

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ABSTRACT

The crystallisation properties of milk fat emulsions containing dairy-based ingredients as functions of emulsion droplet size, cooling rate, and emulsifier type were investigated using a differential scanning calorimeter (DSC). Anhydrous milk fat and its fractions (stearin and olein) were emulsified with whey protein concentrate, sodium caseinate, and Tween80 by homogenisation to produce emulsions in various size ranges (0.13–3.10 μm). Particle size, cooling rate, and types of emulsifier all had an influence on the crystallisation properties of fat in the emulsions. In general, the crystallisation temperature of emulsified fats decreased with decreasing average droplet size and was of an exponent function of size, indicating that the influence of particle size on crystallisation temperature is more pronounced in the sub-micron range. This particle size effect was also verified by electron microscopy.

1. Introduction

Crystallisation of lipids in emulsions is of interest not only in foods but also pharmaceuticals, nutraceuticals, cosmetics, petroleum, and agrochemicals. Many studies on the crystallisation of lipids in food emulsions and fat-containing food products have shown that crystallisation behaviour, expressed as crystallisation temperature, crystal polymorphism, solid fat content and/or lipid destabilisation, is influenced by numerous factors such as the nature of oil and aqueous phases, processing conditions, thermal treatment (cooling/heating rate and temperature), and temperature history (Lopez et al., 2002a; Lopez, Bourgaux, Lesieur, & Ollivon, 2002b; Martini & Tippetts, 2008; Palanuwech & Coupland, 2003). The emulsion droplet size is also known to impact crystallisation properties of dispersed fat phase (Montenegro, Antonietti, Mastai, & Landfester, 2003; Walstra, van Vliet, & Kloek, 1995). Owing to the emulsified state in which each droplet needs a nucleus or impurity to initiate nucleation, extensive supercooling is required for lipid droplets in a dispersed system to induce crystallisation compared to its bulk counterpart (Walstra et al., 1995). Variations in emulsion droplet size may alter the crystallisation and structural behaviour of emulsified fats, particularly with "nanoemulsions" (droplet diameter below 0.2 μm) (Bugeat et al., 2011; Bunjes, Koch, & Westesen, 2000; Gulseren & Coupland, 2007; Lopez et al., 2001; McClements, Dungan, German, Simoneau, & Kinsella, 1993; Montenegro et al., 2003). It was reported that submicron-size droplets exhibited at lower crystallisation temperature than micron-size ones in *n*-hexadecane oil-in-water emulsions (Dickinson, Mcclements, & Povey, 1991) whereas crystallisation temperature of tripalmitin, tristearin, and trilauroylglycerol nanoparticles just slightly changed with decreasing average size in nanometre-size range (Bunjes et al., 2000; Higami, Ueno, Segawa, Iwanami, & Sato, 2003). Using edible emulsions, Lopez, Bourgaux, Lesieur, Bernadou, et al. (2002), Lopez, Bourgaux, Lesieur, & Ollivon (2002) observed that crystallisation temperature of β-lactoglobulin-stabilised milk fat emulsions decreased with decreasing droplet size (d_{32} 1.25–0.38 μm) at a cooling rate of 1 °C min^{-1}. A recent study of Bugeat et al. (2011) showed that there was a slight decrease in melting enthalpy in small droplet size (0.18 μm) versus larger droplet size (1.67 μm) of unsaturated fatty acids enriched milk TAGs in emulsion state.

Milk lipids, which comprise 98% triacylglycerols (TAGs), are regarded as the most complex of edible fats owing to the wide variation in both TAG structure and fatty acid composition (Christie, 1995). The melting range of milk lipids spans from around −40 to 40 °C, typically giving three overlapping melting curve endotherms (Christie, 1995; Lopez, Bourgaux, Lesieur, Riaublanc, & Ollivon, 2006). These correspond to the three incompletely miscible, low-, middle-, and high-melting point fractions of milk fat.

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Single step fractionation of milk fat produces two primary fractions – stearin (hard) and olein (soft). As in initial milk fat, TAGs account for more than 98% of both stearin and olein fractions; however, the TAG components are different, with the stearin fraction comprising mainly long-chain fatty acids while the olein fraction is enriched in short-chain and unsaturated fatty acids (Lopez et al., 2006). There is a renewed interest in employing olein-rich dairy fractions as food ingredients (Bazmi & Relkin, 2009) and despite the higher proportion of saturated fatty acids, it has been proposed that the stearin fraction might be particularly suitable for encapsulating the bioactive components in some functional food ingredients (Relkin, Yung, Kalnin, & Ollivon, 2008). The crystallisation properties of milk fat unsaturated fatty acid-enriched emulsions upon storage at 4 °C have been reported recently (Bugeat et al., 2011) but apart from this, there appear to be little information on the crystallisation behaviour of milk fat nanoemulsions or even more generally on the effects of droplet size on lipid crystallisation in milk fat emulsions. A better understanding of droplet size-dependent crystallisation properties of milk fat emulsions, particularly nanoemulsions, is needed in order to evaluate the potential industrial application of these materials, for example as food texture modifying agents. New knowledge in this area may also find application in encapsulation technology, where crystallised fat nanoparticles in the form of solid lipid nanoparticles or nanostructured lipid carriers could be used as carriers to encapsulate and deliver lipid-soluble nutrients (Relkin et al., 2008).

This study aimed to examine the effect of emulsion droplet size, from micron- to nanometric-scale, on the crystallisation behaviour of dairy-based oil-in-water emulsions. The dairy ingredients used were anhydrous milk fat, stearin or olein (oil phase) in combination with whey protein or sodium caseinate (aqueous phase). Tween80 was also used to investigate the effect of a small molecular weight surfactant in comparison with the macromolecular ones (whey protein concentrate and sodium caseinate). In this study, Differential Scanning Calorimetry (DSC) was employed to monitor changes in thermal properties and phase transitions in the emulsion systems at various size ranges during cooling at different rates. In addition to determining crystallisation temperatures, solid fat contents of the dairy-based emulsions were also derived from the DSC thermograms.

2. Materials and methods

2.1. Materials

2.1.1. Anhydrous milk fat (AMF) and its primary fractions (stearin and olein)

Dry fractionation of melted milk fat: In this work, three kinds of dairy-based fat/oil, i.e. anhydrous milk fat (AMF) and its two fractions (St: stearin and O: olein), were employed as the oil phase. Stearin and olein fractions were obtained by dry fractionation; i.e. crystallisation from melted milk fat, in a single step procedure. Anhydrous milk fat (AMF) was supplied by Tatura Milk Industries Limited (Tatura, Victoria, Australia). According to the manufacturer, the AMF contained 99.92% butter fat, 0.08% moisture, 0.13% free fatty acid (expressed as oleic acid), with a peroxide value of 0.1 meq 0_2/kg fat. The AMF was placed in a stainless steel container and melted at 60 °C for 2 h in recirculated water bath. A completely melted milk fat at 60 °C was filtered through a Whatman #4 filter paper under vacuum in a Buchner funnel to remove proteinaceous material (adapted from Kaylegian & Lindsay, 1995). The melted milk fat was then allowed to cool to 21 °C in an incubator (cooling rate recorded ~0.2 °C min^{-1}) and held at crystallisation temperature (21 °C) for the total residence time of 24 h (at 21 °C) without agitation. The liquid olein fraction was separated from the solid stearin fraction by vacuum filtration, using a Buchner funnel. The original milk fat and fractionated samples were kept at 4 °C before use.

Determination of fatty acid composition: To quantify fatty acid components, stearin, AMF, and olein were transmethylated for analysis as fatty acid methyl esters (FAMEs) by gas chromatography (GC). For each sample, heptanoic acid (1 mg) was used as an internal standard that was added to a test tube containing approximately 15–20 mg of the lipid sample. Addition of 0.5 mL methanolic NaOH to each sample was undertaken, following by flushing samples with nitrogen gas and covering tubes with Sub-Seals (Sigma–Aldrich). The covered tubes were placed on a steam bath at 95 °C for 3 min to undergo saponification of the lipid sample. After cooling step, 2.5 mL of boron trifluoride (BF_3) in methanol (14%, v/v) were added to the cooled tubes. Esterification of the fatty acid was done by heating tubes again on the steam bath for 5 min and then allowed to cool to room temperature. Subsequent treatments included addition of 2.0 mL of heptane and 5.0 mL of saturated sodium chloride solution, and agitation using a vortex mixer. The heptane added test tubes were then allowed to settle to obtain two layers. Clear heptane solution (1.5 mL) was recovered into the auto sampler vial.

Derivatisation of fatty acids was carried out by GC on a DB-23 60 m × 0.25 mm × 0.25 μm capillary column using a flame ionisation detector (GC-17A, Shimadzu Co., Japan). The injection port was maintained at 250 °C, detector at 285 °C, and the carrier gas helium at 110 kPa, linear velocity 2.2 mL min^{-1}. The column temperature gradient started at 100 °C, rising to 180 °C at 10 °C min^{-1}, and then 240 °C at 5 °C min^{-1}. Identification of methyl esters of specific fatty acids was done by referencing their retention times to those of an authentic standard of methylated fatty acids (Nu-Chek Prep Inc., Minnesota, USA). Quantification of fatty acid composition was determined according to the area of the internal standard peak. Average values were calculated out of determinations of fatty acid compositions of two independent batches. Among the three samples, the stearin fraction had highest level of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids (42.4% $C_{16:0}$, 14.5% $C_{18:0}$), followed by the AMF (37.6% $C_{16:0}$, 11.9% $C_{18:0}$). The olein fraction was enriched in oleic acid (30.3% $C_{18:1}$) while a lower proportion of unsaturated fatty acids was found in the AMF and stearin samples, 28.0% and 21.8% of $C_{18:1}$, respectively. Full details of fatty acid composition of the stearin, AMF, and olein can be found in Supplementary data.

2.1.2. Emulsifiers

Dairy-based emulsifiers including sodium caseinate (NaCN: 92.6% protein, 0.25% lactose–casein, 0.7% fat, 1.2% sodium) and whey protein concentrate (WPC80: 80% protein, 6% lactose and 7% fat) were obtained from Murray Goulburn Co-op (Melbourne, Victoria, Australia). Tw80 (Tween80 LR CAS # 9008-65-5; polyoxyethelene sorbitan monooleate $C_{64}H_{124}O_{26}$; Labtek Pty Ltd., Brendale, Queensland, Australia) was also chosen to investigate the effect of a small molecular weight surfactant on crystallisation behaviour of dairy-based emulsions.

2.2. Preparation of dairy-based emulsions

The aqueous phase (90% wt.) of each sample comprised of 2% emulsifiers (NaCN, WPC or Tw80) dispersed in distilled water with the addition of 0.02% sodium azide as an antimicrobial agent. The aqueous phase was stirred using an overhead mixer for 2 h and stored overnight to allow complete protein hydration. The pH of the aqueous phase was then adjusted to 6.7 by adding aliquots of 1 M NaOH solution. Before mixing with the aqueous phase, 10% wt. of oil phase (AMF, stearin, or olein) was heated well above their highest melting points (as measured by DSC) for 30 min at 55–60 °C to completely melt the fat. The aqueous phase was also