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Phytosterol crystallisation within bulk and dispersed triacylglycerol matrices as influenced by oil droplet size and low molecular weight surfactant addition

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ABSTRACT

Phytosterols can lower LDL-cholesterol and are frequently used by the functional food industry. However, little is known regarding how phytosterol crystallisation can be controlled, despite solubilised phytosterols having improved bioaccessibility. This study investigates phytosterol crystallisation in bulk milk fat and in model dairy emulsion systems at two average droplet sizes, 1.0 and 0.2 μm. The effect of lecithin and monoacylglycerol addition on phytosterol crystallisation for both emulsion and bulk systems was also evaluated. Results demonstrated that lecithin and monoacylglycerols enrichment into the bulk system minimised phytosterol crystallisation. However, in emulsions, phytosterol crystallisation was mainly influenced by decreasing the droplet size. Smaller emulsion droplets containing lecithin showed the greatest potential for decreasing phytosterol crystallisation and had improved physicochemical stability. This information can be employed by the functional food industry to minimise phytosterol crystallisation and possibly improve bioaccessibility.

1. Introduction

Phytosterols are natural plant-derived compounds found within nuts, seeds, fruits, and vegetables. Phytosterols, which are found within plant cell membranes, are structurally similar to cholesterol (found in animal cell membranes) only differing in the presence or absence of a double bond and an R group at the twenty-fourth carbon. The structural similarities between cholesterol and phytosterols allow phytosterols to reduce absorption of cholesterol by competitive solubilisation in the low-density lipoprotein (LDL) chylomicrons, which are adsorbed by the enterocyte cells in the small intestine (Ostlund, 2002). Higher levels of LDL-cholesterol are associated with coronary heart disease, cerebrovascular accidents or strokes, and gallbladder stone disease (Moreau, Whitaker, & Hicks, 2002).

While phytosterols have been found to be effective in lowering LDL cholesterol levels, their efficacy is limited by their compositional variety, physical state and dose (Carden, Hang, Dussault, & Carr, 2015; Ostlund, Spilburg, & Stenson, 1999). To detect a significant decrease in LDL cholesterol levels ≥1.5 g of phytosterols needs to be consumed, which is not possible without the use of medication or functional foods. Due to the low solubility of phytosterols in oil, the hydroxyl group is normally esterified at the third carbon to improve solubility (Engel & Schubert, 2005). Although esterified phytosterols are easier to formulate into food products, they have unpredictable absorption rates, leading to variations in finding from human clinical trials (Clifton, Noakes, Ross, & Nestel, 2004). This variation in absorption levels likely reflects the requirement to hydrolyse the esterified phytosterol before absorption. Phytosterol hydrolysis during digestion is subject to inter-individual variability, causing phytosterol absorption rates to vary between 40 and 96% (Carden et al., 2015).

The efficacy of phytosterols is also limited by the physical state in which they are consumed. Solubilised phytosterols have been shown to be more effective than crystalline phytosterols at lowering LDL cholesterol (Ostlund et al., 1999). Decreasing the crystal size and degree of crystallinity has been proven to influence phytosterol bioavailability, yet, limited research has been conducted on how to control phytosterol crystallisation within functional food systems (Ostlund et al., 1999).

This study seeks to reduce phytosterol crystallisation within a mixed triacylglycerol (TAG) system, which is the typical composition of most food lipids. The model TAG system chosen for the study was milk fat,

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which forms β polymorphs after cooling and storage. β polymorphic structures are common in foods and occur in other lipids such as palm oil, cocoa, coconut oil, and suet (Akoh & Min, 2008). In addition, the digestion process for milk fat results in extensive changes to the structure of the lipid. During digestion the original lamellar packing of milk fat is observed to transition to more complex packings including a bicontinuous cubosome formation. The high surface area associated with these organised lipid structures may improve the bioaccessibility of bioactives dispersed within the lipid matrix (Salentinig, Phan, Khan, Hawley, & Boyd, 2013). Promising research has also shown that milk enriched with phytosterols (1.8 g/day) using a proprietary crystal-retardation method can result in a 29.1 ± 4.1% reduction in LDL-cholesterol levels (Pouteau, et al., 2003). In a previous study by the authors, the underlying mechanism of phytosterol crystallisation within milk fat matrices was examined (Zychowski, et al., 2016). However, to date, no work has explored possible ways to decrease phytosterol crystallisation within a milk fat system.

Thus, this work seeks to decrease phytosterol crystallisation in both bulk and emulsified milk fat systems, with a concomitant increase in phytosterol bioaccessibility, by varying the formulation and processing parameters of the phytosterol-enriched system. Milk fat formulations were produced with or without added phytosterols and/or low molecular weight surfactants, phospholipids from lecithin or monoacylglycerol (MAG). All lipid formulations were studied in bulk milk fat, and were emulsified with whey protein isolate at two different homogenisation pressures to create droplets approximately 1.0 or 0.2 μm in diameter. Lecithin and MAG were chosen as they have been shown to impact the formation, growth, and location of crystalline compounds within oil-in-water systems (Bin Sintang, et al., 2017; Li, Zheng, Xiao, & McClements, 2012). Engel and Schubert (2005) demonstrated that lecithin and MAG decrease phytosterol crystallisation in both bulk and emulsion systems. However, in this study, detection of crystalline phytosterols was via polarised light microscopy, and their findings may therefore have been limited by the detection limits of the light microscope. As recent studies have demonstrated that phytosterol crystals can be as small as 36.88 Å nm in size and thus higher resolution imaging techniques and analytical tools, such as X-ray diffraction, are required to effectively quantify the level of crystallinity (Bin Sintang, et al., 2017).

Droplet size selection (~1.0 and 0.2 μm) was based on previous research demonstrating that milk fat-based, sub-micrometer-sized (< 400 nm) emulsion droplets were successful in limiting the crystallisation of β-carotene (Zhang, Hayes, Chen, & Zhong, 2013); results from this study will increase our fundamental understanding of the impact of droplet size and addition of surfactants on phytosterol crystallisation within bulk and emulsion systems. This information could potentially benefit the food industry in development of functional food products with increased bioaccessibility.

2. Materials and methods

2.1. Chemicals and ingredients

Crystalline phytosterol consisting of β-sitosterol (≥70%), with residual campesterol and β-sitostanol, was purchased from Sigma Aldrich (Wicklow, Ireland). Glass capillaries with a wall thickness of 0.1 mm were purchased from Charles-Supper (Natick, Massachusetts) for synchrotron analysis. Soy lecithin (Adlec) was kindly donated by Archer Daniels Midland Co. (Chicago, Illinois) and distilled monoglycerides (Dimodan R-T PEL/B Kosher) were purchased from Danisco Australia Pty Ltd. (Banks Meadow, Australia). Whey protein isolate (WPI) (ALACEN© 895, protein content 92.0%) was obtained from Fonterra (Maungaturoto, New Zealand), sodium azide was purchased from Sigma Aldrich (Castle Hill, Australia) and commercial grade anhydrous milk fat was purchased from Marsh Dairy Product (Footscray, Australia; Table S1). For polarised images taken in Ireland at the National Imaging Centre, anhydrous milk fat was purchased from Corman Miloko (Carrick on Suir, Ireland).

2.1.1. Preparation of bulk and emulsion samples

Bulk anhydrous milk fat was combined with or without lecithin or MAG at 3% wt/wt. The bulk mixture was then heated to 110 °C while stirring on a magnetic hot plate at 300 rpm. Once a temperature of 110 °C was achieved, phytosterols were added at 3 and 6% and the mixture was stirred for 2 min. A bulk mixture of each formulation without phytosterols was also made and was subjected to the same thermal and shear treatment. After the holding period, the oil was cooled to 80 °C before loading into capillaries or glass slides for synchrotron analysis or polarised light microscopy, respectively. Samples were then allowed to cool statically to 4 °C and held at this temperature for 48 h.

Oil-in-water emulsions (10% oil: 1% protein: 89% H_20) were prepared by homogenising the described milk fat-based formulations with an aqueous protein phase. The aqueous phase was created by reconstituting whey protein isolate (WPI) at 11.11% protein with stirring at 600 rpm in an ice bath. After 2 h, the solution was then refrigerated at 4 °C overnight to allow for complete hydration. Before homogenising the two phases, aliquots of WPI solution were heated to 55 °C for 20 min. After heating, the WPI solution was mixed with Milli-Q water at 70 °C to create a 1% protein solution. This process, as employed previously, was utilised to minimise denaturation of the whey protein caused by excessive heating or high temperatures (McClements, 2004; Zychowski, et al., 2016).

The two phases were first combined into a pre-emulsion utilising a Silverson rotor–stator mixer set at 3200 rpm for 1 min. The mixture was then homogenised with an EmulsiFlex-C5 (Avestin, Mannheim, Germany) with two different processes. Larger emulsion droplets (~1.0 μm in average diameter) were generated with 1 pass at 300 bar pressure, while smaller droplets (~0.2 μm in average diameter) required 3–5 passes (depending on the formulation) at 1000 bar (Fig. S1). All emulsions were homogenised at 60 °C and then cooled and stored at 4 °C. After cooling, 0.02% of sodium azide was added to each emulsion to prevent microbial growth.

2.1.2. Synchrotron X-ray analysis

Scattering experiments were performed on the small- and wide-angle X-ray scattering (SAXS/WAXS) beamline at the Australian Synchrotron (Clayton, Australia) with a camera length of 0.9 m and a beam of wavelength λ = 0.89 Å (14.0 keV). A Dectris Pilatus 1 M captured small-angle measurements (q = 0.017–1.18 $Å^{-1}$), while a Pilatus 200 K detector recorded wide-angle measurements (q = 0.95–3.19 $Å^{-1}$) (Kirby et al., 2013). Samples were taken from a refrigeration unit directly into a pre-cooled capillary holder at 4 °C. Samples equilibrated at 4 °C for 10 min before SAXS and WAXS patterns were collected. A series of three, 3 s shots were taken for each emulsion in duplicate capillaries over a 15 mm gap. Snapshots of the material references were also collected on the crystalline phytosterol (with and without MilliQ water), the aqueous phase with WPI, the lecithin and MAG after storage at 4 °C for 24 h. The beamline was calibrated using silver behenate and all diffractions patterns of averaged shots were background-subtracted using the Australian Synchrotron SAXS/WAXS software (ScatterBrain, V2.71, Australia). Bulk and emulsion diffraction peaks were analysed utilising Gaussian peak analysis (Eq. (1)) with MatLab (Math Works Inc., Matlab R2014b, USA).

$$f(x) = ae^{-(x-b)^2}/2c^2 \quad (1)$$

In this equation, a is the maximum height of the diffracted peak, b is the peak position, and c is the full width at half-maximum (FWHM) of the peak, as used previously for SAXS/WAXS analysis (Zychowski, et al., 2016).