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Monitoring the progression of calcium and protein solubilisation as affected by calcium chelators during small-scale manufacture of casein-based food matrices

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ABSTRACT

Calcium and protein solubilisation during small-scale manufacture of semi-solid casein-based food matrices was investigated and found to be very different in the presence or absence of calcium chelating salts. Calcium concentrations in the dispersed phase increased and calcium-ion activity (A^{++}_{Ca}) decreased during manufacture of the matrices containing calcium chelating salts; with ~23% of total calcium solubilised by the end of manufacture. In the absence of calcium chelating salts, these concentrations were significantly lower at equivalent processing times and remained unchanged as did A^{++}_{Ca}, throughout manufacture. The protein content of the dispersed phase was low (≤3% of total protein), but was significantly higher for matrices containing calcium chelating salts. This study elucidates the critical role of calcium chelating salts in modulating casein hydration and dispersion and gives an indication of the levels of soluble calcium and protein required to allow matrix formation during manufacture of casein-based food structures e.g. processed and analogue cheese.

1. Introduction

In milk systems calcium is partitioned between colloidal and aqueous phases. Approximately 70% of calcium is present as undissolved complexes in casein micelles, named colloidal calcium phosphate (CCP) (Holt, 1997); the remainder exists as free Ca^{2+} ions or soluble complexes with citrate and HPO_4^{2-} in the aqueous phase (Gao et al., 2010). Calcium ion equilibria are known to play an important role in the structure and stability of casein micelles (Horne, 1998; Walstra, 1990) and alterations to these equilibria have effects on the physico-chemical properties of casein (de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2011). Upon addition of calcium chelating salts (CCS) such as disodium phosphate (DSP) and trisodium citrate (TSC) to casein micelles, solubilisation of CCP and decreased activity or concentration of ionic calcium is observed (Choi, Horne, & Lucey, 2007; de Kort, Minor, Snoeren, van Hooijdonk, & van der Linden, 2009; McIntyre, O'Sullivan, & O'Riordan, 2016a; Udabage, McKinnon, & Augustin, 2000, 2001). The calcium-binding anion of the CCS competes with the phosphoseryl residues and CCP in the casein micelle for Ca^{2+} ions (de Kort et al., 2011). This reduces the extent of calcium-mediated protein cross-linking in casein micelles or the aggregated *para*-casein network of rennet casein during processed and analogue cheese manufacture (Ennis & Mulvihill, 1999) and consequently enhances the functionality (i.e. water-binding capacity, solubility, viscosity, emulsifying capacity) of the casein (de Kort et al., 2011; McIntyre et al., 2016a; O'Sullivan & Mulvihill, 2001).

Although CCS perturb casein-mineral equilibria affecting Ca^{2+} activity, CCP concentration, and the proportion of casein in the micelle (Udabage et al., 2000), the relative contribution of each of these factors to the formation of a hydrated and functional processed or analogue cheese matrix is unclear. Previously, the impact of CCS on calcium equilibria has been studied in detail but only in simplified aqueous systems (de Kort et al., 2009; McIntyre et al., 2016a) or semi-concentrated protein dispersions (Ennis & Mulvihill, 1999; Ennis, O'Sullivan, & Mulvihill, 1998; McIntyre et al., 2016a; O'Sullivan & Mulvihill, 2001). These studies addressed model systems because of the difficulty of working with concentrated casein-based matrices containing lipid, however, this can make extrapolation to real food matrices such as processed and analogue cheese difficult. Therefore, despite the fact that some of the protein dispersions previously used were relatively concentrated (Ennis & Mulvihill, 1999; Ennis et al., 1998; McIntyre et al., 2016a), an even more concentrated model system is required to better simulate CCS behaviour during manufacture of the aforementioned products. Recently, a range of processed cheese products have been successfully manufactured on a small-scale using

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a Thermomix blender cooker (Lee, Klostermeyer, & Anema, 2015; McIntyre, O'Sullivan, & O'Riordan, 2016b; Salek et al., 2015; Černí ková et al., 2008). This mixing system facilitates the manufacture of small batch sizes and allows for rapid sample collection to monitor the changes in calcium equilibria and protein solubility during the hydration, viscosity building and structure development stages of manufacture, enabling a realistic insight into the formation of a casein-based food matrix.

The aim of this study was to enhance our knowledge of how ionic species e.g. calcium, partition in concentrated semi-solid casein-based food matrices by developing a small-scale manufacturing protocol for these systems. An additional aim was to validate previous investigations of the effects of CCS on calcium distribution and protein solubility in dilute model systems as carried out previously in this laboratory (McIntyre et al., 2016a) and to determine if such systems are representative of the true realities of processed and analogue cheese manufacture. The final objective of this work was to evaluate the suitability of the Thermomix as a small-scale mixer for product manufacture relative to pilot scale manufacture.

2. Materials and methods

2.1. Materials

Rennet casein powder (Kerrynor™ R190) with a protein content of 80% was supplied by Kerry Ingredients Ltd. (Listowel, Co. Kerry, Ireland). Rapeseed oil was sourced from Boyne Valley Foods (Drogheda, Co. Louth, Ireland). Novelose (HI-maize 260) resistant starch was obtained from Univar (Ireland) Ltd. (Rathcoole, Co. Dublin, Ireland). The following food grade ingredients were used: anhydrous disodium phosphate (DSP) (Albright and Wilson Ltd., Cheshire, England), trisodium citrate (TSC), citric acid (Jungbunzlauer GmbH., Pernhofen, Austria), sodium chloride (Salt Union, Cheshire, England) and sorbic acid (Hoechst Ireland Ltd., Dublin, Ireland). Calcium reference standard solution for atomic absorption spectroscopy (AAS) (1000 mg/L), lanthanum trichloride heptahydrate ($LaCl_3{\cdot}7H_2O$) and nitric acid (HNO_3) were purchased from Sigma Aldrich (Dublin, Ireland) and were analytical grade. Deionized water was prepared in a Milli-Q water purification system.

2.2. Manufacture of casein-based food matrices

2.2.1. Pilot scale

A Blentech cooker (Model CC-010, Blentech Corporation, California, U.S.A.), was used to manufacture pilot scale batches (4 kg) of low-fat casein-based food matrices containing resistant starch (Novelose). Processing conditions were similar to those described by El-Bakry, Duggan, O'Riordan, and O'Sullivan (2010a) when manufacturing analogue cheese. An operating speed of 100 rpm was used to manufacture casein matrices with a moisture content of 58%. The final moisture content was determined by considering the amount of water added in the formulation combined with the moisture content of rennet casein and also allowing for the amount of steam condensed during manufacture. The casein-based food matrices were manufactured according to the following formulation, which is expressed on a dry matter basis (% w/w): 53.09% rennet casein, 31.19% novelose, 9.52% rapeseed oil, 2.00% TSC, 0.92% DSP, 1.69% NaCl, 1.14% citric acid and 0.19% sorbic acid. Water and rapeseed oil were added to the mixing chamber and agitated for 1 min at 50 °C; the minor ingredients (DSP, TSC, NaCl and sorbic acid) were added and mixed for 1 min. The rennet casein was then added under constant agitation, and the contents blended for 1 min at 50 °C. The temperature was increased to 80 °C using direct steam injection, and mixing was continued until a homogeneous mass was formed and all free water and oil was absorbed. Novelose was then added and the blend mixed for 2 min. Finally citric acid was added and mixing continued for a further 1 min. The hot mass was discharged from the cooker, filled into a rectangular box lined with a polypropylene bag and placed in a freezer at -18 °C and after 1 h transferred to a refrigerator at 4 °C for 24 h before being vacuum packed (Model C10H, Webomatic® Vacuum Packaging Systems, Maschinenfabrik GmbH, Bochum, Germany). The casein-based food matrices were manufactured in triplicate.

2.2.2. Small-scale

Casein-based food matrices were manufactured on a small scale (500 g) using a 2L capacity Vorwerk Thermomix TM 31 blender cooker (Vorwerk & Co. Thermomix; GmbH, Wuppertal, Germany). The Thermomix has a four-blade chopper rotor at the base of the cooker. The cooker is electrically heated at the base and the heating scale ranges from 37 to 100 °C. The casein matrices were processed using a method as similar as possible to that used in the Blentech pilot scale cooker. The variable mixing speed was controlled using the speed selector. The temperature of the mixing bowl was regulated by the temperature controls and monitors which were adjusted accordingly to give temperatures of 50 °C or 80 °C. Ingredients were added to the mixing bowl at 50 °C in the same sequence and mixed for the same times as described in Section 2.2.1. The increase in mixture temperature from 50 °C to 80 °C was achieved by adjusting the Thermomix temperature controls, and verifying the temperature using a hand-held thermometer (HANNA instruments, H19041C, Bedfordshire, UK). This increase in mixture temperature was complete in ~2.25 min. Mixing was continued until a homogeneous mass was formed, after which Novelose was added and the blend mixed for 2 min. Finally citric acid was added and mixing continued for 1 min. Small scale casein-based food matrices were manufactured in triplicate.

2.3. Compositional analysis

Moisture content was determined gravimetrically after drying the sample in a laboratory oven at 101 °C to constant weight (IDF, 2004); ash content was also determined gravimetrically after the complete incineration of the sample in a muffle furnace (B180, Nabertherm, GmbH, Germany) at 550 °C/6 h (IDF, 2007); the calcium content of the ash residue was assessed using a flame atomic absorption spectroscopy method (IDF, 2007). Fat content was evaluated using the Gerber method (National Standards Authority of Ireland, 1955), and total nitrogen was measured by the Kjeldahl method with a conversion factor of 6.38 for crude protein (IDF, 2014). The potentiometric method (Fox, 1963) was used to evaluate sodium chloride (NaCl) content. Values of pH were determined at ambient temperature by inserting a glass tip electrode of a calibrated pH-meter (EL20, Mettler Toledo, Schwerzenbach, Switzerland) directly into the casein matrix at three randomly chosen locations. All analyses were completed in triplicate.

2.4. Functional testing

2.4.1. Texture profile analysis (TPA)

The texture profile of cylindrical samples (25 mm diameter, 20 mm height) were analysed using an Instron Universal Testing machine (Model 5544, Instron Corp., Canton, Mass., USA) fitted with a 1000 N load cell. Cylindrical samples were cut using a cork borer, wrapped in cling film to prevent dehydration, and allowed to equilibrate to 22 °C for 30 min prior to analysis. Afterwards, the cling film was removed and samples were compressed by 80% of initial height using a 35 mm diameter plate at a crosshead speed of 50 mm min^{-1}. The uniaxial compression test was performed in two successive cycles, and the textural parameters, hardness and