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FOOD STRUCTURE

Combined confocal microscopy and large deformation analysis of emulsion filled gels and stirred acid milk gels

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ABSTRACT

The microstructural breakdown properties of heat-induced whey protein (WP) gels prepared at pH 7.0 or 5.4 and containing emulsified sunflower oil (7%, w/w) were studied using notch propagation tensile testing in combination with dynamic confocal laser scanning microscopy (CLSM). In addition, the microstructural breakdown properties of stirred acid milk gels containing added emulsified oil (0–15%, w/w) or Konjac glucomannan (0.05%, w/w) were studied using compressive rheological deformation in combination with dynamic CLSM imaging. The structural breakdown properties (Young's modulus and stress/strain at fracture), the microstuctural behaviour of the protein phase, the emulsified oil phase and the pattern of notch propagation during large deformation tensile testing of the WP emulsion filled gel prepared at pH 7.0 (fine stranded gel) differed from that of the WP emulsion filled gel prepared at pH 5.4 (particulate gel). The protein aggregates, emulsified oil droplets and the Konjac phase in the stirred acid milk gels flowed on compression; however, the flow pattern changed from ''frictional'' flow with no emulsified oil or Konjac to ''slip'' flow in the presence of emulsified oil or Konjac. Also, compression of stirred acid milk gels below a certain critical height led to disintegration of the aggregated protein structure, serum release and reduced stability of the oil droplets in the stirred gels. The critical compression height was influenced by emulsified oil level and the presence of added Konjac.

1. Introduction

Fat imparts highly desirable creamy sensory perception to many food products. However, it also increases calorific content leading to obesity related health problems. Reduction of the fat content of foods while maintaining their creamy sensory perception is a challenging task for the food industry and is a topic of growing research interest. Designing new low fat foods while maximising fat-related desirable sensory attributes requires an understanding of the mechanism of food structure breakdown and fat release in the mouth during mastication which contributes to the perceived attribute (Frøst & Janhøj, 2007). Such mechanisms are highly product-specific.

During mastication, the mechanical action of the teeth breaks the food into small pieces, while the combined actions of the tongue and the palate exert compressive, extensional and shear forces which lead to disintegration of the food structure and fat release from the food matrix (Malone, Appelqvist, & Norton, 2003). This process is further complicated by the effects of mixing with saliva, temperature variation and enzymic activity. Evaluation techniques that combine rheological measurements with simultaneous

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microscopic observations, have been developed to study the structural properties of model multicomponent food systems subjected to small and large deformation, making it possible to gain an insight into the breakdown properties of foods during mastication (Olsson, Langton, & Hermannson, 2002; Nicolas & Paques, 2003; Nicolas et al., 2003).

Milk proteins, principally caseins and whey proteins, are often used as the main structuring component in semi-solid dairy foods due to their unique gelling properties. Under appropriate physiochemical conditions, casein proteins have the ability to aggregate on reducing the pH towards their isoelectric point (pH 4.6) and form a particulate gel network (Cayot, Fairise, Colas, Lorient, & Brule, 2003; Lucey & Singh, 1997). Globular whey proteins unfold on heating (denaturation) at elevated temperatures (>65 °C) and aggregate to form a fine stranded or a particulate gel network depending on the physiochemical parameters at the time of heating. Denatured whey proteins, in particular β-lactoglobulin, can also interact with casein micelles in heat-treated milk via disulphide linkages (Mulvihill & Kinsella, 1987). The structural properties of acid induced milk gels having a casein network structure have been found to strengthen on heat-treating milk prior to acidification due to increased inter-connectivity of the protein network structure in the gels as a result of casein-denatured whey protein or denatured whey protein-denatured whey protein interactions (Lucey, Munro, & Singh, 1999; Lucey & Singh, 1997). Milk protein based food products often contain other components such as fats or neutral polysaccharides and the final structure of the food product is influenced by the interactions between the protein matrix and the fat or the polysaccharides in the system. The effects of fat or polysaccharide addition on the structural properties of milk protein gels have also been widely investigated The effect of gelling agent concentration, droplet size on the mechanical properties of filled gels has been studied by Chojnicka, Sala, de Kruif, and van de Velde (2009) and Sala, van Vliet, Stuart, van de Valde, and van Aken (2009) who found that increasing gelling agent concentration increased gel strength. Chojnicka et al. (2009) measured lubrication properties of emulsion filled gels and found that the addition of oil droplets to the gel had a profound effect on the lubricant properties and that bound vs. unbound droplets also differed in their effects. The relationships between filled gels and sensory properties has been investigated by several workers (Mosca, Rocha, Sala, van de Velde, & Stieger, 2012; Sala, de Wijk, van de Velde, & van Aken, 2008; Sala, van de Velde, Stuart, & van Aken, 2007) who showed that fat level, fat interfacial properties and matrix effects all had an effect on sensory perception, in particular creaminess. Addition of fat emulsified with milk proteins can reinforce the structural properties of milk protein gels due to strong interactions between the protein adsorbed on the surface of fat droplets and the protein gel matrix (Barrantes, Tamime, Sword, Muir, & Kaláb, 1996; Lucey, Munro, & Singh, 1998; Pereira, Matia-Merino, Jones, & Singh, 2006). The presence of neutral polysaccharides may strengthen or weaken the rheological properties of undisturbed or mechanically stirred milk protein gels depending on the polysaccharide concentration in the system due to segregative interactions between the protein aggregates and the polysaccharide (Everett & McLeod, 2005) and also concentration, sequence of addition and whether the polysaccharide is in the continuous or dispersed phase (see for example Sala et al., 2009). Abhyankar, Mulvihill, and Auty (2011) recently investigated the microstructural changes occurring in fine stranded whey protein gels containing milk protein emulsified oil droplets during large rheological deformation and fracture and observed that the microstructural behaviour of oil droplets in the gels was influenced by the structural properties of the whey protein gel matrix and the proximity of the oil droplets to the fracture path. However, the microstructural changes and differences between fine stranded particulate milk protein gels (stirred or unstirred) containing added fat or polysaccharides during large rheological deformations has not been widely investigated by confocal microscopy since the original article by Plucknett et al. (2001). In particular, studying the mobility of the fat phase during breakage could help in the design of new reduced fat products with optimised fat release properties.

Konjac glucomannan is a neutral food polysaccharide extracted from the tubers of *Amorphophallus konjac* and has been widely used in food products as a thickening agent (Nishinari, Williams, & Phillips, 1992). Konjac consumption is associated with health benefits such as obesity control, improving intestinal activity and lowering of blood cholesterol, making it an attractivel ingredient for use in food products. However, the microstructural and rheological properties of Konjac glucomannan in acidified dairy products have not been studied.

Dynamic confocal scanning laser microscopy (CSLM) techniques are powerful tools for studying microstructure formation and breakdown in dairy systems (see, for example Auty, Fenelon, Guinee, Mullins, & Mulvihill, 1999; Olsson et al., 2002). The overall hypothesis of this study is that combining large deformation of solid and semi-solid food models with dynamic CSLM analysis can give new insights into the release of fat from foods during deformation. There are therefore two objectives of this study: (1) to monitor the fracture behaviour and microstructure of fat-filled particulate and fine-stranded whey protein gels in extension using microtensile stretching and (2) to study the microstructural behaviour of stirred acid milk gels containing either added oil or Konjac glucomannan during compression.

2. Materials and methods

2.1. Materials

Low heat skim milk powder was supplied by Kerry Food Ingredients, Co. Kerry, Ireland. Whey protein isolate [BioPro; manufacturer specifications: 96.8% protein (on a dry matter basis), 0.7% fat, 2.4% ash, 4.7% moisture] was purchased from Davisco Food International, MN, USA. To remove possible effects of solid fat crystals on mechanical properties of the filled gels a liquid oil was used. Commercial sunflower oil containing 10% (w/w), saturated fat as declared on the label, was purchased from SuperValu Supermarkets Ltd., Co. Cork, Ireland. Konjac flour (Amalan, Food grade, E425; >90% total dietary fibre (glucomannan); <10% moisture) was provided by De Oxy Limited, Cork, Ireland. Nile Red, Fast Green and fluorescein isothiocyanate (FITC) were supplied by Sigma Chemicals, MO,