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The inhibitory roles of native whey protein on the rennet gelation of bovine milk

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ABSTRACT

Rennet gelation is used to produce many types of cheese. The effect of native whey protein on rennet gelation kinetics was investigated. Milks with a wide range of whey protein:casein (WP:CN) ratios (with standardised casein concentrations) were made from powders produced by microfiltration. Measurements of casein macro peptide release showed that native whey protein inhibited the enzymatic action of chymosin, which delayed the onset and reduced the subsequent rate of gelation. Experiments in which increased chymosin concentrations compensated for the inhibition, demonstrated that other factors also contributed to the reduced gelation rate. Neither an increase in viscosity nor a reduction in soluble calcium was responsible, leading to the conclusion that in addition to inhibiting chymosin, native whey proteins present a physical barrier to para-casein aggregation. This study demonstrates and explains how casein-enriched retentates from microfiltration gel faster than regular cheese milk that contains higher amounts of native whey protein.

1. Introduction

Rennet gelation is a key process in cheese making that transforms liquid milk to a coagulum. Much research has been undertaken to understand the mechanisms of rennet gelation (Guinee & Wilkinson, 1992). This has facilitated improvement of cheese production processes to manufacture better quality cheese at a lower cost. More recently, the advent of membrane filtration technology has enabled control over the protein composition of the milk used for cheese making (Maubois, 2002). While the overall mechanisms of rennet gelation are well established (Lucey, 2002), a more detailed understanding of the mechanisms in relation to protein composition is required to take full advantage of membrane technology in the manufacture of renneted cheese.

Milk is a complex system, comprising of an emulsion of fat globules, a colloidal suspension of two distinct types of proteins (caseins & whey proteins), and an aqueous solution of lactose and various minerals (Jenness, 1999). Whereas, whey proteins are soluble in water, caseins (CN) are present in milk in the form of large colloidal aggregates (50–200 nm in diameter) known as casein micelles. The surface of casein micelles is rich in κ-casein, which has a hydrophilic section that protrudes outwards like a 'hairy layer', stabilising the micelles through electrostatic and steric repulsion (Walstra, 1999). Rennet gelation destabilises the casein micelles by selectively cleaving the 'hairy layer' using a proteolytic enzyme called 'chymosin' that is found in rennet (Vasbinder, Rollema, & De Kruif, 2003). As this enzymatic reaction progresses, the cleaved C-terminal of the κ-CN known as casein macro peptide (CMP) solubilises in the milk serum. The casein micelles become progressively more hydrophobic allowing them to aggregate to form a coagulum (Fox, Guinee, Cogan, & McSweeney, 2017; Vasbinder et al., 2003; Walstra, 1990).

Casein micelles are central to the rennet gelation process and the rate of coagulation increases with increasing casein concentration (Fox et al., 2017). Ultrafiltration (UF) can be used to concentrate milk proteins in cheese milk (Nelson & Barbano, 2005), which increases the rate of gelation as well as the volumetric productivity of the cheese vats. Whereas UF concentrates all of the milk proteins, microfiltration can separate larger casein micelles from the smaller whey proteins (Maubois, 2002; Papadatos, Neocleous, Berger, & Barbano, 2003; Zulewska, Newbold, & Barbano, 2009). This enables the production of cheese milks from MF retentates with increased casein:whey protein (CN:WP) ratios.

Although the overall mechanisms of rennet gelation are well established, the role of whey proteins has yet to be fully elucidated. As

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separate soluble proteins, the whey proteins are not directly involved in the rennet gelation process. However, when combined with heat treatment above the denaturation temperature (~65–70 °C), whey proteins have been found to impair rennet gelation leading to elongated gelation time and weaker gels (Vasbinder et al., 2003). This behaviour was attributed to the formation of disulphide bridges between thiol groups of the κ-CN and denatured β-lactoglobulin (β-LG), the most abundant of the whey proteins (Jang & Swaisgood, 1990). Although it was initially thought that this disulphide bridging blocked the κ-casein hairs from being cleaved (Hinrichs, 2001), a more recent study has shown that heat treatment has a more significant effect on the aggregation of the renneted casein micelles (para-casein micelles) than on the actual κ-casein hydrolysis (Vasbinder et al., 2003). In a contrasting study, supplementation of reconstituted skim milk powder with whey protein prior to heating at 65 °C for 30 min and subsequent rennet gelation was found to result in firmer gels (Meza-Nieto, Vallejo-Cordoba, González-Córdova, Félix, & Goycoolea, 2007). Although this degree of heat treatment was shown not to result in extensive attachment of denatured whey protein to the casein micelles, the increased gel strength was attributed to the cross-linking between the casein micelles by whey proteins (Meza-Nieto et al., 2007). A more likely explanation, however, is that the addition of whey powder simply increased the soluble calcium levels, which were not standardised and are otherwise lacking in reconstituted skim milk powder (Martin, Williams, & Dunstan, 2007), thereby limiting the rate of rennet gelation (Martin, Williams, Choong, Lee, & Dunstan, 2008).

In cheese production, milk does not typically undergo severe enough heat treatment for whey proteins to be denatured. Therefore, it is the absence of native whey protein in MF retentates that is of interest from a cheese making perspective. However, to date there have been only a few studies (Lelievre, Creamer, & Tate, 1990) investigating the effect of native whey proteins on the rennet gelation process focusing mainly on the overall gelation properties of milk systems. Lelievre and colleagues observed an increased clotting time with added whey protein isolate (WPI) and an inhibition of α_{s1}-casein hydrolysis, which develops flavour during the cheese maturation stage. However, the effects native whey proteins have on the enzyme hydrolysis of κ-CN and the aggregation phases post-renneting have not yet been distinguished clearly. Therefore, in this study we investigate for the first time the effect of native whey protein on the kinetics of the enzymatic hydrolysis and the aggregation phases of rennet gelation. This was done by comparing the gelation of milks prepared with a wide range of WP:CN ratios (with a constant CN concentration) using casein concentrate and whey protein concentrate (WPC) powders produced through microfiltration.

Establishing the role of native whey protein on the kinetics and mechanisms of rennet gelation will enable the cheese industry to optimise the formulation of cheese milks with different WP:CN ratios to maximise productivity and reduce the total production cost. Further, a detailed kinetic study of rennet gelation will provide new insights, especially into the secondary aggregation stage of casein micelles that is less understood to date.

2. Materials and methods

2.1. Production of casein and whey powders

All casein and whey protein solutions were made from reconstituted powders that were isolated using a microfiltration process. The microfiltration consisted of a 3-stage batch concentration process adapted from Nelson and Barbano (Nelson & Barbano, 2005), designed to isolate casein from whey proteins in skim milk. ISOFLUX™ microfiltration membranes with a nominal pore size of ~0.15 μm were operated in a membrane filtration pilot plant (GEA, 2012 Model Type R). The microfiltration process was operated with a nominal permeate flux of between 60 and 90 kg/h, feed pressure of 1 bar, and recirculation pressure between 2.5 and 2.9 bar.

700 kg of pasteurized skim milk was processed through the microfiltration pilot plant in stage 1. Casein was concentrated in the retentate as whey proteins were selectively removed in the permeate. Retentate was bled from the holding tank at a rate of ~30 kg/h. Approximately 3X protein concentration of the retentate occurred at the completion of each stage of processing. Prior to stage 2 and 3 diafiltration, a volume of filtered water was added to the retentate to return it to ~1X protein concentration. Temperature was maintained at 55 °C throughout the process.

The permeate (native whey protein stream) was collected and separately concentrated using a multistage ultrafiltration (UF) process. The UF was operated at a nominal permeate flux of between 240 and 300 L/h, feed pressure of 3 bar, and recirculation pressure of between 4.0 and 4.1 bar. Approximately 20X protein concentration was achieved by the UF process. 2 stages of diafiltration with 90 kg of water were performed. Temperature was maintained at 10 °C throughout the ultrafiltration process.

The concentrated liquid retentate and permeate streams were collected and spray dried (GEA-Niro, Mobile Minor). Liquid was fed into the spray dryer via a rotary atomizer set with an outlet temperature of 80 °C. Water was evaporated at a rate of ~2 kg/h.

2.2. Compositional analysis of casein and whey powders

Protein analysis of powders was performed based on the Dumas Combustion method using a LECO Trumac CNS analyser (LECO Corporation, Michigan, USA) following a method that complies with the standard ISO 1489. Samples of powder (~0.1 g) were weighed onto a ceramic boat and dried in the oven overnight (104 °C). The boats were then loaded into the LECO analyser and combusted at a temperature of 1100 °C within the furnace. Protein values were determined from the measured nitrogen % by multiplying by 6.38 (Mariotti, Tomé, & Mirand, 2008). The protein compositions of the casein powder and native whey protein powder were 84.9% w/w and 76.3% w/w respectively.

The proportions of casein and whey protein were also assessed using the LECO Trumac CNS analyser, using reconstituted liquid samples after casein and/or whey proteins were precipitated by acid to determine the non-casein nitrogen and non-protein nitrogen. The protein in the casein powder was determined to be 97.0% w/w casein and 3.0% w/w whey protein. The protein in the native whey protein powder was determined to be 1.5% w/w casein and 98.5% w/w whey protein.

2.3. Standardised cheese milks

Batches of concentrated casein (10% w/w) and whey protein (10% w/w and 20% w/w) were prepared by reconstituting microfiltered casein and native whey protein concentrate powders (preparation and composition provided in Sections 2.2 and 2.3 respectively). Sodium azide (Chem-Supply, assay: 99%) was added to each solution at 0.02% w/w to inhibit microbial action and stored at 4 °C. Four milk systems were prepared by mixing the concentrated solutions to achieve WP:CN ratios of 0.03:1, 0.25:1 (the ratio in natural milk), 1:1 and 4:1 w/w while keeping the casein concentration constant at 0.0264 g/g to match the casein content of skim milk. Calcium chloride (Chem-Supply, assay: 93%) was added to each milk system to achieve a final concentration of 4.7 mM. The pH (measured from Mettler Toledo Education Line pH meter) of every milk system was maintained between 6.6 and 6.8. Replicate samples made from concentrated casein and whey protein batches were tested during each experiment. The number of replicates of each experiment are denoted in the caption of figures illustrated in the results and discussion section.

2.4. Determination of gelation kinetics by rheometry

Samples (20 mL) of each milk system were brought to 31 °C in a