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Effect of storage time and temperature of milk protein concentrate (MPC85) on the renneting properties of skim milk fortified with MPC85

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ABSTRACT

This study investigated the effect of storage temperature (20–50 °C) and time (0–60 days) on the renneting properties of milk protein concentrate with 85% protein (MPC85). Reconstituted skim milk was fortified with the MPC85 (2.5% w/w) and the renneting properties of the skim milk/MPC85 systems were investigated using rheology. It was found that the final complex modulus (final G^*) and the yield stress of the rennet-induced skim milk/MPC85 gels decreased exponentially with storage time of the MPC85 for storage temperatures greater than 20 °C, with a greater effect at the higher storage temperatures. Changes in the solubility of MPC85 with storage time were correlated with the rheological properties. The primary phase of renneting (cleavage of κ-casein) was not affected by the storage of the MPC85; hence the effect was related to the secondary stage of renneting (aggregation/coagulation of rennet-treated casein micelles). Using a temperature–time superposition method, a master curve was formed from the final G^*, yield stress and solubility results. This suggested that the same physical processes affected the solubility and rennet gelation properties of the milks. It is proposed that the MPC85 protein in rennet-treated skim milk/MPC85 solutions may transform from an interacting material, when solubility is high, to an inert or weakly interacting material, when solubility is low, and that this results in the reduced final G^* and yield stress of the rennet gels when MPC85 is stored at elevated temperatures for long periods.

1. Introduction

Storage of milk protein concentrate with 85% protein (MPC85) at temperatures greater than 20 °C reduced its solubility in water, with a more rapid decrease in solubility at higher temperatures (Anema, Pinder, Hunter, & Hemar, 2006). The solubility results were analysed using a temperature–time superposition method, and all the data could be collapsed onto a single master curve, indicating that the same physical process affected the solubility at all temperature/time combinations. Recent studies proposed that rehydration of MPC85 was due to the slow dissolution kinetics and that, given sufficient hydration time, MPC85 could be almost fully dispersed (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2009). This was suggested to occur even for stored MPC85 samples (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, in press); however, the powders used in the latter study were not stored under particularly severe conditions and would not have attained minimum solubility.

Electrophoresis results showed that it was only the casein micelles that became insoluble, and that the whey proteins and other small molecules were in the solution phase (Anema et al., 2006; Havea, 2006). It was proposed that the casein micelles may cross-link at the surface of the powder particles through either covalent or non-covalent (hydrophobic) interactions, forming a particle that allows small molecules including whey proteins to diffuse out but retains the casein micelles (Anema et al., 2006). This proposal has been supported by recent studies that show that the casein micelles are involved in the insolubility (Mimouni et al., in press) and that a network of casein micelles forms at the surface of the particles, producing a porous barrier that allows diffusion of small molecules but retains the casein micelles (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010).

The clotting of milk by rennet forms the basis for the manufacture of most cheese varieties. The rennet coagulation process may be divided into three separate but overlapping stages (Dalgleish, 1992). The primary phase of rennet coagulation is essentially a single-step enzymatic reaction involving the hydrolysis of κ-casein at the phenylalanine^{105} to methionine^{106} bond, which is the connection between the para-κ-casein and glycomacropeptide (GMP) moieties, by the enzymes in rennet. The GMP is released to the serum and the para-κ-casein remains with the casein micelles. The secondary phase of the renneting process involves aggregation of the casein micelles once their stabilities have been reduced via hydrolysis of sufficient levels of the κ-casein. It has

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been reported that, when approximately 85% of the total κ-casein has been converted to para-κ-casein and GMP, the micelles can begin to aggregate. This aggregation requires the presence of calcium ions and slightly elevated temperatures (~30–40 °C). The tertiary stage involves processes such as syneresis, non-specific proteolysis of the caseins in the rennet curd and structural rearrangements of the renneted gel network once it has been formed (Dalgleish, 1992).

When MPC85 is used as a source of protein for cheese manufacture (cheese milk extension), it is required to participate in the renneting reaction and the casein protein should be incorporated in the curd (Guinee, O'Kennedy, & Kelly, 2006; Harvey, 2006; Kuo & Harper, 2003; Ur-Rehman, Farkye, Considine, Schaffner, & Drake, 2003a; Ur-Rehman, Farkye, & Yim, 2003b). It is important that the inclusion of MPC85 in this reaction does not have any detrimental effects on the cheese forming process.

The renneting properties of MPC85, when dispersed in water (Ferrer, Hill, & Corredig, 2008; Martin, Williams, & Dunstan, 2010) and in skim milk (Ferrer et al., 2008), have been studied. MPC85 solutions produced weaker gels in water than in skim milk at equivalent protein concentrations. This was related to the ionic composition of the serums as addition of calcium (Martin et al., 2010) or dialysis of the MPC85 solutions against skim milk (Ferrer et al., 2008) could restore the renneting properties to those of the skim milk. For the MPC85 dispersed in skim milk, the gelation time was shorter and the stiffness (G') of the gel was greater than those of reconstituted skim milk at equivalent protein concentrations. These differences were again attributed to the ionic environment as dialysis against skim milk returned the gelling properties to those of the skim milk (Ferrer et al., 2008).

Although it is known that the solubility of MPC85 changes on storage, there have been few studies examining the effect of the storage conditions and the solubility of MPC85 on its functional properties. As MPC85 is often used as a protein source for cheese milk extension, the changes in its solubility may adversely affect its use in cheese manufacture. Therefore, the aim of this work was to study the effect of varying both the storage time and the storage temperature of MPC85 on the renneting properties of skim milk fortified with MPC85 (RSM/MPC). The MPC85 was stored at temperatures ranging from 20 to 50 °C for periods of up to 60 days. The solubility of the MPC85 in skim milk was monitored, as were the rheological properties of the skim milk/MPC85 solutions when rennet was added, so that relationships between the renneting properties and the solubility of the skim milk/MPC85 solutions could be established.

2. Materials and methods

2.1. Materials

The skim milk powder (SMP) was obtained from Fonterra Co-operative Group Ltd., New Zealand. The milk powder was low heat (WPNI >6.0), was less than 6 months old, and had ~35% protein on a dry basis. The MPC85 was the same sample as used in our previous study (Anema et al., 2006). This MPC85 was stored at −18 °C immediately after manufacture until it was required for further study. Double strength calf rennet (80 RU/ml) was obtained from the Rennet Company, Eltham, New Zealand. The rennet was diluted with water to 10% of its original concentration just prior to use.

2.2. Storage of MPC85

Subsamples of MPC85 (~200 g) were sealed in airtight aluminium foil bags and stored in incubators at the desired temperature (20–50 °C) for periods of up to 60 days. After the samples had been incubated at the required temperatures, they were returned to −18 °C storage until required.

2.3. Preparation of RSM/MPC samples for renneting experiments

RSM/MPC samples of ~5.6% total protein content were prepared by dispersing SMP (10% w/w), MPC85 (2.5% w/w) and azide (0.01% w/w) in purified water and stirring at 20 °C for 2 h. The solutions were then left at 4 °C for 24 h to allow for equilibration before testing commenced.

2.4. Determination of the solubility of RSM/MPC and RSM samples

RSM/MPC samples for solubility testing were prepared by reconstituting MPC85 (5% w/w) in RSM (10% w/w) to give samples with ~7.7% total protein. The RSM for solubility testing was prepared by reconstituting skim milk powder (10% w/w) to give a solution with ~3.5% protein. The RSM/MPC and RSM samples were held in a water bath set to 30 °C with continuous stirring for 30 min to ensure complete dispersion. A subsample of the RSM/MPC or RSM solution was centrifuged at 700 *g* for 10 min using an MSE Mistral 1000 centrifuge (Sanyo-Gallenkamp, Loughborough, England). A sample of the supernatant was placed in a preweighed moisture dish, weighed and then dried overnight at 105 °C, cooled in a desiccator and then reweighed. The percentage of soluble material (σ) in the RSM/MPC or RSM sample was calculated using Eq. (1).

$$\sigma = \frac{(\text{weight of dry material})}{(\text{weight of solution})} \times 100 \quad (1)$$

2.5. Polyacrylamide gel electrophoresis

The changes in the proteins on renneting the RSM/MPC solutions were determined using a sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) technique under reducing conditions, as has been described previously (Anema & Klostermeyer, 1997; Anema et al., 2006). After staining and destaining, the gels were scanned using a Personal Densitometer SI (Molecular Dynamics Inc., Molecular Dynamics World Headquarters, Sunnyvale, CA, USA) and integrated using the Imagequant software associated with the densitometer.

2.6. Rheological properties of rennet-treated RSM/MPC samples

The methodology for the rheological experiments was modified from that used to follow the acid gelation of milk, with rennet added to the milk in the current experiments instead of the glucono-δ-lactone that was used in the acid gelation experiments (Anema, 2008). The rheological properties of renneted RSM/MPC samples were determined using an AR2000 rheometer (TA Instruments Ltd., New Castle, DE, USA). A 4°, 4 cm diameter cone (with solvent trap) and plate geometry was used for all rheology experiments. The temperature of the rheometer was maintained at 30 °C for all experiments. A subsample of RSM/MPC was removed from the refrigerator and allowed to equilibrate at room temperature for 1 h. Diluted rennet (100 μl) was added to the RSM/MPC (50 ml), the sample was stirred, the required volume was transferred to the rheometer plate and then the cone was lowered into position and the measurement tests were started. The first experiments were small strain rheological tests and involved following the changes in the rheological properties with time after rennet addition. A strain of 0.5% and an oscillation frequency of 0.1 Hz were used. Measurements were taken every 60 s for 3 h. After the time sweep was complete, large strain properties were