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The effect of pH at renneting on the microstructure, composition and texture of Cheddar cheese

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ABSTRACT

The effect of milk pH at renneting on the microstructure, composition and texture of Cheddar cheese was investigated. Four different Cheddar cheeses were made by the addition of rennet to milk pre-acidified to pH 6.7, pH 6.5, pH 6.3 or pH 6.1. The gel renneted at pH 6.1 showed a dense protein network when observed using confocal laser scanning microscopy and cryo scanning electron microscopy. This structure became more compact after cooking, forming an irregular and coarse matrix with lower porosity than in other treatments. There was less fat lost to the whey during the draining of curd after cooking and higher fat lost to the whey during pressing in samples made from milk renneted at a lower pH. The texture of the Cheddar cheese made using milk renneted at pH 6.1 was altered, with lower chewiness, gumminess, cohesiveness and springiness when compared to cheese made using milk renneted at pH 6.7 or pH 6.5. The yield in dry matter of cheese renneted at pH 6.1 or pH 6.3 was 11–13% higher than for cheese renneted at pH 6.7. These results indicate that the pH of milk at renneting is a process variable that can be used to increased yield and alter the texture of Cheddar cheese.

1. Introduction

The pH of milk is an important factor during the gelation phase of cheese making, where milk coagulates to form a gel. Milk typically has a pH of 6.7 but this pH may vary due to the action of the starter culture, pre-ripening of the milk or addition of acid or acidulants. The effect of a low pH in milk is three-fold; it accelerates rennet activity (Zoon, van Vliet, & Walstra, 1989), reduces the electrostatic repulsion between casein micelles (CM) and alters the distribution of calcium between the micelle and serum phases.

The proteolytic activity of rennet varies with milk pH. The maximum proteolytic activity on casein is reported at a pH of 6.0 (Van Hooydonk, Hagedoorn, & Boerrigter, 1986), although this optimum varies between different rennet preparations. Cleavage of the macropeptide units of κ-casein by rennet reduces the surface potential and steric repulsion between the CM, permitting the closer approach of the micelles and facilitating aggregation. The faster action of rennet at low pH therefore results in faster coagulation (Hannon, Lopez, Madec, & Lortal, 2006). At lower pH, the rennet also tends to absorb onto the para-casein micelle and once adsorbed may remove κ-CN hairs over a certain area before diffusing away leaving bare patches on the surface, permitting aggregation of micelles at a lower degree of κ-casein conversion (Walstra, 1990).

The pH of milk also influences the aggregation of casein micelles during cheese making due to the effect of pH on Ca^{2+} activity (Mishra, Govindasamy-Lucey, & Lucey, 2005). The structure of CM still has not been unequivocally determined and there are different models of CM. However, the structure is commonly believed to be held together by colloidal calcium phosphate (CCP) (Fox & Brodkorb, 2008). A decrease in pH solubilises CCP, thereby increasing the Ca^{2+} activity in the solution phase. This increase in Ca^{2+} ions in solution is suggested to reduce the surface potential of the para casein micelle (Kim & Kinsella, 1989; Lucey & Fox, 1993). However, this variation in the surface potential is unlikely to have a significant effect on the aggregation rate. The increased aggregation rate is most likely due to the greater number of Ca^{2+} ions that may provide salt bridging between the micelles (Mishra et al., 2005). Extensive solubilisation of CCP at very low pH may cause disassociation of casein micelles. CM retain their integrity between a pH of 6.6 to 5.7 but the rate of CM solubilisation greatly increases at pH 5.6 and is complete at a pH of ~5.0–5.2 (Kim & Kinsella, 1989; Lucey & Fox, 1993).

The influence of milk pH at renneting (often referred to in other studies as the gelation or coagulation pH) on the properties of rennet-induced gel has been reported by various groups (Daviau, Famelart, Pierre, Goudedranche, & Maubois, 2000; Esteves, Lucey, Wang, & Pires, 2003; Hannon et al., 2006; Lucey, Johnson, & Horne, 2003; Mishra et al., 2005; Zoon et al., 1989). These reports offer valuable insights but are often limited to gels made using skim milk (Daviau et al., 2000; Esteves

Abbreviations: CLSM, confocal laser scanning microscopy; CCP, colloidal calcium phosphate; CN, casein; CM, casein micelles; ICP-OES, inductively coupled plasma optical emission spectroscopy; SEM, scanning electron microscopy; YDM, yield in dry matter.

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et al., 2003), non-fat milk (Mishra et al., 2005), ultrafiltered milk (Hannon et al., 2006) or reconstituted skim milk powder (Mishra et al., 2005). Furthermore, the microstructure of the gel, curd or cheese and the composition of the final cheese product has not been the focus in the majority of these studies. Given the importance of milk renneting pH in the early stages of cheese making, we sought to examine the effect of pH in full-fat milk at renneting on the microstructure of the gel, curd and cheese during Cheddar cheese production and the composition of the final cheese product.

A previous study by Hannon et al. (2006) using ultrafiltered milk gives some indication that the milk pH at renneting may alter the microstructure of the final cheese made from full fat milk. Cheese produced at a higher renneting pH of 6.6 had a dense protein structure with fat globules of different sizes distributed within the aqueous phase when analysed using Confocal Laser Scanning Microscope (CLSM). Conversely micrographs of cheese produced using milk renneted at pH 5.2 revealed a protein network that was less dense with evenly distributed fat globules. The effect of pH on the microstructure of a non-fat dry reconstituted skim milk gel made from different coagulants provides further insight (Esteves et al., 2003). The microstructure of the gels made using plant coagulants and chymosin had no major differences at a high renneting pH of 6.7. The microstructure of the gels formed at a pH of 6.0 or 6.3, however, showed a protein network that was denser; consequently the storage modulus (G') for these gels was also higher.

Other studies have focused mainly on altering the pH of the final product and not the milk pH at renneting. Decreasing the pH of shredded cheese and cheese blocks to pH 5.0 impaired protein-to-protein interactions due to increased calcium solubilisation but protein interactions were significantly improved at a pH of 4.7 due to reduced electrostatic repulsion (Pastorino, Hansen, & McMahon, 2003). Cream cheese samples adjusted to a pH of 4.0 or 4.9 with acetic acid displayed a very open protein network with limited contact occurring between the protein and the fat phases (Monteiro, Tavares, Kindstedt, & Gigante, 2009). This decreased pH reduced the electrostatic repulsion and increased protein-to-protein interactions, resulting in a protein network structure with reduced volume thereby leaving a more open cheese structure with reduced contact between fat globules and the protein network.

In the current study, advanced microscopy techniques including CSLM and Cryo-SEM are coupled with dynamic oscillatory low-strain rheology to study the microstructure and rheological properties of the gel, curd and cheese samples. The primary aim is to investigate the influence of milk pH at renneting on the microstructure of gel, curd and cheese collected during Cheddar cheese production. The influence of renneting pH on the composition and texture of the Cheddar cheese is also investigated.

2. Materials and methods

2.1. Manufacture of Cheddar cheese

Cheddar cheeses were made using four different milk preparations each with a different pH value at rennet addition: pH 6.1, pH 6.3, pH 6.5 or pH 6.7. This pH range was chosen so that Cheddar cheese can still be obtained without altering other cheese-making variables. The coagulation pH for a standard Cheddar cheese-making is pH 6.5. If the coagulation pH is too low the draining pH would subsequently need to be lowered and this in turn will require other processing variables to be altered e.g. the Cheddaring time, preventing useful comparisons between the different experimental treatments. Each cheese was made with 4 L of pasteurised cheese-milk (Murray Goulburn Co-Operative Co. Ltd, Cobram, Vic, Australia), where cheese-milk is defined as milk prepared for Cheddar cheese manufacture. The milk was standardized by blending raw milk with raw ultrafiltered milk (UF) retentate before pasteurization at 72 °C for 15 s. The cheese making was performed within 2 days of milk delivery. The cheese-milk was tempered to 33 °C before inoculation with 0.05 $g.kg^{-1}$ of freeze dried mixed strain direct vat set (DVS) mesophilic starter culture, *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* (Chr. Hansen, Bayswater, Vic, Australia). The milk was allowed to ripen until it reached a pH of 6.1, pH 6.3, pH 6.5 or pH 6.7 generating the four different milk preparations. The pH of the milk was measured using a pH meter (Orion 720 A, Orion Pacific Pty Ltd., Frankston, Vic, Australia) after calibrating with freshly prepared pH 4.0 and 7.0 standard buffers. Rennet (Hannilase; Chr. Hansen) was added at a concentration of 0.1 $mL.L^{-1}$ of milk which initiated the coagulation process. The coagulation process continued for a period equal to the cutting time determined in Section 2.2. Gel cutting to 1 cm^3 was performed with cheese-wire knives by inserting a horizontal and vertical wire knife of 1 cm spacing into the gel simultaneously and slicing the sample from left to right with one knife and right to left with another knife. After cutting, the cheese-making process continued according to methods described in a previous study (Ong, Dagastine, Kentish, & Gras, 2011). Three batches of cheese were made for each pH treatment in random order.

2.2. Determination of cutting time by rheological analysis

The viscoelastic properties of the gels formed from the milk with different pH at renneting were analysed before the cheese making experiment using an Advanced Rheometric Expansion System (ARES) rheometer (TA Instruments, New Castle, USA) equipped with a cup (34 mm diameter) and bob (32 mm diameter, 33 mm length) accessory. A sample of cheese-milk (15 mL) that had been inoculated with starter culture (0.05 g L^{-1}) and ripened to reach a pH of 6.1, pH 6.3, pH 6.5 or pH 6.7 was added to the cup immediately after the addition of rennet (0.1 mL L^{-1}). The temperature of the milk was maintained at 33 °C. A dynamic time sweep (7200 s) analysis at angular frequency of 5 $rad.s^{-1}$ (0.8 Hz) and 1% strain was used to analyse the changes in storage modulus (G') as the milk coagulated (Jaros, Seitler, & Rohm, 2008). The time taken for the cheese-milk to first reach a G' of 140 Pa was recorded and used as the cutting time in cheese making experiments.

2.3. Determination of gel setting time

The setting time for the cheese milk ripened to a pH of 6.1, pH 6.3, pH 6.5 or pH 6.7 was determined during the cheese making process. After the addition of rennet, 15 mL of the cheese-milk was transferred to 15 microfuge tubes (Eppendorf, North Ryde, NSW, Australia) so that each tube contained 1 mL of sample and the samples were incubated in a water bath at 33 °C. Single samples were then taken out from the water bath and inverted at 2 min intervals until the milk coagulated within the tube. The setting time of the milk was defined as the time needed for the milk to set within the tube so that the sample was self supporting and would not collapse when the tube was inverted.

2.4. CSLM and cryo SEM of gel, curd and cheese samples

Gel, curd and cheese samples were prepared for observation by CLSM as described previously (Ong, Dagastine, Kentish, & Gras, 2010). Briefly, protein was labelled with Fast Green FCF and fat with Nile Red. Dual channel images were obtained using 488 nm and 633 nm laser excitation to visualise the protein and fat, respectively. An aliquot of 12 μL of the ripened (pH 6.1, pH 6.3, pH 6.5 or pH 6.7), renneted and stained milk was transferred to a cavity slide (ProSciTech, Thuringowa, Queensland, Australia) and covered with a 0.17 mm thick glass coverslip (ProSciTech), so that the sample was flush with the coverslip. The slide was then incubated at 33 °C for a period equal to the cutting time (determined in Section 2.2) before observation using an inverted CLSM (Leica Microsystems, Heidelberg,