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Effects of gelation temperature on Mozzarella-type curd made from buffalo and cows' milk: 2. Curd yield, overall quality and casein fractions

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ABSTRACT

The overall quality of Mozzarella-type curds made from buffalo and cows' milks were measured at gelation temperatures of 28, 34 and 39 °C, and cutting times of 45, 60, 75 and 90 min after chymosin addition. The curd yield and moisture content decreased with increasing gelation temperature, while whey fat losses increased. The effect of higher gelation temperature (39 °C) was more pronounced in cows' milk than buffalo milk. This results in more fat losses and lower yields in both milk samples at a gelation temperature of 39 °C. The minimum losses of fat and protein in rennet whey occurred at a gelation temperature of 34 °C in both milk samples. The curd yield was higher in buffalo milk as compared to cows' milk. This is due to difference in total solids (fat and protein contents) of the two types of bovine milk. The different cutting times had a small effect on the yield and overall quality of curds made from both milk types. Curd moisture and loss tangent have a strong relationship with respect to effects of gelation temperature. Two different curd drainage methods (centrifugation and Buchner funnel filtration) were used to compare the final overall quality of Mozzarella-type curds made from both milk types. The α_{s1} and β casein fractions were found to be in different proportions in the two milk types. The total- and casein bound-calcium were higher in buffalo milk than cows' milk. The total protein, casein and fat were also found to be higher in buffalo milk than cows' milk.

1. Introduction

Buffalo and cows' milk are commonly used for the manufacture of dairy products on a commercial scale. Buffalo milk has become popular in the UK and Europe over the last decade. It contains approximately 18% total solids and 40–60% more protein, fat and calcium than cows' milk. Buffalo milk is popular due to its sweet taste and white colour, and it is also suitable for cheese and yogurt making. It is particularly useful for Mozzarella manufacture and there is no need to concentrate it prior to processing (Sindhu & Arora, 2011).

Mozzarella-type curd is made from buffalo and cows' milk at a higher gelation temperature (approximately 39 °C) than most other cheese types. The gelation temperature is a very important factor and has a great influence on the quality of curd made from both milk types. Chymosin activity is highly temperature sensitive. It is possible that there is more proteolysis at higher gelation temperatures such as 39 °C. Additional proteolysis could assist in the reduction in the interconnectivity of the curd network and encourage more rearrangements to take place (Castillo, Lucey, Wang, & Payne, 2006a). Due to its higher solids contents, buffalo milk has better curd formation and curd moisture retention at higher gelation temperatures when compared with cows' milk.

Thermophilic bacterial cultures are usually used to reduce cheese milk pH to 6.5 before the addition of chymosin in commercial Mozzarella manufacture. Chemical acidification of milk is also a very common practice. This is done by the use of organic acids, including lactic and citric acid, in the manufacture of Mozzarella for pizza topping. However, scientific literature regarding the effect of different gelation temperatures on the overall quality and yield of Mozzarella-type curd is very limited.

The curd moisture is temperature dependent, but it is also affected by fat and protein contents. Ultrafiltered milk undergoes less curd shrinkage, which results in better moisture retention in the curd. Higher gelation temperatures lead to increased whey syneresis (Lucey, Munro, & Singh, 1998). In rennet curds, the rearrangements of particle strands can occur, which leads to greater syneresis (Van Vliet, Lucey, Grolle, & Walstra, 1997). The loss tangent is a very good parameter to assess syneresis in rennet induced curds. It has been observed in previous studies that the value of loss tangent in cows' milk is higher than buffalo milk (Hussain, Bell, & Grandison, 2011). This suggests that cows' milk is more liable to whey syneresis than buffalo milk under similar processing conditions such as milk pH and gelation temperature.

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Mozzarella curd is usually cut into 10–12 mm pieces 45 min after chymosin addition, then healed and pressed. Higher gelation temperatures give rise to more whey syneresis as compared to low gelation temperatures and affect curd yield, resulting in lower yields. The cutting time also greatly affects moisture, curd yield, quality and whey fat losses. Delayed cutting produces an overly firm curd, in which the network is unable to rearrange, which thus increases curd moisture content (Castillo, Lucey, Wang, & Payne, 2006b).

The objectives of this study were to determine the effects of different gelation temperatures and cutting times on curd yield, curd moisture and whey fat losses of rennet induced curds made from buffalo and cows' milk. The curds were made according to Mozzarella cheese processing conditions (Hussain et al., 2011).

2. Materials and methods

2.1. Materials

Pasteurised whole buffalo and cows' milk were supplied by Waitrose (Bracknell, UK). Fermentation produced chymosin (Chymax) was supplied by CHR. Hansen (Hungerford, UK). Lactic acid (88% w/w) was supplied by BDH (Poole, UK) and was of analytical grade. Purified α_s- (70%), β- (90%), κ- (70%) casein, sodium azide (99.5%), sodium citrate (99.9 %), tris-(hydroxymethyl) amino methane (99.8%), 2–mercaptoethanol (98%), and urea were purchased from Sigma–Aldrich (Dorset, UK). HPLC grade trifluoroacetic acid (TFA), water and acetonitrile were purchased from Fisher Scientific (Leicestershire, UK).

2.2. Chemical composition of bovine milk

Pasteurised whole buffalo and cows' milks were analysed for fat, protein, milk solids non-fat (MSNF) and lactose contents using a Dairy Lab 2 milk analyzer (Foss UK Ltd, York, UK). The pH of milk was recorded using a portable pH meter (Oakton Instruments, Vernon Hills, USA). Ionic calcium was determined using a Ciba Corning 634 Ca^{2+}/pH analyzer (Bayer Plc. Diagnostics Division, Newbury, UK) (Lin, Lewis, & Grandison, 2006). The casein was determined by the micro-Kjeldahl method (Ahmad et al., 2008).

Buffalo and cows' milk were both coagulated by the addition of chymosin and then centrifuged at 5500*g* for 15 min to produce rennet whey. Soluble calcium in milk samples was defined as calcium content in rennet whey multiplied by a correction factor (0.89 and 0.94 for buffalo and cows' milk, respectively (Davies & White, 1960; Hussain et al., 2011) to account for the volume of casein precipitate. Casein bound calcium was determined as the total calcium in the milk minus the soluble calcium. For the measurement of total calcium and soluble calcium, the milk and serum samples were deproteinised by mixing with an equal volume of 24% (w/v) trichloroaceteic acid and kept for 30 min at room temperature, followed by centrifugation at 15,000*g* for 30 min, and subsequently filtered. The calcium content of deproteinized filtrate was determined by atomic absorption spectrometry (Analytik Jena, Germany) (Hussain et al., 2011).

2.3. Sample preparation and rennet curd formation

Both buffalo and cows' milk samples were warmed at 25 °C in a temperature controlled water bath with gentle agitation for 30 min. The pH was adjusted to 6.5 at 25 °C using 1:4 diluted lactic acid. Samples were incubated for a further 15 min and the pH checked and readjusted if necessary.

After the pH adjustment, milk samples were placed at the gelation temperatures of 28, 34 and 39 °C in a temperature controlled water bath for 30 min. Chymosin (1:10 in distilled water) was then added (0.1 mL^{-1}). The milk samples were then thoroughly mixed and incubated for 90 min at different gelation temperatures. After 45, 60, 75 and 90 min of chymosin addition, curds were cut into 10–12 mm size pieces using a specially designed laboratory scale Mozzarella cheese cutter. Curd samples were healed at a similar gelation temperature for 10 min and stored at 4 °C for further analysis.

2.4. Physicochemical parameters of curd and rennet whey

Fat content of rennet whey was measured using a Dairy Lab 2 milk analyzer (Foss UK Limited, York, UK). Approximately 5 g of whey and curd were accurately weighed into dried and tared china dishes for the measurement of total solids (TS) of curd and whey at different gelation temperatures from both milk. The samples were placed in a hot air oven at 102 °C to constant weight (~18 h). The curd yield on wet (CY_{wb}) and dry basis (CY_{db}), whey fat losses (WFL, g) and curd fat retention (CFR) were calculated using a method adapted from Fagan, Castillo, Payne, O'Donnell, and O'Callaghan (2007).

2.5. Curd drainage methods

Rennet whey was drained from healed curd samples using two different methods. In the first method, curd samples (100 g) were centrifuged at 5500*g* for 15 min at 20 °C. In the second method, curd samples (100 g) were filtered in a Buchner funnel overnight at 4 °C using nylon cloth. The resulting rennet curd and whey were stored at 4 °C for further analysis.

2.6. Quantification of casein fraction in milk samples

Individual protein solutions were prepared separately by dissolving 125 mg of purified α_{s1}-casein, 100 mg of β-casein or 25 mg of κ-casein in 5 ml of a solution containing 8 M urea, 165 mM Tris, 44 mM sodium nitrate and 0.3% β-mercaptoethanol. A mixed standard solution was prepared by mixing 1 ml of each individual protein solution and adding 2 ml of urea solution, and then adding urea solution to a final volume of 5 mL. Further dilution was carried out to produce a final casein concentration of 2 mg mL^{-1}. Twenty microlitre was then directly injected into the HPLC system.

The casein fractions were quantified using RP-HPLC system adapted from Bonizzi, Buffoni, and Feligini (2009) and Feligini, Bonizzi, Buffoni, Cosenza, and Ramunno (2009). The milk samples were prepared for analysis by diluting 0.5 mL skimmed milk with 1.5 mL of urea solution. The diluted samples were filtered with a 0.45 µm PVDF filter and analysed in a Dionex system consisting of P680 HPLC pump, ASI – 100 automated sample injector, thermostatted column compartment TCC100, PDA – 100 photodiode Array Detector with a Jupiter C4 column (250 mm × 4.6 mm, 300 Å – sized pores, 5 µm sized particles; Phenomenex, Macclesfield, Cheshire, UK). The elution was performed at 25 °C with linear gradient B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) from 30–50% in 45 min at a flow rate of 1.0 mL min^{-1}. Peaks were detected at the wavelength of 220 nm.

2.7. Statistical analysis

The results were expressed throughout as mean ± standard deviation of data obtained from three curd samples. ANOVA was carried out using SPSS 17 software to investigate whether there was a significant effect of different gelation temperatures and cutting times on curd yield, curd moisture, whey fat losses and casein fractions of the curds made from three different batches of both