

Rheology and Microstructure of Mozzarella Type Curd Made from Buffalo and Cows' Milk

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ABSTRACT

Mozzarella curds were made from buffalo and cows' milk at the gelation temperature of 39°C using pH 6.5. The curd formation and development process was monitored using small amplitude oscillation rheometry (SOAR) and large deformation properties of both types of milk.

The dynamic moduli (G' , G'') values were found to be higher in the buffalo curd than in the cows' curd after 90 minutes coagulation. The loss tangent however was found to be lower in buffalo curd than that observed for the cows' curd (0.42 and 0.48 respectively). The frequency profile of each type of curd was recorded 90 minutes after the enzyme addition (0.1-10 Hz); all of samples were found to be "weak" viscoelastic frequency dependant gels. The yield stress was also measured 95 minutes after the enzyme addition, and a higher value was observed in buffalo curd when compared to cows' curd. The cryo SEM and CLSM micrographs showed that curd structure appeared to be more "dense" and less porous in buffalo curd than cows' curd. The ionic and soluble calcium contents were found to be similar in all milk samples studied. The total and casein bound calcium were higher in buffalo milk than in cows' milk. Casein micelles and fat globule size was found to be different in the both types of milk. Buffalo milk was found to contain a higher concentration of α_s and β casein than cows' milk. Gel strength and fracture forces was found to be higher in the resultant buffalo curd than for curds made from cows'

milk. Differences in the rheology and microstructure observed between the buffalo and the cows' curd appear to result from the differences in casein composition and overall micelle structure, rather than casein concentration alone.

Key words: mozzarella curd, rheology, microstructure, buffalo, cows'

INTRODUCTION

Mozzarella cheese is usually made from buffalo and cows' milk in commercial environment. Buffalo milk contains 40-60 % more fat, protein and calcium than cows' milk. These differences in chemical composition would suggest a different rheology and microstructure of mozzarella curd made from both types of milk suggested Hussain et al.¹. Ahmad et al.² stated that the molecular and chemical changes were qualitatively similar in buffalo and cows' milk but quantitatively different.

Buffalo and cows' milk are not different chemical composition alone. They have some other differences like casein micelles & fat globule size, casein fractions and casein bound calcium etc. These differences suggest that processing conditions for cheese making (gelation temperature, chymosin concentration, pH and cutting time) which are well established for cows' milk, cannot be directly extrapolated to buffalo milk. Some modifications and changes are compulsory to optimize the processing conditions for cheese making from buffalo milk recommended by Hussain et al.¹ Buffalo milk is better suited for

mozzarella cheese making when compared to cows' milk due to their unique characteristics.

MATERIALS AND METHODS

Materials

Pasteurized whole buffalo and cows' milk were supplied by Waitrose (Bracknell, UK). Fermentation produced chymosin (Chy-max) was provided by CHR. Hansen (Hungerford, UK). Lactic acid (88% w/w) was supplied by BDH (Poole, UK) and was of analytical grade.

Sample preparation, analysis and rennet curd formation

Pasteurized whole buffalo and cows' milks were analyzed for fat, protein, milk solids non-fat (MSNF) and lactose content using a Dairy Lab 2 milk analyzer (Foss UK limited, York, UK). The pH of milk was recorded using a portable pH meter (Oakton Instruments, USA). Ionic calcium was determined using a Ciba Corning 634 Ca⁺⁺/pH analyzer (Bayer Plc. Diagnostics Division, Newbury, UK) followed method of Lin et al.¹⁰ The casein was determined by the Kjeldahl method using a correction factor of 6.38 for milk protein according to method described by Ahmad et al.²

Buffalo and cows' milk were both coagulated by the addition of chymosin (0.1mL L⁻¹) and then centrifuged, after the 90 minutes, at 5500g for 15 minutes to produce the rennet whey. Soluble calcium in milk samples was defined as calcium content in rennet whey multiplied by a correction factor (0.89 & 0.94 for buffalo and cows' milk respectively. This was to allow for the volume of casein precipitated. Casein bound calcium was taken 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) trichloroacetic acid and kept for 30 minutes

at room temperature, followed by centrifugation at 15000g for 30 minutes, and then subsequently filtered. The calcium content of deproteinized filtrate was determined by atomic absorption spectrometry (Analytik Jena, Germany).

Rheological Properties

The rheological properties of rennet induced curds made from pasteurized buffalo and cows' milks were studied both by dynamic small amplitude oscillatory rheology (SAOR) and large deformation measurements using a high resolution CVOR Bohlin controlled stress rheometer (Malvern instruments ltd, Worcestershire, UK). The storage modulus (G'), loss modulus (G''), loss tangent ($\tan \delta$) and shear stress (σ) were measured. The concentric cylinder measuring system consisted of a moving "bob" (inner cylinder) located in a fixed "cup" (outer cylinder) with the sample contained in the annular gap between them. In order to follow the curd formation, a frequency of 0.1 Hz and 1% strain was applied to the samples, and rheological measurements were taken as a function of time.

A milk sample (13 mL) was warmed to gelation temperature 39°C for 15 minutes, 13.0 μ L of 1:10 diluted enzyme (chymosin) was added, mixed thoroughly for 30 seconds and transferred immediately to the rheometer cylinder. A thin layer of low viscosity oil was spread on the exposed surface to prevent any water loss.

Samples were measured every 10 minutes after chymosin addition for 90 minutes (0.1 Hz, 1% strain). The frequency range from 0.1-10.0 Hz was used to study the rheological properties of the buffalo and cows' curd systems, 90 minutes after chymosin addition. The large deformation properties of these rennet induced curds were determined 95 minutes after chymosin addition at a constant shear rate of 0.01s⁻¹. Yield stress was defined as the point when the shear stress attained the maximum value.

The rennet coagulation time was determined after the addition of chymosin in milk samples. Time was recorded when storage modulus attained value of 1Pa.

Cryo scanning electron microscopy (Cryo SEM)

The cryo sample preparation system PolarPrep 2000 (Quorum Technologies, UK) consists of three main parts: a slushing chamber, a vacuum transfer device (VTD) and a sample preparation chamber. The curds samples were cut into small piece (5x5x2 mm) by razor blade according to size of aluminum cryo stub and mounting on stub. The stub was attached to the VTD that fits directly onto the slushing chamber. The method was used with some modification described by Ong et al.³

Confocal laser scanning microscopy (CSLM)

To observe the fat globule and protein in curd samples, a fat specific stain Nile red (Sigma-Aldrich, Dorset, UK) and a protein specific stain fast green FCF (Sigma-Aldrich) were used. A 100 mL batch of all milk samples were pre warmed at 25°C in water bath for 30 minutes. Then pH was reduced to 6.5 by using the diluted lactic acid. All milk samples were pre warmed at different gelation temperatures before the addition of chymosin. A 100 µL of chymosin (1:10) was added in batch and mixed well. The renneted milk (10 mL) was mixed with Nile red (0.005 gL⁻¹) and fast green FCF (0.012 gL⁻¹). Subsequently a 100 µL aliquot of the stained samples were transferred to a cavity dish (0.7 mm in depth), covered with a glass coverslip (0.17 mm thick) that was also flushed with sample and secured with transparent color sealant. The dished were then incubated at different gelation temperatures for 90 minutes. The samples were then inverted for CSLM analysis immediately after staining. This method was used with little modification described by Ong et al.⁴

Measurement of the casein micelle and fat globule size

These were determined by laser light scattering using a Mastersizer 2000 (Malvern instruments Ltd, Worcestershire, UK) as described by Ahmad et al.². The casein micelles were determined by the method of Ahmad et al.² using a Zetasizer (Malvern instruments Ltd, Worcestershire, UK). A 125 µL skimmed milk sample was dispersed in 25 mL of Milli Q-water at 20°C. The average diameters of the casein micelles were calculated by the Zetasizer software.

Statistical analysis

The results were expressed throughout this paper as the mean ± the standard deviation of data obtained from three curd samples. Each batch was analyzed in triplicate. ANOVA was carried out using SPSS 17 software to test for significance. The differences in the means were determined using the Duncan test. Significance was established at a level of P<0.05.

RESULTS AND DISCUSSION

Chemical composition of milk samples

The total protein, casein proteins, fat, lactose and MSNF were found to be higher in buffalo milk than cows' milk (Table 1) as expected (Hussain et al.¹). The values of the ionic and soluble calcium were found to be at a maximum in the cows' milk sample when compared to buffalo milk. However the actual concentrations (mg100mL⁻¹) of all of the calcium fractions were higher in buffalo milk when compared to cows' milk (Table 1). The overall levels of the calcium associated with casein micelles were found to be 84 and 76% of the total in the buffalo and cows' milks respectively.

Buffalo milk contained larger casein micelles and fat globules when compared to cows' milk but lower values of overall casein micelle hydration (Table 1). The

Table 1. Physico-Chemical Properties of Pasteurized Whole Buffalo and Cows' Milk.

Physico-chemical properties	Buffalo milk	Cows' milk
Protein %	4.76±0.17 ^a	3.29±0.11 ^b
Casein %	3.82±0.20 ^a	2.64±0.17 ^b
Fat %	8.27±0.38 ^a	4.08±0.06 ^b
Lactose %	4.80±0.12 ^b	4.50±0.08 ^b
MSNF* %	10.12±0.22 ^a	08.45±0.16 ^b
pH (25°C)	6.80±0.02 ^a	6.70±0.02 ^b
Total solids %	18.41±0.60 ^a	12.64±0.20 ^b
Ash %	0.72±0.06 ^b	0.68±0.09 ^b
Total calcium (mg 100mL ⁻¹)	205±08 ^a	115±08 ^b
Ionic calcium (mg 100mL ⁻¹)	8.2±0.3 ^a	7.3±0.3 ^b
Ionic calcium (% of total calcium)	4±0.3 ^b	6±0.3 ^a
Soluble calcium (mg 100mL ⁻¹)	32±2 ^a	28±2 ^b
Soluble calcium (% of total calcium)	16±1 ^b	24±2 ^a
Casein bound calcium (mg 100mL ⁻¹)	173±7 ^a	87±4 ^b
Casein bound calcium (% of total calcium)	84±3 ^a	76±3 ^b
Casein micelles hydration (gH ₂ Og ⁻¹)	1.60±0.10 ^b	2.03±0.12 ^a
Casein micelles size (nm)	190±3 ^a	180±3 ^b
Fat globule size (μm)	5.05±0.20 ^a	3.55±0.10 ^b

All values are Mean±s.d. Data represents the average of 3 batches, each analyzed in

triplicate (n=9). ^{a-b} Means with different superscripts letters within same row are significantly different (P < 0.05).

*milk solids non-fat

buffalo milk contained bigger casein micelles than those in the cows' milk.

Dynamic rheology of mozzarella type curd as a function of time

The curd formation was observed as a function of time (90 min., Figure 1, Table 2). The dynamic moduli (G', G'') were plotted as a function of time after the chymosin addition and in all cases, the dynamic moduli increased with increasing time. This was accompanied by a concurrent decrease in the value of the loss tangent. This is consistent with a change from essentially a "liquid state" to a more viscoelastic "gel system". The dynamic moduli (G', G'') values of buffalo and cows' curd systems were found to be significantly different from each other (P<0.05). This suggests that the "networks" formed although of a similar "type" differ from each other in terms of their overall levels of "interaction" (either with an increased number of interactions and/or an increase in their actual strength).

In general, the inclusion of the casein micelles within the gel network proceeds at

a faster rate in the milk samples with the higher protein content. This higher protein concentration caused the gels to be more rapidly formed and "stronger" than those created from the low protein milks (Figure 1 and Table 2). This effect may be due to casein micelle size differences and/or the different proportions of the various casein fractions present in the different milks. It may also be due to differences in the fusion or "packing" of the differing sizes of casein micelle within the curd structure.

Table 2. Rheological Properties of Mozzarella Type Curds Made from Pasteurized Whole Buffalo and Cows' Milk.

Rheological properties	Buffalo milk	Cows' milk
Storage modulus (Pa)	160±10 ^a	37±04 ^b
Loss modulus (Pa)	65±5 ^a	18±1 ^b
Loss tangent (Tan δ)	0.42±0.01 ^b	0.48±0.01 ^a
Yield stress (Pa)	120±8 ^a	44±5 ^b
Yield strain (-)	0.53	0.63
Rennet coagulation time (min)	25±1 ^b	38±2 ^a

All values are Mean±s.d. Data represents the average of 3 batches, each analyzed in triplicate (n=9). ^{a-b} Means with different superscripts letters within same row are significantly different (P < 0.05). All values were determined after 90 minutes of chymosin addition except yield stress which was determined after 95 minutes.

The Tan δ profiles as a function of time were quite different in both types of milk. The maximum values of loss tangent attained were 0.42 and 0.48 in buffalo and cows' curd respectively (90 minutes after chymosin addition at a gelation temperature of 39°C, Table 2). The elevated values of loss tangent in cows' milk curd as compared to those in the buffalo milk curd, would suggest that cows' milk curd is more liable to shrinkage and whey syneresis and more viscous behaviour when compared to curd made from buffalo milk, probably due to the lower protein and milk solids content in the cows' milk.

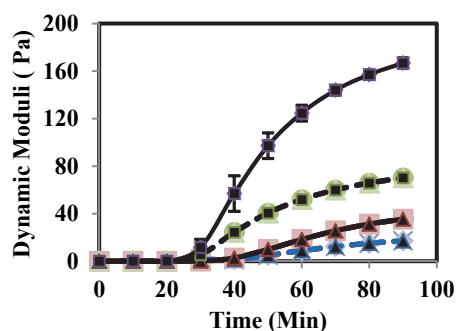


Figure 1. Storage modulus (—) and loss modulus (- - -) as a function of time for mozzarella type curds made from pasteurized whole buffalo milk (■) and pasteurized whole cows' milk (▲). Results are means of 9 values with error bars for standard deviation.

Dynamic rheology of mozzarella type curd as a function of frequency

When the frequency dependence of the mozzarella type curds (0.1-10.0 Hz, strain 1%) were examined 90 minutes after chymosin addition for both milks, the dynamic moduli (G' , G'') of curds were found to be frequency dependent over all of the measured range (Figure 2). These findings were agreement with findings of Lucey.⁵ All frequency sweeps were characteristic of weak viscoelastic materials. All of the samples examined showed that the dynamic moduli increased with

increasing frequency (0.1-10.0 Hz). Such behavior is characteristic of an "amorphous" weakly cross linked system and similar to finding of Ferry⁶.

In common with all viscoelastic systems, any interactions or "bonds" formed in the curd/protein matrix have less time to relax if the time of applied stress is shorter. Therefore, the dynamic moduli appeared to be higher at the higher frequencies in both curd samples. Lower values of the dynamic moduli were found at the same frequency in cows' curd when compared to those in the curd made from buffalo milk. This is probably due to different "relaxation spectra" of bonds/interactions in the different curds. This suggests the matrixes formed by the two types of milks are not identical due to differences in overall gross chemical composition of both milks.

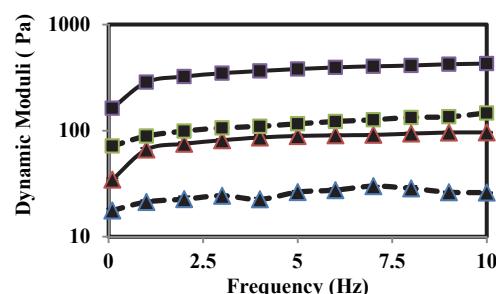


Figure 2. Storage modulus (—) and loss modulus (- - -) as a function of frequency for mozzarella type curds made from pasteurized whole buffalo milk (■) and pasteurized whole cows' milk (▲). Results are means of 9 values with error bars for standard deviation.

The large deformation properties of mozzarella type curd

The values and graphs of yield stress (σ) of mozzarella curds are shown in table 2 and figure 3.

The maximum value of yield stress was obtained in curds made from the buffalo milk rather than in those made from the cows' milk (Figure 3 and Table 2). This may be as a result of an increased fusion and

packing of the micelles and their different overall size range in the buffalo milk. This resulted in an increase in the strength of final buffalo curd. The casein micelles in the protein strands appear to fuse to a greater extent in these samples (Figure 3) resulting in the higher yield stress required to break the strands. These findings were similar to Mishra et al.⁷

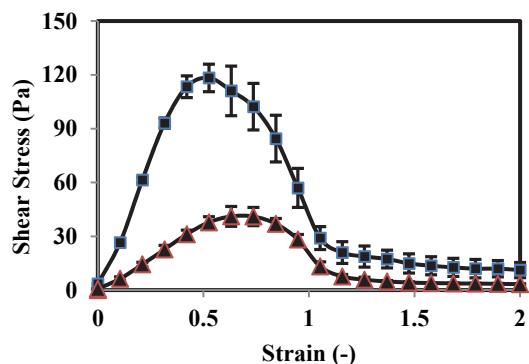


Figure 3. Shear stress as a function of applied deformation (strain) at constant shear rate (0.01s^{-1}) for mozzarella type curds made from pasteurized whole buffalo milk (■) and pasteurized whole cows' milk (▲). Results are means of 9 values with error bars for standard deviation.

Table 3. Porosity of Mozzarella Type Curd Made from Pasteurized Whole Buffalo and Cows' Milk.

Milk type	Curd porosity		Average pore area (μm)	
	CLSM**	Cryo-SEM*	CLSM*	Cryo-SEM*
Buffalo	$0.36 \pm 0.01^{\text{b}}$	$0.15 \pm 0.04^{\text{b}}$	$2.10 \pm 0.06^{\text{b}}$	$0.28 \pm 0.03^{\text{b}}$
Cows'	$0.45 \pm 0.04^{\text{a}}$	$0.36 \pm 0.01^{\text{a}}$	$4.00 \pm 0.29^{\text{a}}$	$2.16 \pm 0.40^{\text{a}}$

All values are Mean \pm s.d. Data represents the average of triplicate (n=3). ^{a-b}

Means with different superscripts letters within same column are significantly different ($P < 0.05$).

* Cryo- scanning election microscopy

** Confocal laser scanning microscopy

(porosity/pore density) again suggesting subtle differences, not simply based on the “gross” composition of the milk.

The CLSM and cryo SEM micrographs of mozzarella type rennet curd made from whole buffalo and cows' milk (Figure 4 and 5) showed that majority of milk fat globules were located within the serum phase of protein matrix. Previous studies (Hassan and

Microstructure of mozzarella type curd made from different milks

The effect of manufacture using different types of bovine milk on the microstructure of mozzarella type curd was determined using both CLSM and Cryo SEM (Figure 4 & 5). The mozzarella curd made from buffalo milk was found to be less porous when compared to the curd made from the cows' milk (Table 3). This is likely to occur as a result of the different protein concentrations in the original bovine milks. A higher protein concentration lowers the volume fraction of the aqueous phase, which consequently lowers mean distance between casein micelles and so increases the extent of their subsequent aggregation. The buffalo milk having the higher protein content (Table 1) also has the lower gel porosity, with more dense protein “fibers”, compared to those observed in the cows' milk curd.

The “stronger” gel structures observed in the buffalo curd samples gave rise to “thicker” protein strands with an overall less porous structure. This resulted in the much higher yield values observed for these samples (Figure 3). The overall characteristics were not identical

Award⁸) using cryo SEM showed that unhomogenized fat globules are surrounded by serum “cavities” in samples of fresh cheddar cheese. This suggested that the fat globules were acting as an “inert” filler material. However, other studies (Everett and Auty⁹) suggest that the native fat globules are held within the casein matrix by some sort of interaction with a “globule membrane”. In either case the fat is more

likely to contribute to the “liquid” character of the system (G'') rather than the “elastic network” (G').

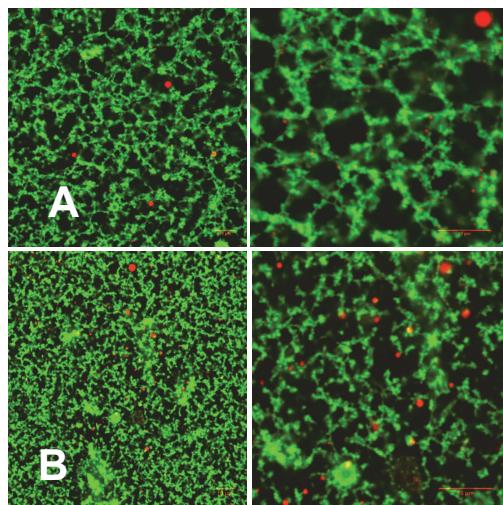


Figure 4. CLSM micrographs of mozzarella type curd made from cows’ milk (A) and buffalo milk (B). Images were captured at 2X (left) and 5X (right) magnification with a 63X lens. The scale bars are 10 μm in length.

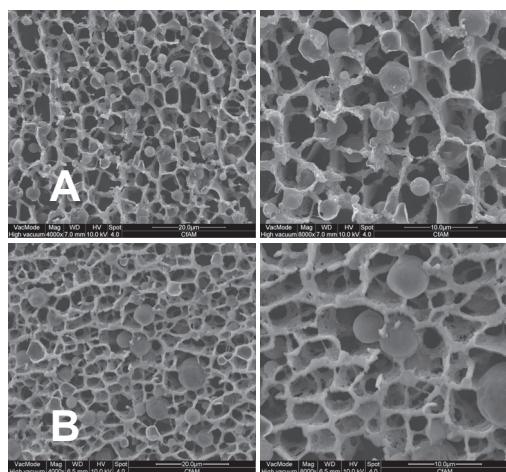


Figure 5. Cryo – SEM micrographs of mozzarella type curd made from cows’ milk (A) and buffalo milk (B). Images were captured at 4000X (left) and 8000X (right) magnification. The scale bars are 20 μm (left) and 10 μm (right) in length.

The sizes of fat globules found in the buffalo curd were bigger than those found in the cows’ milk curd (Figures 4 and 5). Some of fat globules appear to be attached to the actual protein matrix but can also be observed to “protrude” into the serum phase. However, it is likely that some of the fat globules may have become detached during the freeze fracture process, with some of fat globules being “lost”, left behind in the serum. It has been suggested that chemical bonds may exist between the native fat globule/ membrane and the protein matrix.

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