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EFFECT OF DRAW pH ON THE DEVELOPMENT OF CURD STRUCTURE DURING THE MANUFACTURE OF MOZZARELLA CHEESE

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Abstract

The impact of varying the pH of whey at whey drainage (5.9, 6.15 or 6.4) on the development of curd structure during the manufacture of Mozzarella cheese was investigated using scanning electron microscopy. Dramatic changes in curd structure were apparent with stage of manufacture, in particular the stretching step which aligned the protein fibers.

Of additional interest is the effect of draw pH on the structure of curd at whey drainage. When whey was drained at pH 6.4, an open poorly fused network of paracasein particles was observed in the cheese curd. In contrast, lowering the draw pH (6.15 and 5.9) increased fusion of paracasein particles producing a more continuous three dimensional network. The degree of paracasein fusion in curd at whey drainage may be related to overall calcium levels and particularly to the calcium/total protein ratio.

Key Words: Mozzarella, cheese, curd, scanning electron microscopy, calcium, casein, whey, micelle, structure, fibers.

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Introduction

Since microstructure is a major determinant of cheese texture and consistency (Stanley and Emmons, 1977; Emmons *et al.*, 1980; Green and Manning, 1982; Green *et al.*, 1986), structural investigations of a wide variety of cheese types using transmission (TEM) and scanning electron microscopy (SEM) are numerous (Holcomb, 1991). Different cheese varieties represent variations (either of bio-ingredients or make conditions) of a common manufacturing scheme and by varying processing conditions, composition and structure within a particular cheese type can be manipulated. Thus, many authors (Kimber *et al.*, 1974; Kalab, 1977; Kalab and Emmons, 1978; Eino *et al.*, 1979; Green *et al.*, 1981; Findlay *et al.*, 1984; Omar, 1988; Rousseau, 1988; Rousseau and LeGallo, 1990) have investigated the impact of bio-ingredients, manufacturing stage and ripening stage on the development of curd and cheese structure.

The manufacture of cheeses using starter and rennet includes the drainage of whey from curd once the appropriate pH (draw pH) has been reached. The latter varies with the type of cheese being produced. Because of a pH and temperature dependent dynamic equilibrium between micellar and serum casein and ions (Ca^{2+} and phosphate in particular), the extent of cheese milk acidification is pivotal in determining mineral (e.g., Ca, P) and protein (e.g., β-casein) retention in rennet curd as well as retention of sugar (lactose) and proteases (e.g., coagulant, plasmin) (Lawrence *et al.*, 1984). The relative proportions of these constituents together with moisture and fat determine basic cheese structure. Although some structural alterations can occur with subsequent processing (e.g., cheddaring and salting), these steps are not considered to have a major impact on the spatial arrangement of the casein components (Lawrence *et al.*, 1984).

Characteristic to Mozzarella cheese manufacture is a hot water stretching step which results in orientation of protein fibers (Kalab, 1977; Masi and Addeo, 1986). Such orientation is probably important in relation to the stretching and melting properties exhibited by Mozzarella when baked on pizza. Recently, a new Mozzarella

Table 1. Conditions during Mozzarella manufacture (n = 1).

Manufacture stage	Treatment	Time[1] (min)	Temperature (°C)	pH Curd	pH Whey
Start cook	6.40	108	34.7	-	6.46
	6.15	107	34.5	-	6.42
	5.90	109	34.6	-	6.42
Start drain	6.40	128	41.3	6.23	6.39
	6.15	147	41.0	5.95	6.13
	5.90	169	40.8	5.68	5.94
Start mill	6.40	193	40.8	5.22	5.33
	6.15	177	40.1	5.22	5.30
	5.90	189	39.3	5.24	5.27
Start stretch	6.40	221	57.0	-	-
	6.15	207	57.0	-	-
	5.90	217	57.0	-	-
Cheese[2]	6.40	-	-	5.17	-
	6.15	-	-	5.13	-
	5.90	-	-	5.08	-

[1]represents time elapsed after addition of culture.
[2]three days old.

cheese making scheme was developed which differs from the traditional format in that the milled curds are dry salted and stretched in hot 10% NaCl solution, thus eliminating brining after stretching (Barbano *et al.*, 1991). This scheme has been used to systematically investigate the impact of manufacturing variables on Mozzarella cheese composition, rheological behavior, melting properties, etc. The latter two properties are expected to be influenced by cheese microstructure. However, while systematic investigations of cheese structure have been performed for Cheddar (Kimber *et al.*, 1974; Green *et al.*, 1981), Saint Paulin (Rousseau, 1988), Emmental (Rousseau and LeGallo, 1990) and possibly a few other cheese varieties, a systematic investigation of curd structure development during Mozzarella cheese manufacture is lacking. Thus, the changes in structure of Mozzarella curd during manufacture and the effect of draw pH thereon were investigated.

Materials and Methods

Cheese manufacture

Three vats of low moisture part skim (LMPS) Mozzarella cheese (approximately 40% fat in dry matter) were manufactured at Cornell University on the same day using the same standardized (2.25% fat) and pasteurized (72 °C X 16 seconds) milk and cheese making was replicated on 3 different days according to a 3 X 3 Latin square design. Cheese manufacture was conducted according to a scheme outlined in an earlier report (Barbano *et al.*, 1991). Cheese milk was heated to 36 °C and after 30 minutes was inoculated with starter culture which consisted of a 1:1 mixture of *Streptococcus salivarius ssp. thermophilus* (Thermococcus C120, Rhone-Poulenc, Madison, WI) and *Lactobacillus delbrueckii ssp. bulgaricus* (Thermorod R160, Rhone-Poulenc, Madison, WI). Ripened milk was coagulated with pure (fermentation-produced) chymosin (Chy-Max, Pfizer Inc., Milwaukee, WI). The coagulum was cut approximately 30 minutes later and curds were cooked to 41 °C. Cooking time varied with pH of whey at draw (i.e., 20 minutes for draw pH 6.4, 40 minutes for draw pH 6.15, and 60 minutes when whey drainage was at pH 5.9). Following whey drainage, curds were cheddared until the curd pH decreased to pH 5.25. The time from the end of draw to mill ranged from 51 minutes for draw pH 6.4 to 19 minutes and 5 minutes for draw pH 6.15 and 5.9 respectively. Following milling, curds were dry-salted at a total rate of 1.8% (w/w) and stretched in hot water (57 °C) that contained 10% NaCl (w/w) and extruded by a twin screw Mozzarella mixer (Model 640, Stainless Steel Fabricating, Columbus, WI) into stainless steel cylinders (7.5 cm i.d. X 30 cm long). Cylinders of cheese were cooled immediately to an internal temperature of 20 °C by immersion in ice water and were vacuum packaged and stored at 4 °C.

Chemical composition of curd

Samples of curd were analyzed at draw (whey drainage), immediately before milling and immediately after salting for Ca, moisture and protein. Similar analyses were performed on 3 day old cheeses. All measurements were conducted in duplicate (except moisture) on finely ground samples. Moisture contents were determined in quadruplicate. Curd and whey pH was determined by direct immersion of a Xerolyt electrode (Model HA405, Ingold Electrode, Wilmington, MA). Moisture content was calculated as the loss in weight of a 2 - 3 g sample of curd, dried for 24 h at 100 °C in a forced air oven (Model OV-490A-2, Blue M, Blue Island, IL). Calcium concentration was determined by complexometric titration (Kindstedt and Kosikowski, 1985) and protein by Kjeldahl determination of N (IDF, 1989).

Samples for scanning electron microscopy

Approximately 1 mm^3 samples of curd were taken at draw, immediately before milling, immediately after salting and from the stretched curd as it exited the auger. Samples were fixed by immersion for 2 hours in 2.5% glutaraldehyde buffered with 0.13 M sodium phosphate buffer, pH 7.3 (Millonig, 1961). After fixation, cheese samples were washed four times in this buffer and dehydrated in a graded ethanol series that consisted of 15 minutes in each of 10, 20, 35, 50, 70, 85 and 95% (v/v) ethanol. Samples were then frozen in liquid nitrogen and fractured. Fractured samples were thawed in 100% ethanol (2 changes, 15 minutes each) and critical point dried with liquid CO_2 using a Samdri PVT-3B critical point drier (Tousimis Instruments, Rockville, MD). The dried fractured cheese samples were mounted on copper specimen holders with fracture faces uppermost

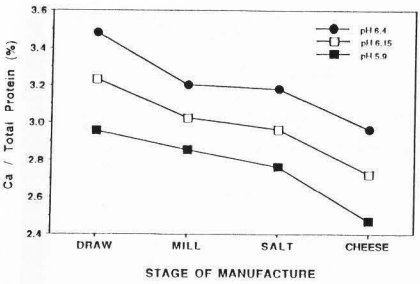


Figure 1. The Ca/total protein ratio of Mozzarella curd during manufacture (average of 3 trials).

Table 2: Mean squares and P values for calcium/total protein ratio at different manufacturing stage and draw pH.

Factor	M.S.	P
Draw pH	0.599	0.0005
Error I	0.012	
Mfg. Stage	0.389	0.0001
Draw pH X Mfg. Stage	0.005	0.9785
Error II	0.027	

and sputter coated with gold/palladium in a DC Sputter Coater E5100 (Polaron Instruments Inc., Doylestown, PA). The morphological appearance of fracture faces was examined in a JEOL 100 CX II TEMSCAN equipped with a high resolution ASID scanning module using an accelerating voltage of 20 kV.

Statistical Analysis

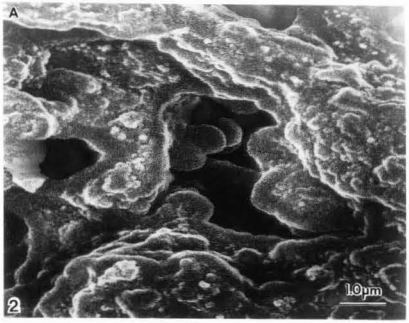
Differences in Ca/total protein ratios were analyzed as a 3 X 4 analysis of variance (ANOVA) in randomized block format, with stage of manufacture and draw pH as independent variables.

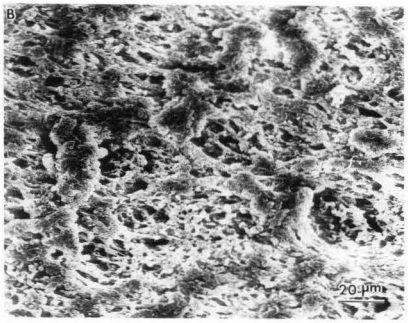
Results and Discussion

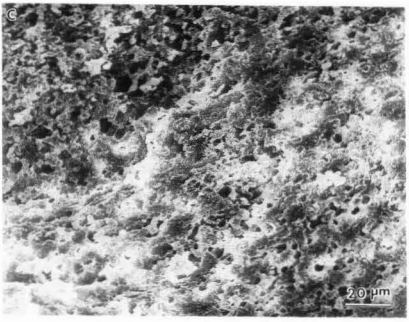
The time, temperature and pH conditions of cheese manufacture are shown in Table 1. Total make times were similar for all three cheeses regardless of draw pH. As expected, the time required to reach the appropriate pH of whey at draw differed among cheeses i.e., 169 minutes after culture addition to reach draw pH 5.9 to 128 minutes to reach draw pH 6.4. However, since curds were milled at approximately the same pH, the time between draw and milling was greater for the high draw pH cheese. Thus, for the present discussion, acid production may be considered to occur in two stages, i.e., before and after drain. Since the equilibrium between many micellar and serum components, e.g., Ca, P and casein is pH dependent (Lawrence *et al.*, 1987), curds drained at the lower pH are expected to sustain higher losses of micellar calcium phosphate to the whey. Thus, the ratio of calcium to total protein in curd samples was significantly influenced by draw pH (Table 2), with lower ratios occurring at lower draw pH (Figure 1). Curd samples continued to lose calcium throughout manufacture (Table 2), resulting in progressive decrease in calcium/total protein ratio (Figure 1). These data are of interest since Lawrence *et al.*, (1987) suggest that the stretching characteristics of Mozzarella cheese are related to the relatively high concentrations of intact casein and critical concentrations of Ca and P (25% in colloidal form) in the finished cheese. In this respect, the Ca/casein ratios in the finished cheeses are of interest. Assuming that casein constitutes approximately 85% of solids-not-fat (Lawrence *et al.*, 1984), the ratio of Ca/casein in cheeses manufactured using a draw pH of 5.9, 6.15 and 6.4 are 34, 38 and 42 mg Ca/g casein respectively. Furthermore, Lawrence *et al.*, (1987) indicate that at cheese pH 5.2, approximately 75% of total calcium is located in cheese serum with the remaining 25% presumably colloidal. If this is the case, the ratio of Ca directly associated with casein (expressed as mg colloidal Ca/g casein) can be calculated to be 8.5, 9.5 and 10.5 for cheeses manufactured using draw pH 5.9, 6.15 and 6.4 respectively.

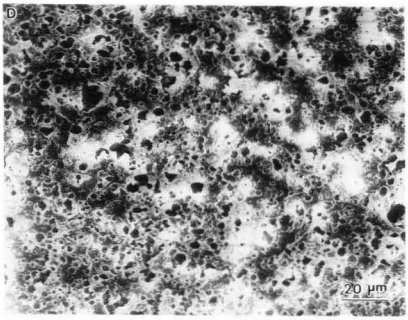
The effects of varying degrees of curd demineralization on curd structure are shown in Figs. 2-4. Curd drained at pH 6.15 had an open meshwork appearance throughout which holes or cavities were uniformly distributed (Fig. 3B). In contrast, curd drained at pH 6.4 appeared to be less dense with a more non-uniform distribution of irregularly sized cavities (Fig. 4B) while the fracture face of curd drained at pH 5.9 appeared to be least regular and although exhibiting a somewhat spongy morphology, had sinuous nodes of casein occurring in an apparently random fashion throughout (Figure 2B). As these samples were not postfixed in osmium and were dehydrated in an ethanol series, these cavities between paracasein aggregates/strands could be considered to represent areas originally occupied by fat and water. Also, since we are unaware of any other study presenting scanning electron micrographs of Mozzarella curd at whey drainage, comparisons with other studies are not possible. However, in gross morphology, Mozzarella curd drained at whey pH 6.15 (Fig. 3B) is similar to renneted Cheddar curd at whey drainage (Eino *et al.*, 1976) and to Cheddar curd at drain, made from concentrated milk (Green *et al.*, 1981). Although Rousseau (1988) presented structural data on Saint Paulin curd prior to whey removal, direct comparisons are invalid due to differences in curd treatment and image magnification.

A better understanding of gross morphology can be obtained by examination of higher magnification micrographs of curd samples at whey drainage (Figs. 2A, 3A and 4A). When whey was drained at pH 6.4, partially fused paracasein particles of irregular shape could be observed in an open matrix (Fig. 4A). In contrast, at









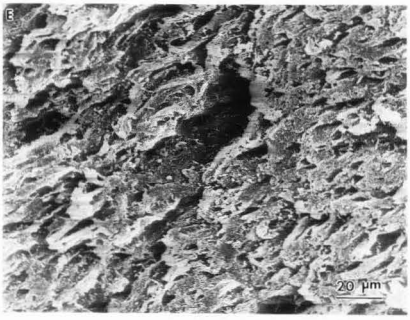
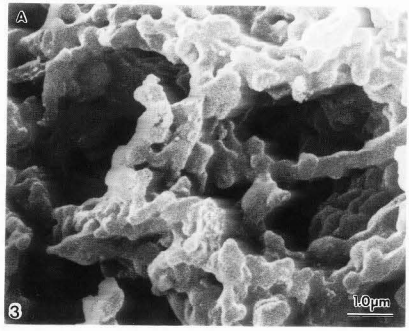
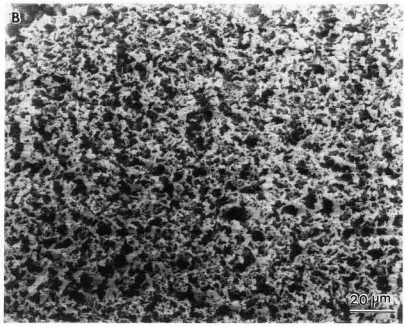
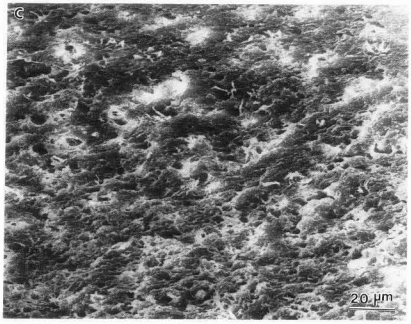


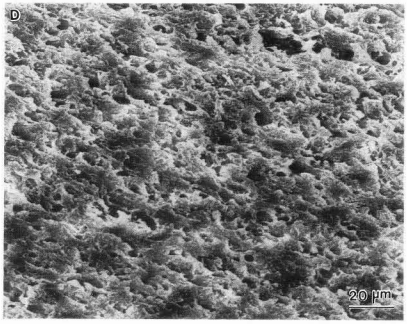
Figure 2 (this page). Mozzarella curd structure during manufacture (draw pH 5.9). Paracasein particles appear highly fused at whey drainage (A and B). Subsequent processing, i.e., milling (C) and salting (D) led to a compaction of the protein network. The stretching step imparted a directional orientation to protein fibers shown here in oblique section (E).

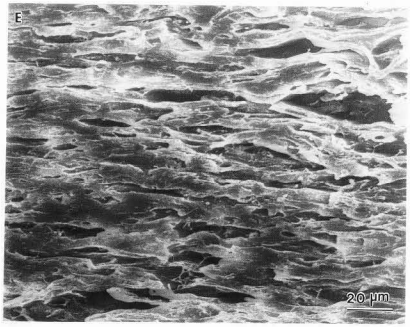
Figure 3 (facing page). Mozzarella curd structure during manufacture (draw pH 6.15). Paracasein particles although discrete in appearance are beginning to exhibit considerable fusion and strand formation at whey drainage (A and B). The protein matrix seemed to contract and become denser after milling (C) and salting (D). Stretching the curd resulted in longitudinally directed fibers that interweave extensively (E).











whey pH 6.15 (Fig. 3A), the beginning of a three-dimensional network with a higher degree of fusion of paracasein particles could be observed. Although the matrix still contained discrete particles, inter-particle fusion had progressed to a point where continuous protein strands evolved. At pH 5.9 (Fig. 2A), fusion had progressed to a point where the strands of the protein matrix appear as an almost completely fused amorphous mass. In addition, subjective evaluation (hand feel) indicated that the curd at pH 6.4 had a paste-like consistency unlike curd at pH 5.9 which was rubbery and firmer in nature.

The observed gradations in the degree of fusion of paracasein particles are probably due to differences in curd pH and the extent of dissolution of colloidal calcium phosphate from paracasein micelles which shows a pH dependency similar to that of native micelles (Van Hooydonk *et al.*, 1986).

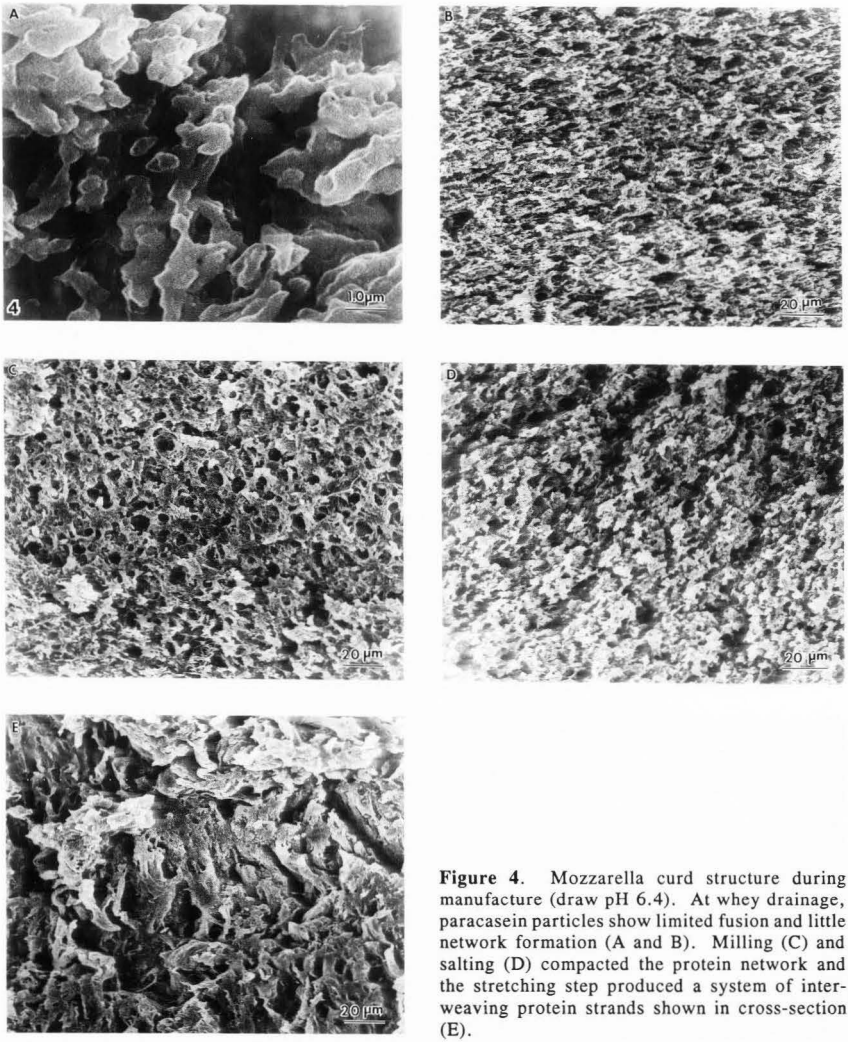


Figure 4. Mozzarella curd structure during manufacture (draw pH 6.4). At whey drainage, paracasein particles show limited fusion and little network formation (A and B). Milling (C) and salting (D) compacted the protein network and the stretching step produced a system of interweaving protein strands shown in cross-section (E).

Table 3. Average composition[1] of Mozzarella curd at each manufacturing stage.

	% Moisture			% Calcium			% Protein		
	5.9[2]	6.15[2]	6.4[2]	5.9[2]	6.15[2]	6.4[2]	5.9[2]	6.15[2]	6.4[2]
Draw	64.17	65.48	72.19	0.484	0.528	0.440	16.51	16.34	12.83
Mill	50.05	50.16	50.59	0.690	0.740	0.785	24.24	24.49	24.54
Salt	50.91	51.40	50.56	0.640	0.685	0.739	23.17	23.15	23.61
Cheese	45.84	46.28	45.73	0.694	0.750	0.830	28.07	27.59	28.03

[1]N = 3 [2]pH of whey at draw

Striking changes in curd morphology were observed between the draining of whey and milling (Figs. 2C, 3C and 4C). In general, curds became more compact and exhibited varying degrees of fusion. This compaction of the protein matrix is an obvious consequence of syneresis. The continued aggregation of casein causes the curd to shrink (Green and Grandison, 1987). Even though curds differed with respect to Ca/protein ratio at milling (Fig. 1), a sponge like protein matrix exhibiting continuity in three dimensions can be discerned in all samples at mill with no obvious treatment effects (draw pH) apparent.

After salting, curds drained at pH 5.9 (Fig. 2D) and 6.4 (Fig. 4D) appeared to incur some structural modifications. As no significant compositional differences arose due to salting (Table 3), we are unsure as to the nature of these alterations. The final step in Mozzarella cheese manufacture involving physical manipulation is stretching. This was conducted in hot water (57 °C) that contained 10% NaCl. It is this step that imparts the particular orientation to protein fibers in Mozzarella cheese. Such orientation has been reported previously (Kalab, 1977; Masi and Addeo, 1986). The longitudinal orientation of protein fibers in freshly extruded Mozzarella cheese is clearly demonstrated in Figure 3E. The fibers, of varying thickness, exhibit extensive interweaving and although oriented in a longitudinal direction, show extensive continuity in cross (Fig. 4E) and oblique section (Fig. 2E). Unfortunately, we could not control for plane of fracture, so direct comparison between treatment cheeses is difficult. Stretching of milled curd in hot water imparted a directional orientation to the protein fibers. The irregularly shaped voids and vaults occurring between these fibers probably represents the location sites of fat and bulk phase water.

Thus, irrespective of the observed differences in a) Ca/total protein ratio due to differences in draw pH and b) differences in curd structure at whey drainage, it appears that the present manufacturing scheme ultimately yielded Mozzarella cheese of similar morphological appearance. However, the structural features of curd at draw were affected either directly or indirectly by pH. These effects presented visibly as gradations in the degree of fusion of paracasein particles. Subsequent processing of curd led to a more compacted protein network which was relatively similar in all the treatment cheeses. The thermal, textural and rheological behavior of these cheeses during ripening will be the subject of subsequent papers.

Acknowledgements

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Discussion with Reviewers

H.D. Goff: Your conclusion seems to be that, although draw pH has a significant effect on calcium content of the Mozzarella cheese, it has no effect on the structure of the cheese. Should the reader also draw the conclusion that the cheeses were similar in other aspects. Was the yield of cheese affected by these variations in draw pH? Please comment.

Authors: The data presented in this paper only document the influence of draw pH on Mozzarella cheese structure. Thus conclusions regarding other aspects of Mozzarella cheese properties are unfounded. The impact of the pH of whey at draw on the textural and functional properties of these cheeses will be addressed in future papers. Cheese yield as a function of draw pH was not measured.

M. Rousseau: In **conclusion** (end of **Discussion**) the authors state: "Thus, irrespective ... of similar morphological appearance" and "subsequent processing ... similar in all the treatment cheeses". This is in contradiction with Figs. 2E, 3E and 4E in which marked differences in the cheese structure are observed. The authors did point out in the **Abstract** "Dramatic changes ... which aligned the protein fibbers" and in the **Discussion** "... the curd at pH 6.4 ... was rubbery and firmer in nature". To me that these remarks seem contradictory. Rheological measurements at the "cheese" step of Fig. 1 might be beneficial to elucidate this point.

Authors: This point is well taken. We feel that the observed structural differences in curd at draw (Figs. 2A, 3A and 4A) were consistent with our subjective (hand feel) evaluation of curd texture at draw. However, in our opinion, the structural differences between treatment cheeses did not (despite the observed differences in chemical composition) persist after the stretching step, i.e., in the finished cheeses. From previous experience of fracturing the same cheese sample at different angles we feel that Figs. 2E, 3E and 4E represent the same structure but at different fracture angles. We do agree that the rheological characteristics of the finished cheeses are very significant and these will be dealt with in a future paper.