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PROCEEDINGS
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29TH ANNUAL MARSCHALL ITALIAN CHEESE SEMINAR

September 16 & 17, 1992

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Rod-to-Coccus Ratio: Change In Cell Count During Mozzarella Manufacturing And Impact On Proteolysis During Refrigerated Storage

By

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Abstract

Three 375 lb vats of cultured low moisture part skim Mozzarella cheese were produced from the same batch of standardized milk and rennet, but using three different rod (*Lactobacillus bulgaricus* - Marschall Products - Thermorod) and coccus (*Streptococcus thermophilus* - Marschall Products - Thermococcus) ratios (9R:lC, 5R:5C, and lR:9C). The total volume and total colony forming units of culture added was kept constant for all ratios (i.e., 102 ml/375 lb). Cheese was made with the "no-brine" cheese making method using a 106°F (41°C) cook temperature, 6.4 draw pH *whey), 5.25 milling pH, and 135°F (57°C) stretching temperature. Cheese making was replicated on three different days as a 3 X 3 Latin square design. Titratable acidity increase and pH decrease were slower, while make time was longer with the 9R:1C ratio. The number of both rod and coccus per gram increased during cheese making, but differential enumeration of rod and coccus revealed that coccus was the dominant organism in the curd at milling regardless of initial rod:coccus ratio added to the milk. The number of viable coccus was not decreased by stretching and remained constant during 50 days of refrigerated storage. The number of viable rods seemed to decrease as a result of stretching and may continue to decrease during storage. The amount of inoculation (given constant ripening time) may be as important or more important than the ratio of rod-to-coccus.

There were no significant differences in initial chemical composition (moisture, fat, protein, salt) and pH among cheeses made using different rod:coccus ratios. Soluble protein content (i.e., pH 4.6 acetate soluble - extent of proteolysis; 12% TCA soluble depth of proteolysis) in the cheese increased with time for all cheeses, but proteolysis of cheese with higher rod:coccus ratio increased at a faster rate. Rod:coccus ratio had a greater impact on 12% TCA soluble protein, than on pH 4.6 soluble protein. The highest rod:coccus ratio produced more 12% TCA soluble nitrogen during refrigerated storage. Thus, the higher the number of viable rods present during cheese manufacturing, the greater the depth of proteolysis.

Introduction

Mozzarella cheese texture and functional properties change with time of refrigerated storage (1). Immediately after traditional Mozzarella cheese manufacture, the cheese has poor meltability. However, with time of refrigerated storage the meltability of the cheese increases (1). Experience in the cheese industry has shown that Mozzarella cheese for institutional use in the preparation of pizza has optimum functional characteristics after about 2 to 3 weeks of refrigerated storage. It is assumed that the major contribution to changes in unmelted cheese

texture and melting characteristics of Mozzarella cheese during refrigerated storage is due to the action of proteolytic enzymes.

The possible sources of proteolytic activity in Mozzarella cheese are coagulant, starter culture, milk, and nonstarter bacteria. A recent study designed to identify the relative contribution of these sources of proteolytic enzymes reported that the coagulant and starter culture are the primary source of proteolytic activity in Mozzarella cheese (2). A contribution of native milk enzymes was not detected. The level of nonstarter bacteria in the cheese was very low and did not increase with time of refrigerated storage and therefore was not a major source of proteolytic activity. Thus, the coagulant and the starter culture are the major contributors of proteolysis during refrigerated storage of Mozzarella cheese.

At the 1991 Marschall Conference, Dr. Paul Kindstedt presented data indicating that the proteolytic activity of most milk coagulants is not heat inactivated during the Mozzarella cheese manufacturing process (3). Proteolysis was monitored during refrigerated storage by measuring the increase in pH 4.6 acetate soluble nitrogen and 12% trichloroacetic acid (TCA) soluble nitrogen. The pH 4.6 acetate soluble nitrogen is a measure of both the small and large peptides produced by the action of proteases and peptidases. The 12% TCA soluble nitrogen is a measure of the very small peptides and amino acids released by proteases and peptidases. Thus, the pH 4.6 acetate soluble nitrogen is a reflection of the extent of proteolysis, while the 12% TCA soluble nitrogen is a reflection of the depth of proteolysis. Large differences in production of pH 4.6 acetate soluble nitrogen from one coagulant to another were reported. Smaller differences in 12% TCA soluble nitrogen due to different coagulants were also reported.

The starter cultures used in manufacture of Mozzarella cheese also provide proteases and peptidases that break down casein in Mozzarella cheese during refrigerated storage. Generally, it has been found that the rod contributes more to proteolytic activity in the cheese than the coccus. Oberg et al. (4), has reported differences in the rate of change in functional properties of Mozzarella cheese during refrigerated storage when protease positive and negative strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* were used for cheese making. Commercial strains are mostly protease positive, but may vary in their proteolytic activity from strain to strain.

It has been common practice in the Mozzarella cheese industry to use different strains of cultures for Mozzarella cheese manufacture and to use different ratios of rod-to-coccus. Cheese makers use this approach to try to fine tune the characteristics of their cheese. The objectives of our study were to determine the influence of initial rod-to-coccus ratio on a.) the pH, titratable acidity, cheese making time, and viable counts of rod and coccus in low moisture part skim Mozzarella and b.) initial chemical composition and proteolysis during storage at 4°C for 50 days.

Methods

Cheese Making.

Three 375 lb vats of cultured low moisture part skim Mozzarella cheese were produced from the same batch of standardized milk and rennet, but using three different rod (*Lactobacillus bulgaricus* - Marschall Products - Thermorod) and coccus (*Streptococcus thermophilus* - Marschall Products - Thermococcus) ratios (9R:lC, 5R:5C, and lR:9C). The total volume and total colony forming units of culture added was kept constant for all ratios (i.e., 102 ml/375 lb). Cheese was made with the "no-brine" cheese making method using a 106°F

(41°C) cook temperature, 6.4 whey drawing pH, 5.25 milling pH, and 135°F (57°C) stretching temperature, as described previously (1). If the vats made with different rod:coccus ratios developed acid at different rates, they were held until they reached a milling pH of 5.25. Cheese making was replicated on three different days as a 3 X 3 Latin square design.

Rod and Coccus Counts.

Viable differential plate counts of rod and coccus were done using the method of Lee et al. (5). Spread plates of appropriate dilutions were made and incubated anaerobically at 38°C for 48 h. Differential counting of rod and coccus was done on the same plate based on the difference in colony morphology between rod and coccus. Confirmation and validation of the method was done by spiking samples with known amounts of pure rod or pure coccus. In addition, representative colonies were picked and the presence of rod or coccus was confirmed by gram stain and microscopic examination. The detection limits of the method are from about 30:1 to 1:30 in rod:coccus ratio. Rod-to-coccus ratio was determined for milk at the time of inoculation, curd at draw, curd at milling, cheese at three days, and cheese at 50 days of refrigerated storage.

pH, TA. Cheese Making Time.

The pH of milk, whey, and cheese were measured using a pH electrode (Xerolyt, model HA405, Ingold Electrodes, Inc., Wilmington, MA) and an Accument pH meter (Model 915, Fisher Scientific, Springfield, NJ). The electrode was immersed in a 3M KC1 storage solution at 38°C between pH measurements to improve the stability of pH readings. Temperature of whey and cheese during cheese making was 38°C. Reference buffers for pH 4 and 7 were tested at 38°C. The actual pH of the reference buffers were calculated for 38°C based on the recommended temperature coefficients provided by the manufacturer. Titratable acidity (TA) was measured during cheesemaking (6). Cheese making time for the purpose of this experiment was defined as the time from culture addition to achievement of milling pH of 5.25.

Cheese Composition.

The initial chemical composition of the cheese was determined. Cheese samples were ground in a blender to obtain a particle size of about 2 to 3 mm. Ground samples were packed in 50 ml plastic snap-lid vials, without head space, to minimize moisture loss from cheese during storage at 4°C (up to 2-d prior to analysis). Cheese moisture was determined gravimetrically, in quadruplicate by drying 2 g of cheese at 100°C in a forced air oven (Model OV-490-2, Blue M, Blue Island, IL) for 24 h. Salt content of cheese was determined using the Volhard method (6) and fat content by Babcock (6). Total nitrogen was measured by the Kjeldahl method and converted to protein using a factor of 6.38 (7).

Proteolysis.

Both pH 4.6 acetate buffer soluble nitrogen and 12% TCA soluble nitrogen content of cheese were measured at 3, 8, 15, 21, 29, and 50 days of storage at 4°C. Nitrogen was converted to a protein basis using a 6.38 factor. All soluble protein values are expressed as a percentage of the total protein content of the cheese. SDS-PAGE (8) was used to monitor α_s and β-casein

breakdown during refrigerated storage. The results are expressed as the amount of remaining intact α_s-casein and β-casein at various times of refrigerated storage.

Results And Discussion

pH, TA. Cheese Making Time. and Viable Counts.

The rate of decrease in pH and the rate of increase in titratable acidity were slower for the 9R:lC, while there was little difference in pH and TA between the 5R:5C and lR:9C ratios (Figures 1 and 2). As a result, the cheese making time to achieve a milling pH of 5.25 was longer for 9R:lC, than for the other two ratios (Figure 3).

The actual viable counts of rod and coccus at the time of milk inoculation were close to the intended ratios identified in the experimental design (Table 1). Many times cheese makers talk about more extreme ratios of rod-to-coccus than are covered in this study (i.e., 100 to 1, 1000 to 1, etc. or visa versa). However, if you look at the milk at inoculation in terms of colony forming units (CFU), it can be seen that once you reach a ratio of about 9:1 or 1:9, the number of each organism in colony forming units will not change that dramatically, particularly the organism that is present in the highest number. For example, theoretically at a 5R:5C ratio, the colony forming units would be 20 X 10^6 of rod and of coccus, while at a 9.1 rod-to-coccus ratio would result in 36 X 10^6 rods and 4 X 10^6 coccus in the milk at inoculation. If the ratio was changed further from 9R:lC to 99R:lC, the counts would be 39.6 X 10^6 rods and .4 X 10^6 coccus. As can be seen by the comparison of the CFU, the amount of increase in the total count of rod going from a ratio of 5R:5C to 9R:lC is more dramatic than the change in count when the ratio is changed to 99R:lC. Thus, rod-to-coccus ratios may be misleading.

The number of viable rod and coccus both increased during the cheese making process, but the rate of increase in numbers of coccus was much greater than for the rod. No matter what the initial ratio of rod-to-coccus was at the time of inoculation, the coccus was the predominant organism in the cheese at milling (Table 1). The mixing step may have caused some decrease in viable rods in the cheese, but this was difficult to determine because the coccus greatly outnumbered the rod at this point in the manufacturing. In general, the coccus seemed to survive the mixing and the amount of viable coccus in the cheese remained relatively constant during 50 days of refrigerated storage. It was clear that if you started out with a higher amount of rod in the milk at the time of inoculation, you would have a higher amount of viable rod in the cheese during refrigerated storage.

Unlike the conditions in our experiment, in a cheese factory when a different rod-to-coccus ratio is used, there may be more than one factor changing at the same time. If the rod-to-coccus ratio was increased from 5R:5C to 9R:lC and the rate of acid production decreased, then the cheese maker would very likely increase the amount of culture added to maintain a constant make time. Thus, two parameters have changed in the factory; the ratio and the level of inoculation. It is likely that, in practice, when a cheese maker increases the ratio of rod-to-coccus, the level of inoculation is also increased. The cheese maker will assume that all the changes observed in the functional characteristics of the cheese were due to the change in ratio, when in fact they were due to both the ratio change and, more importantly, the inoculation level change. This could cause cheese makers to overestimate the impact of changing rod-to-coccus ratio on proteolysis and changes in functional properties.

Cheese Composition.

The chemical composition of the cheese is shown in Table 2. There were no detectable differences ($P>.05$) in composition of the cheese due to the difference in rod-to-coccus ratio. The mean moisture, fat, protein, and salt content of the cheeses were very similar. The pH of the cheeses at all 3 R:C ratios started out similar. However, the pH of the cheeses made with the lR:9C had a tendency to increase with time of refrigerated storage, while the pH of cheeses made with the 9R:lC and 5R:5C remained constant during 50 days of refrigerated storage (Figure 4). The reason for this difference in pH behavior is not clear.

Proteolysis.

The changes in pH 4.6 acetate soluble protein and 12% TCA soluble protein are shown in Figures 5 and 6. As shown by numerous researchers, both forms of soluble nitrogen increase significantly with time of refrigerated storage. Our results (Figures 5 and 6) indicate that the level of both types of soluble nitrogen increase with increasing ratio of rod-to-coccus. The relative impact of differences in rod-to-coccus ratio was greater for the 12% TCA starter culture and particularly the rod influenced the depth of proteolysis (i.e., TCA soluble nitrogen) more than the extent of proteolysis (pH 4.6 acetate soluble nitrogen). This was confirmed by the electrophoretic data. The amount of residual α_s-casein decreased with time for all treatments but was not different for different rod-to-coccus ratios (Figure 7). The amount of residual β-casein was constant with time, indicating little if any proteolysis of β-casein occurred during 50 days of storage (Figure 8). If the extent of proteolysis was different for different rod-to-coccus ratios, then one would expect that there would be a difference in the amount of intact α_s- or β-casein. This was not the case.

Conclusions

1. The rate of decrease in pH and the rate of increase in TA during cheese making were slower with 9R:lC.
2. The rate of increase in viable counts of coccus was much faster than that of rod during cheese making. As a result, coccus became dominant in milled curd regardless of the initial R:C ratio.
3. Viable counts in cheese did not change much during stretching at 57°C or during 50 days of storage at 4°C.
4. Variation in rod-to-coccus ratio at constant inoculation level did not influence general cheese composition, as long as make time was adjusted to maintain constant drawing pH and milling pH among treatments.
5. Higher rod-to-coccus ratio caused a higher rate of production of pH 4.6 and 12% TCA soluble nitrogen during refrigerated storage.

The data presented last year (3), the data in this study, and other data that is available in our laboratory (2), lead us to believe that the coagulant and the starter culture are the two major

sources of proteases in Mozzarella cheese when proper sanitation has been used to prevent the growth of high numbers of nonstarter bacteria in the cheese. In addition, we find that the coagulant contributes most to the extent of proteolysis, while the culture (particularly the rod) contributes to the depth of proteolysis. Control of the relative contributions of these two sources of proteases could lead to better control of the functional characteristics of Mozzarella cheese and improved cheese quality.

FIGURE 1.

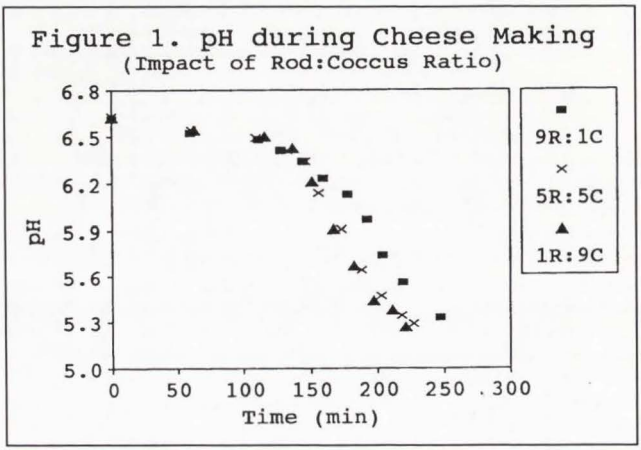

FIGURE 2.

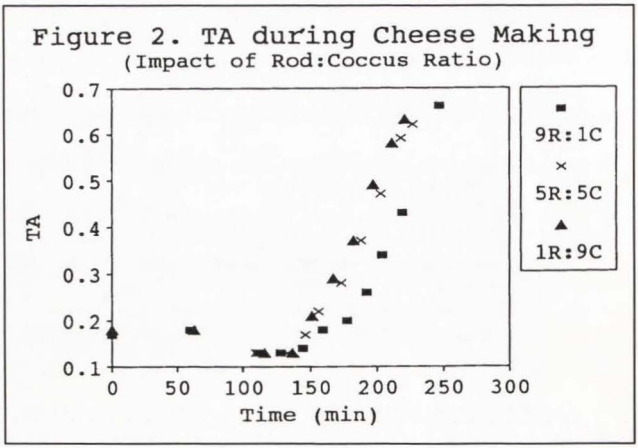

FIGURE 3.

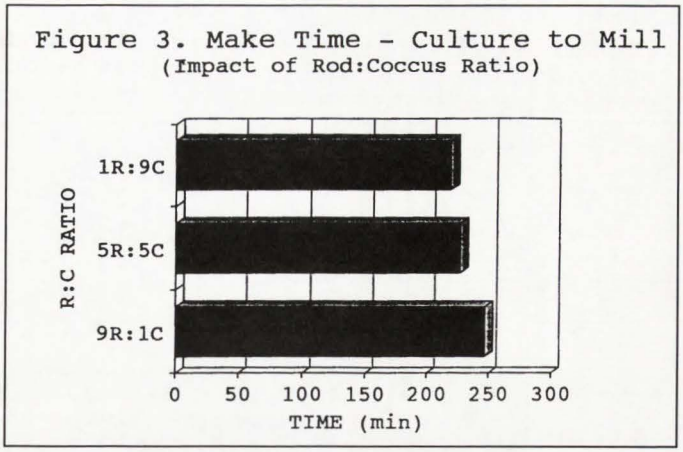

FIGURE 4.

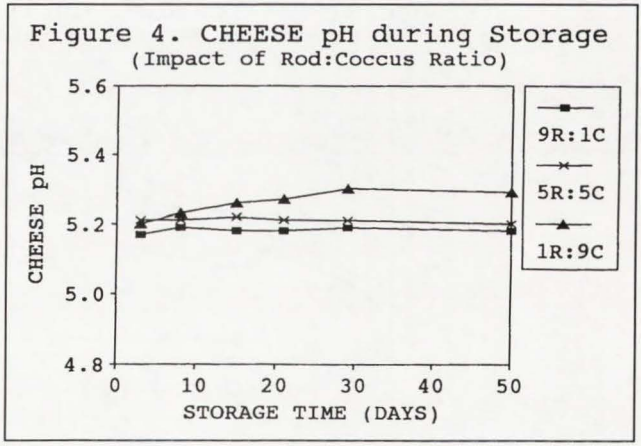

FIGURE 5.

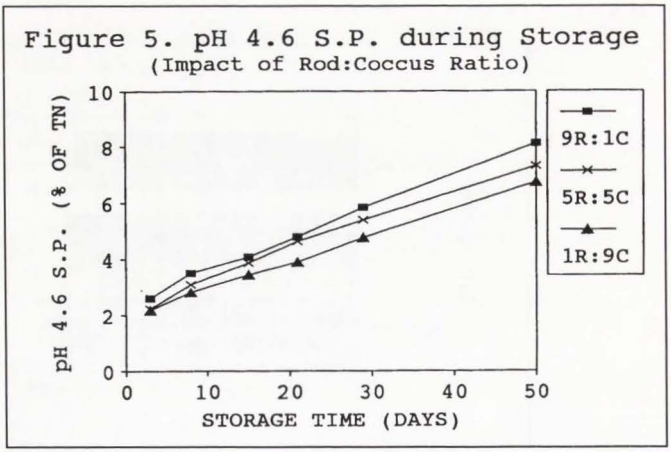

FIGURE 6.

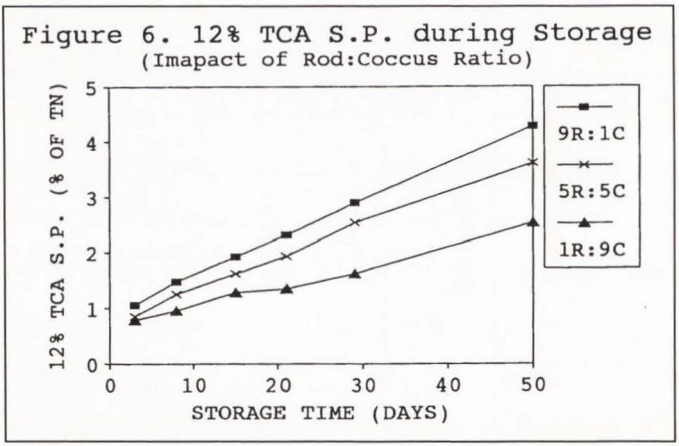

FIGURE 7

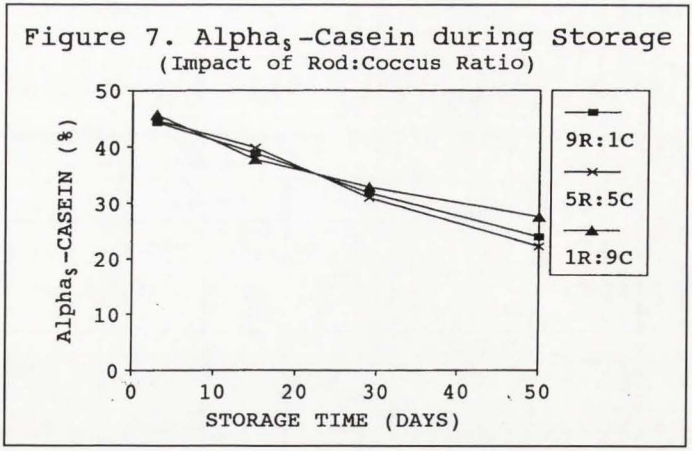

FIGURE 8

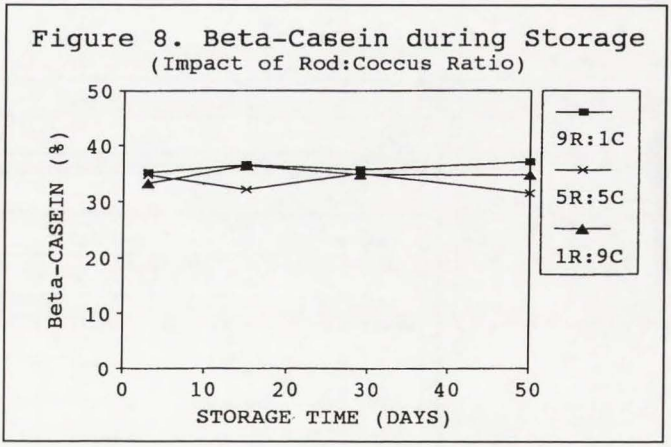

TABLE 1

TABLE 1. VIABLE COUNTS OF ROD AND COCCUS. (CFUx10^6/ml or g)

SAMPLE	R:C RATIO					
	9R:1C		5R:5C		1R:9C	
	ROD	COCCUS	ROD	COCCUS	ROD	COCCUS
MILK AT INOCULATION	33	3	18	23	2	35
CURD AT DRAW	75	68	37	300	<10	410
CURD AT MILLING	100	1100	20	2200	<10	4700
CHEESE AFTER 3 D	50	1100	<10	1900	<10	1700
CHEESE AFTER 50 D	20	1300	<10	2100	<10	2200

TABLE 2

TABLE 2. CHEMICAL COMPOSITION OF MOZZARELLA CHEESE

COMPONENT	R:C RATIO		
	9R:1C	5R:5C	1R:9C
CHEESE pH	5.17	5.21	5.20
% H_2O	45.63	45.81	45.87
% FAT	20.75	20.92	20.88
% FDB	38.16	38.60	38.57
% TOTAL N	27.93	27.97	27.63
% SALT	1.32	1.33	1.34
% SALT/H_2O	2.64	2.60	2.65

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Rod-To-Coccus Ratio: Changes In Functional Characteristics And Microstructure In Mozzarella Cheese During Refrigerated Storage

By

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Abstract

This investigation is a continuation of the previous study on rod-to-coccus ratio (see companion abstract by Barbano et al.). Three 375 lb vats of cultured low moisture part skim Mozzarella cheese were produced under the same conditions, but using three different rod (*Lactobacillus* - Marschall Products - Thermorod) and coccus (*Streptococcus thermophilus* - Marschall Products - Thermococcus) ratios (9R:lC, 5R:5C, and lR:9C). Cheeses were stored at 40°F (4°C) and analyzed for functional characteristics and cheese microstructure 3, 8, 15, 21, 29, and 50 days after manufacture. Unmelted cheese texture was evaluated by Instron Texture Profile Analysis (TPA), meltability by modified Schreiber test, apparent viscosity by helical viscometry, free oil formation by centrifugal separation and microstructure by scanning electron microscopy.

Rod:coccus ratio significantly affected three of the six TPA parameters (springiness, gumminess, chewiness). Cheeses made with 9R:lC were less springy, less gummy, and less chewy than cheeses made with lR:9C. However, the other three TPA parameters (hardness 1, hardness 2, and cohesiveness) were not significantly affected by rod:coccus ratio. In addition, meltability, apparent viscosity, and free oil formation did not differ significantly among cheeses made with different rod:coccus ratios. Thus, rod:coccus ratio had a limited impact on unmelted cheese texture and did not appear to influence cheese melting behavior. These findings are surprising in view of the widespread perception in industry that rod:coccus ratio is an important determinant of cheese functional characteristics. As discussed in the companion abstract, the amount of inoculation may be as important or more important than the ratio of rod-to-coccus. The microstructure of all cheeses, regardless of rod:coccus ratio, showed progressive increase in porosity with age. The cause of these changes is not known but may relate to proteolytic breakdown of the protein matrix, destabilization of cheese fat, or gas formation. No obvious differences in microstructure were observed among cheeses made with different rod:coccus ratios.

Introduction

A thermophilic starter culture consisting of *Lactobacillus delbrueckii* ssp. *bulgaricus* (hereafter abbreviated *L. bulgaricus*) and *Streptococcus salivarius* ssp. *thermophilus* (hereafter abbreviated *S. thermophilus*) is usually used in the making of low moisture Mozzarella cheese. In the industry this is referred to as a rod:coccus culture. The ratio of rod-to-coccus used in Mozzarella cheese making and its influence on functional characteristics has been the subject of

much interest in the industry (3, 14, 15) and has led to the development of process control systems to regulate rod:coccus ratio in commercial bulk cultures (2). Therefore, it is surprising to find that published reports on the impact of rod-to-coccus ratio on Mozzarella cheese are scarce.

There are two principal ways in which rod-to-coccus ratio may influence cheese functional characteristics. The first relates to the production of lactic acid and the formation of the basic curd structure during cheese making. According to Lawrence et al. (8, 9), the rate and extent of acid production and resulting demineralization of the curd during manufacture are critical determinants of cheese structure and texture characteristics, particularly stretching properties.

Acidification during Mozzarella cheese making depends on a symbiosis between *L. bulgaricus* and *S. thermophilus* (10, 12, 13). *S. thermophilus* is weakly proteolytic and cannot alone produce sufficient free amino acids and small peptides from casein to sustain vigorous growth and acid production in milk. In contrast, *L. bulgaricus* is much more proteolytic and thus stimulates the growth of *S. thermophilus* by producing the needed peptides and free amino acids. The invigorated *S. thermophilus* in turn encourages the growth of *L. bulgaricus* by producing stimulatory levels of CO_2 and formic acid, and by lowering the oxidation-reduction potential. This symbiosis between rod and coccus results in a two-stage fermentation in which acid production at the start of cheese making (i.e., before draining) is due largely to *S. thermophilus,* whereas *L. bulgaricus* becomes the dominant acid-producer towards the end of manufacture. Consequently, changing the proportion of rod-to-coccus in the starter culture will change the schedule of acid production which may influence the structure of the cheese curd and the functional characteristics of the final cheese.

The second way that rod-to-coccus ratio may influence functional characteristics relates to proteolytic breakdown of the curd structure during aging. As mentioned above, *L. bulgaricus* is much more proteolytic than *S. thermophilus*. Both *L. bulgaricus* and *S. thermophilus* are fairly heat tolerant, therefore it is doubtful that they are completely inactivated by the high curd temperatures attained during stretching. Thus, it is reasonable to expect that a higher ratio of rod-to-coccus in the starter culture will result in a higher population of proteolytic rods in the final cheese and higher levels of starter-associated proteolysis during aging. As was discussed at last year's Marschall ICS (5), proteolysis strongly influences the functional changes that occur in Mozzarella cheese during aging.

This investigation is a continuation of the previous study on rod-to-coccus ratio by Barbano et al. (see companion paper, 1992 ICS). In this paper we report on the impact of using three different rod-to-coccus ratios on the unmelted texture and the melting characteristics of Mozzarella cheese during refrigerated aging. We also used electron microscopy to examine the microstructural changes that occur during aging in order to better understand the relationship between cheese structure and functional characteristics.

Materials And Methods

Details of the cheese manufacturing procedure are given in the companion paper by Barbano et al. (1992 ICS) and will only be summarized here. Three 375 lb. vats of cultured low moisture part skim Mozzarella cheese were produced at Cornell University under the same conditions, but using three different rod (*Lactobacillus bulgaricus* - Marschall Products - Thermorod) and coccus (*Streptococcus thermophilus* - Marschall Products - Thermococcus)

ratios (9R:lC, 5R:5C, and lR:9C). It is important to note that the total amount of starter culture added to each vat was held constant; only the ratio of rod-to-coccus was varied. The "no brine" procedure was used for all cheese making experiments.

Cheese samples were vacuum packaged and stored at 40°F (4°C) at Cornell University and then analyzed for unmelted texture by Instron Texture Profile Analysis (TPA) (1), and meltability by a modified Schreiber test (7) at 3, 8, 15, 21, 29 and 50 days of storage. In addition, samples of all cheeses were sent on ice via overnight express mail to the University of Vermont and stored at 40°F (4°C) until analysis. Changes in apparent viscosity (by helical viscometry (4)) and free oil formation (by centrifugal separation (6)) were measured at 3, 8, 15, 21, 29 and 50 days of storage. Data were analyzed for statistical significance by the SAS Statistical Software Package. Microstructural changes during aging were examined using scanning electron microscopy. Cheese samples were immersion fixed in 2.5% gluteraldehyde, dehydrated in ethanol, frozen, fractured, critical point dried, and sputter coated with gold/palladium.

Results And Discussion

As discussed in the companion paper by Barbano et al. (1992 ICS), rod-to-coccus ratio did not affect cheese composition (moisture, fat, protein, salt) but did influence proteolysis during aging and appeared to influence acid production during manufacture. Specifically, a higher ratio of rod-to-coccus resulted in greater proteolysis during aging, as measured by the formation of pH 4.6 soluble nitrogen and especially 12% TCA soluble nitrogen. In addition, cheeses made with a higher ratio of rod-to-coccus tended to have slower acid production during cheese making and longer make times.

Unmelted Cheese Texture

All six Instron TPA parameters changed significantly in all cheeses during refrigerated storage. Changes in hardness 1 (i.e., the force required to compress the cheese sample by 50% during the first of two compression cycles) are shown in Figure 1. Hardness of cheese from all three rod:coccus treatments decreased at about the same rate during refrigerated storage, indicating a progressive softening that is probably important to shredding characteristics. Rod-to-coccus ratio influenced the textural changes during storage in a limited manner that is not easily interpreted. Three of the six Instron TPA parameters (hardness 1, hardness 2, and cohesiveness) were not significantly affected by rod:coccus ratio. However, the TPA parameters of springiness, gumminess, and chewiness were significantly influenced by rod:coccus ratio. Data presented in Figures 2 - 4 reveal that cheese made with a ratio of 9 rod:l coccus had lower springiness, gumminess, and chewiness values during storage than cheese made with a ratio of 1 rod:9 coccus. The practical importance of these differences is not clear. Perhaps what is most important is that storage time had a greater impact on unmelted texture than rod:coccus ratio.

Melting Characteristics

Melting characteristics of cheese from all three rod:coccus treatments changed significantly during refrigerated storage but did not appear to be influenced by rod-to-coccus ratio. Changes in meltability during storage are shown in Figure 5. The meltability test

measures the spread of a cheese sample as it melts. Meltability increased significantly with time of refrigerated storage for all cheeses, but there were no significant differences in meltability due to rod:coccus ratio.

Changes in apparent viscosity during refrigerated storage are shown in Figure 6. High apparent viscosity typically indicates a tough, elastic melt with limited fluidity, whereas low apparent viscosity indicates a softer, less elastic and more fluid melt. Apparent viscosity decreased significantly with time of refrigerated storage for all cheeses, but there was no significant differences in apparent viscosity due to rod:coccus ratio.

Figure 7 shows changes in free oil formation, a measure of oiling off, during refrigerated storage. Free oil increased significantly with time of refrigerated storage for all cheeses, but there were no significant differences in free oil formation due to rod:coccus ratio.

In summary, cheeses in this study showed typical patterns of change during refrigerated storage with respect to unmelted textural attributes and melting characteristics. However, rod-to-coccus ratio had only a limited impact on unmelted cheese texture and did not appear to influence melting characteristics during storage. This is surprising in view of the widespread perception in industry that rod-to-coccus ratio is an important determinant of cheese functional characteristics. A possible explanation may relate to the experimental design of this study, whereby rod:coccus ratio was varied while total amount of starter culture was held constant. As discussed in the companion paper by Barbano et al. (1992 ICS), using a higher rod:coccus ratio in the starter while holding total inoculum constant tended to delay acid production and to increase total make time. This would be unacceptable to most industrial cheese makers who must adhere to tight manufacturing schedules. Consequently, when high rod-to-coccus ratios are used in the industry, cheese makers may tend to increase the total amount of starter added to the vat to counteract the slowdown in acid production. This would tend to elevate the population of proteolytic rods in the final cheese, which would favor greater proteolysis and possibly greater functional changes during aging. Thus, whereas in our study the use of a 9:1 ratio of rod:coccus led to a modest elevation in proteolysis during aging, in industrial practice the use of a 9:1 ratio coupled with an increase in the total amount of starter added to the vat might result in larger increases in proteolysis with greater functional consequences. In short, the amount of starter added may be as important or more important than the rod:coccus ratio.

Cheese Microstructure

Results of the present study and numerous earlier investigations clearly demonstrate that the texture and melting characteristics of low moisture Mozzarella cheese undergo large changes during refrigerated storage. Ultimately, cheese functional characteristics derive from the chemical composition of the cheese and the paracasein matrix that provides the structural framework of the curd. Functional changes during aging partly result from proteolytic breakdown of the paracasein matrix. In this study we used scanning electron microscopy to investigate changes in curd microstructure during refrigerated storage.

The microstructure of all cheeses showed a common pattern of change during storage, with no obvious differences among the three rod:coccus treatments. Scanning electron micrographs of a representative cheese made with a 5:5 rod:coccus ratio at 3, 29, and 50 days of storage are compared in Figure 8. Fat and moisture components were removed from the cheese during sample preparation, therefore the solid background of each micrograph represents the protein matrix, whereas the void spaces may represent areas orginally occupied by fat droplets,

whey pockets and true void spaces. It is evident that microstructure showed a progressive increase in porosity with age. The cause of these changes is not known but may relate to proteolytic breakdown of the protein matrix, destabilization of the cheese fat, or gas formation. Paquet and Kalab (11) also observed a highly porous microstructure in Mozzarella cheese which they attributed to fat vacuoles. If the vacuoles in Figure 8 truly represent fat droplets, then their progessive increase in number and size during storage suggests that a substantial destabilization and agglomeration of fat took place. This may relate to the observed increases in free oil formation during storage. An alternative possibility is that carbon dioxide gas produced by *Streptococcus thermophilus* or by heterofermentative nonstarter lactobacilli accumulated in the curd and formed gas pockets at the microstructural level. In either case, the increase in porosity seen in Figure 8 would be expected to contribute to a weakening of the protein matrix and therefore may relate to changes in texture and melting characteristics during storage.

Conclusions

Rod-to-coccus ratio in this study had a limited affect on unmelted cheese texture and no apparent effect on cheese melting characteristics during refrigerated storage. Thus, the ratio alone may be less important than once thought. However, if changing the rod:coccus starter ratio causes the cheese maker to change the amount of starter used in order to maintain a consistent make schedule, then the cumulative effect on functional characteristics may greater than that observed in this study.

FIGURE 1

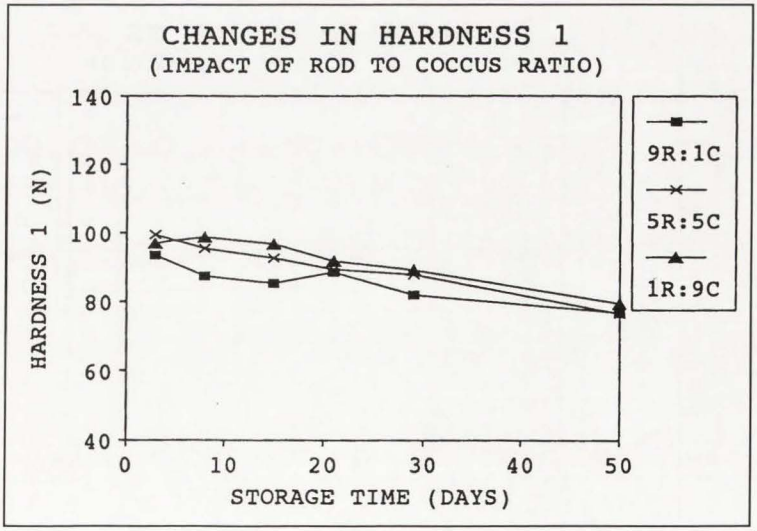


FIGURE 2

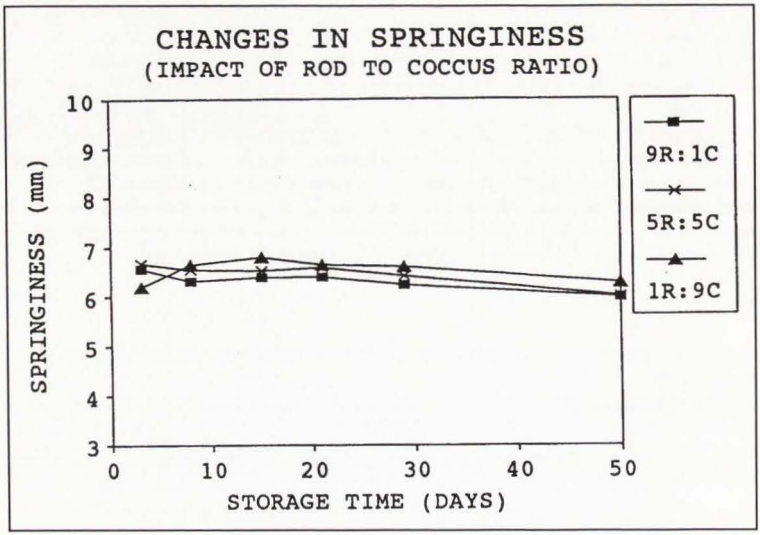


FIGURE 3

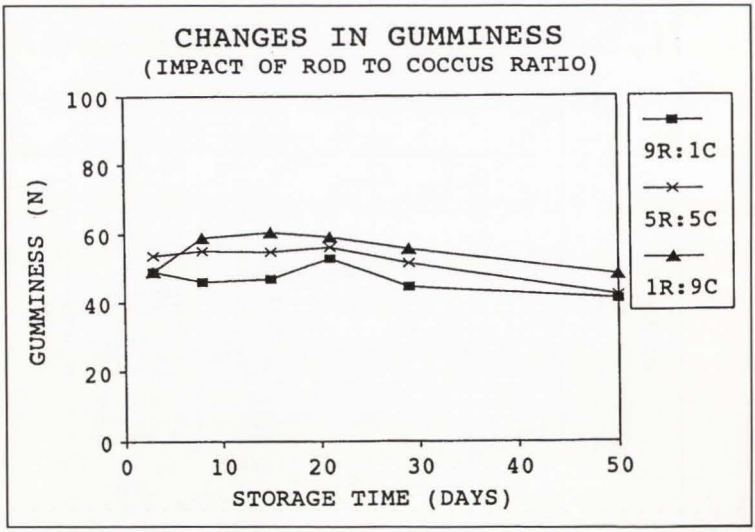


FIGURE 4

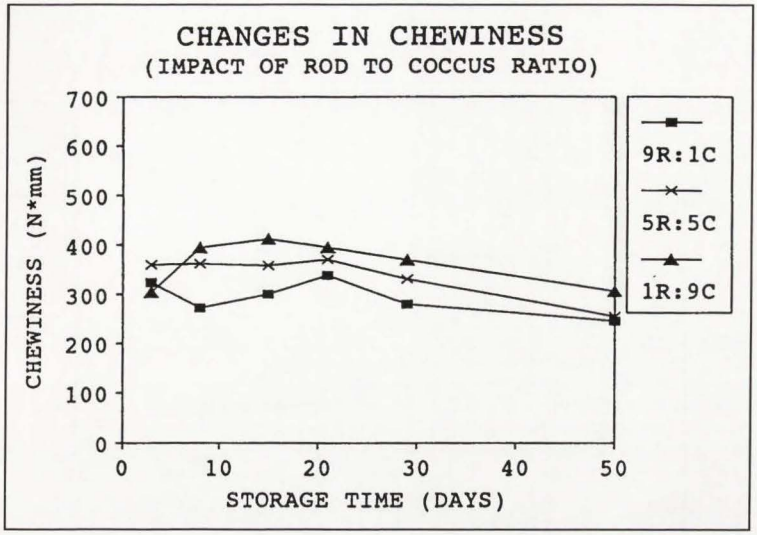


FIGURE 5

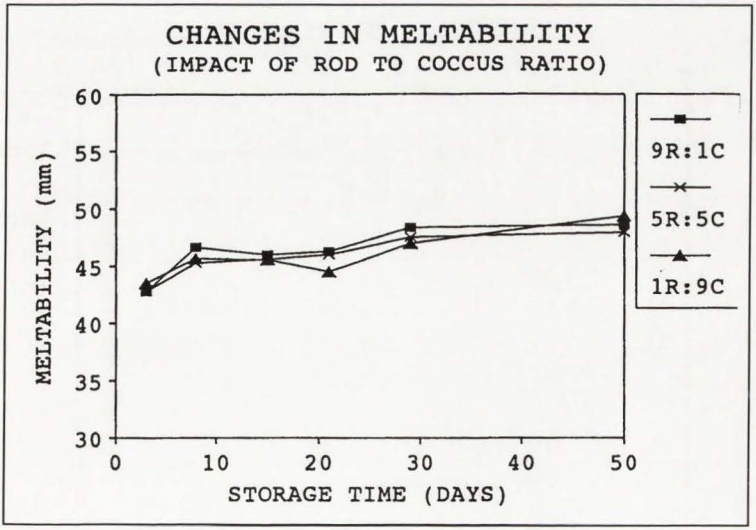


FIGURE 6

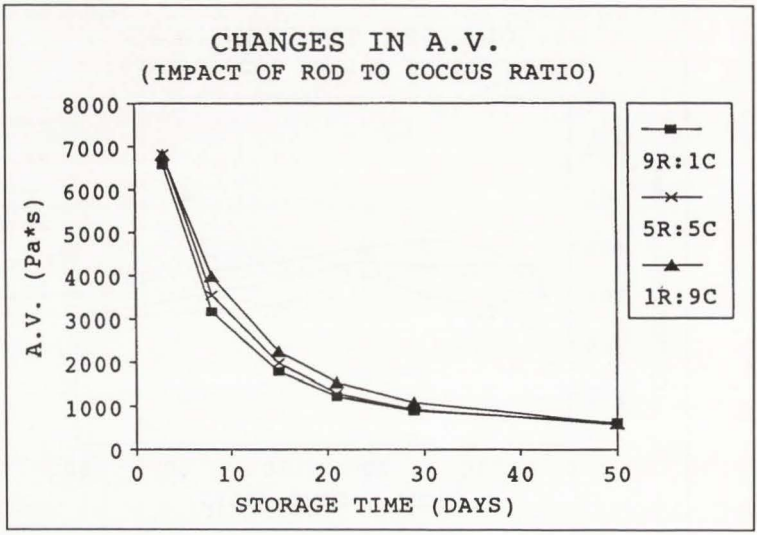


FIGURE 7

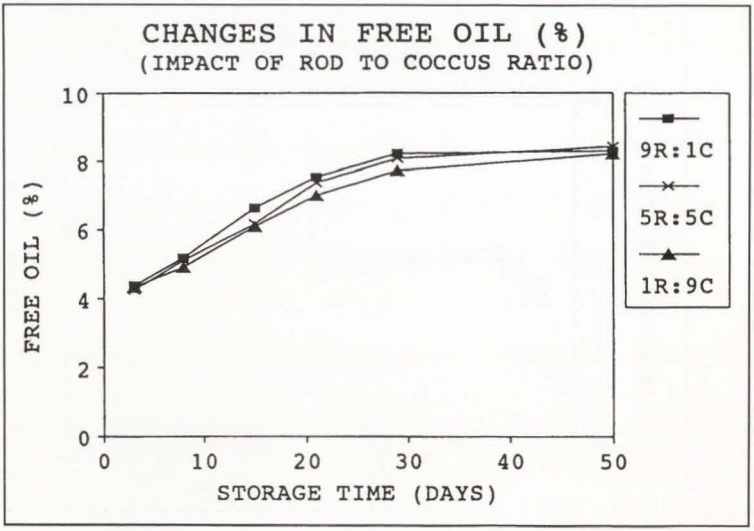
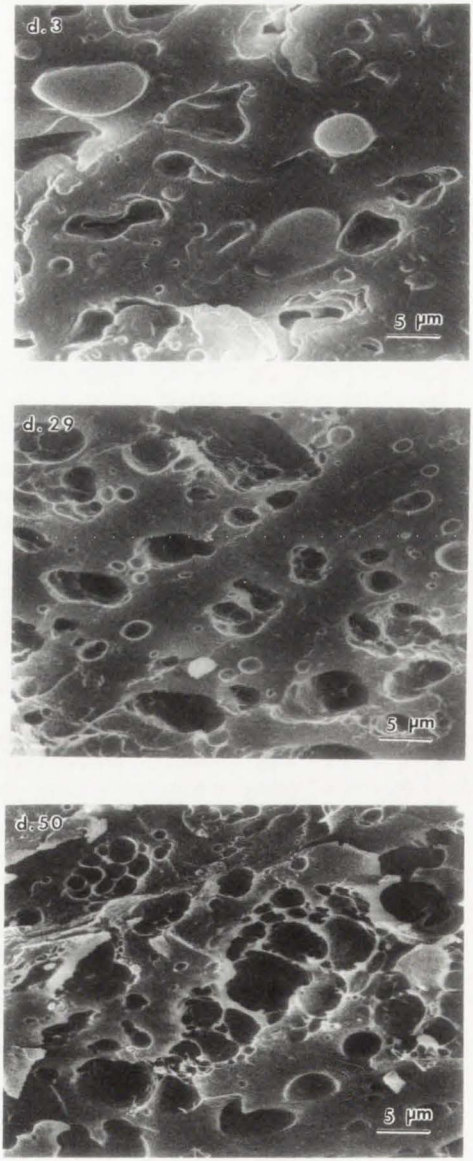


FIGURE 8
Scanning electron micrographs of the microstructure of low moisture part skim Mozzarella cheese at 3, 29 and 50 days of storage at 40°F (4°C).



Acknowledgment

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Microstructure Of Mozzarella Cheese

By

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Introduction

Mozzarella cheese quality is based on its physical properties, particularly stretch, melt, cook color, and oiling off during baking (Alvarez, 1986). These properties are derived primarily from the interaction of casein and fat in the cheese matrix. In addition, composition of the cheese, degree of proteolysis, storage time and storage temperature can influence these physical properties (Oberg et al., 1991; Oberg et al., 1992). Surveys concerning Mozzarella cheese quality indicate that improvement in manufacturing a more consistent product with predictable physical properties is needed (Nilson and La Clair, 1976; Pilcher and Kindstedt, 1991).

Paquet and Kalab (1988) studied the structure of stirred curd and stretched Mozzarella. They noted a parallel orientation of protein fibers in the stretched Mozzarella that was lacking in the stirred curd Mozzarella. In addition, stirred curd Mozzarella appeared to have an even distribution of fat globules, while stretched Mozzarella did not. Taneya et al. (1992), in a study of string cheese, also found a uniform longitudinal orientation of the protein matrix in the finished cheese and associated this with stringiness. Neither study followed curd structure development through the manufacturing process.

Understanding the microstructure of Mozzarella cheese, particularly how the casein and fat interact during and after manufacture, can provide valuable insight into what constitutes a quality product. In addition, as reduced fat Mozzarella cheeses are manufactured, comparison of their microstructure development with the microstructure observed in this study, may show which processing steps are critical and how these steps can be adjusted to produce a high quality product. For example, Tunick et al. (1991) found that a reduction in fat content of part skim Mozzarella cheese significantly changed textural and melt properties. Our study follows the development of curd structure through the manufacture of low moisture, part skim Mozzarella cheese. In addition, it documents the effects of the stirred curd step, dry salting prior to stretching, and changes in curd structure following stretching and molding.

Results And Discussion

The Mozzarella cheese examined in this study is a commercially manufactured low moisture, part skim Mozzarella. The manufacturing process includes a stirred curd step and a dry salting step prior to stretching. The culture used consisted of a 4:1 cocci to rod ratio based on weight. Cultures were grown separately and combined just prior to vat inoculation. Scanning electron microscopy was done according to the method of McManus et al. (1992). Several innovations in sample preparation and microscopy methodology over previous techniques allowed for greater resolution of the curd particles and protein structure. In sample preparation for scanning electron microscopy fat, water, and whey are removed during one of the extraction steps, leaving only the protein matrix and bacteria, along with impressions in the protein matrix to be scanned. Therefore, the supposition is that the vacuoles or empty spaces in the cheese structure are fat globules, whey, water, or a combination thereof.

Figure 1 shows the curd structure 10 minute (la) and 40 minute (lb) after cutting. A 10 minute heal follows cutting, then curd is heated to 105°F over a 30 min period and held at that temperature during cooking. Curd particles are suspended in the whey during this step. Aggregation of micelles is evident, along with uniform dispersion of fat globules. Initially, individual micelle structure can be seen in the coagulated protein matrix. By 40 minute the micelle structure is gone, replaced by a dense protein matrix. In the 10 minute sample (1a), bacteria are difficult to distinguish, probably because of sampling and low numbers in the initial phases of manufacture. At 40 minute (lb) they are entrapped in the protein matrix and appear to be forming micro
colonies.

Figure 2 compares the curd structure at the beginning (2a) and end (2b) of the stirring period. This period lasts for approximately 1.5 hours. During the first 45 minutes, the curd is stirred in the make vat with one third of whey drained out. It is then pumped to a finish vat, the remaining whey is drained, and the curd is stirred until the pH reaches 5.3. Bacteria are seen throughout the matrix in both samples. Compression and knitting of the curd continue. Initially the curd has large whey/fat pockets that are greatly reduced as syneresis occurs. There is a uniform distribution of fat and whey pockets. Vacuoles are round and as syneresis occurs and the protein matrix shrinks, the shape of individual fat globules becomes more evident. Individual micelle structure is gone at the finish of dry stirring and a coalescence of fat globules occurs.

Figure 3 shows the structure of the Mozzarella curd before (3a), after (3b) salting, and following the three hour brining period (3c). When the curd reaches a pH of 5.3 in the finish vat, it is dry salted. These figures show a cross section through the edge of individual curd particles. There is a continued loss of the open structure associated with expulsion of whey. This occurs very rapidly on the surface of the curd particle during the dry salting step as seen by comparing Figures 3a and 3b. In Figure 3a the case layer can be seen, approximately 10 um in thickness. Bacteria are now seen at the fat:water/protein interface instead of evenly dispersed throughout the curd matrix. The body is slightly more compact after brining, but many of the body texture changes are due to the stretching step in manufacture.

Figure 4 contains lateral (4a) and cross section (4b) views of Mozzarella cheese after it has been through the stretcher. During the stretching step, the curd is placed in 143°F water for 10 seconds at which time the curd reaches a temperature of 120 to 130°F. It travels up an auger, pressed in 5 pound loaf molds and placed in cool water (43 to 44°F) for one hour. Long strands of protein are seen with large areas of fat/whey accumulation. It appears that the fat has been squeezed into long tube-like structures as the protein fibers are stretched. Bacteria are seen in large numbers at the interface between the fat and protein layers. Bacteria appear to be intact, suggesting that the stretching temperature did not lyse them. Higher magnification (7000X) shows that the protein surface is very smooth. Also seen are clumps of fibrous material associated with the cocci, suggesting that this material may be exocellular polysaccharide secreted from the bacteria or residues of fat globule membrane.

Figure 5 shows the structure of Mozzarella cheese after one day of storage. A cross section view of the cheese (Figure 5a) shows matting of the protein fibers has continued since stretching. Large, irregular shaped areas of fat/whey are seen randomly dispersed throughout the protein matrix. These are also seen in samples taken from the interior of the cheese loaf. Very few bacteria are seen embedded in the protein matrix with the majority seen at the fat/protein interface. From these pictures, it is difficult to tell if the bacteria are in the fat/whey phase and left when the fat is removed or bound to the surface of the protein. Most of the bacteria are cocci

and they appear to be viable as some show evidence of cell division under higher magnification. Fibers are still seen in conjunction with the bacteria, suggesting possibly exocellular polysaccharide or remnants of the fat globule membrane.

Longitudinal sections of the interior of one day old Mozzarella cheese (Figure 5b) show fibers of protein with long tubes between the fibers (Figure 6). These tubes are probably comprised of fat with some whey. Fat globule indentations are seen imprinted on the protein fibers as compared to the smooth protein surface of the curd after the stretching step (Figure 4a).

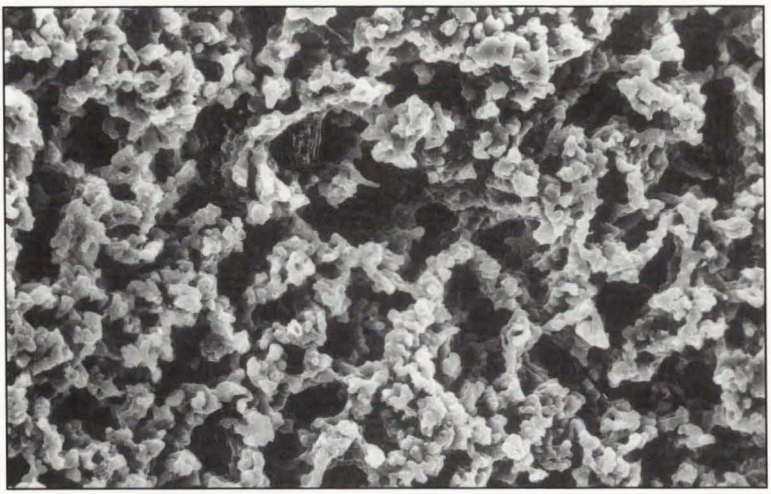
Figure 6a shows the structure of one day old Mozzarella that has been melted using the modified tube test (Oberg et al., 1991). In the modified tube test, four cm of shredded cheese is placed in a test tube, heated upright at 110°C for 60 minutes, then laid horizontal with the flow length measured. The fibrous structure of the protein matrix is gone and a clumping of fat globules into pools or pockets takes place. Although the fat coalesces into clumps, at this temperature the fat does not liquefy enough to form large pockets with a smooth surface. The indentation of individual fat globules is seen on the surface of the protein matrix.

Figure 6b shows the structure of one day old Mozzarella cheese that has been stretched with a helical viscometer (Kindstedt et al., 1989). The fat structure and distribution is different from cheese that has been melted using the modified tube test. The surface of the protein fibers appears smoother as compared to the melted samples. Large accumulations of bacteria are seen in some vacuole areas, perhaps squeezed there as the protein fibers are twisted and stretched.

There are a large number of places in the manufacturing procedure for Mozzarella cheese where a reduction in milk fat could have an effect. As noted by Yang and Taranto (1982), Tuckey (1974), and Tunnick et al. (1991), changes in the fat content of Mozzarella cheese result in changes in the physical properties, particularly melting properties. Changes in fat content will affect protein aggregation as soon as the curd is cut and become increasingly significant as the protein structure develops. In addition, decreased fat levels may affect where bacteria are found in the curd since they are now seen at the fat/protein interface after stretching. As further studies are done with reduced fat Mozzarella, ways to modify manufacture to enhance physical property development will be found.

FIGURE 1 Scanning electron micrographs of Mozzarella curd sampled at 10 (a) and 40 (b) minutes after cutting cheese curd.

1-A



1-B

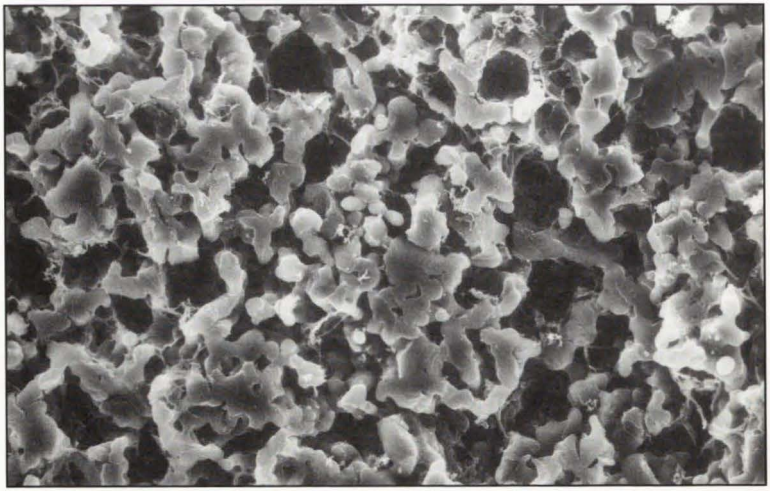
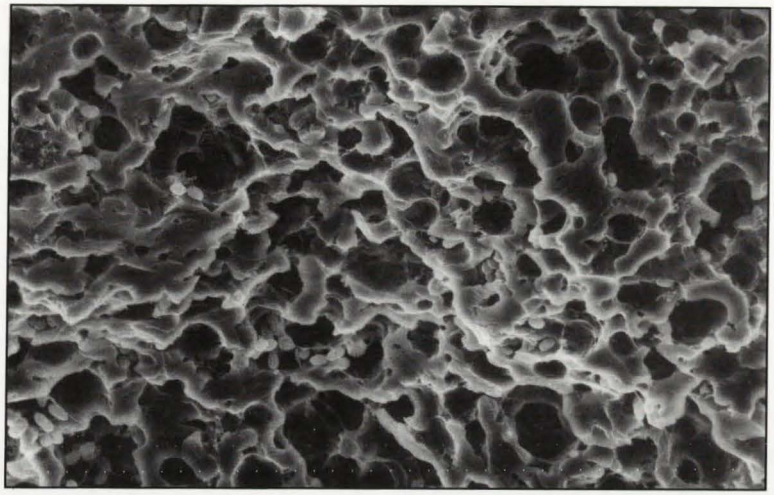


FIGURE 2 Scanning electron micrographs of Mozzarella curd taken just before (a) and after (b) the dry stirring step.

2-A



2-B

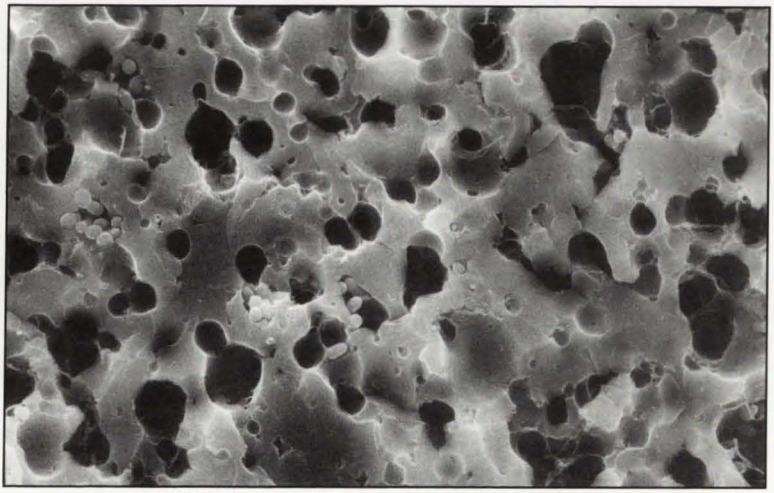
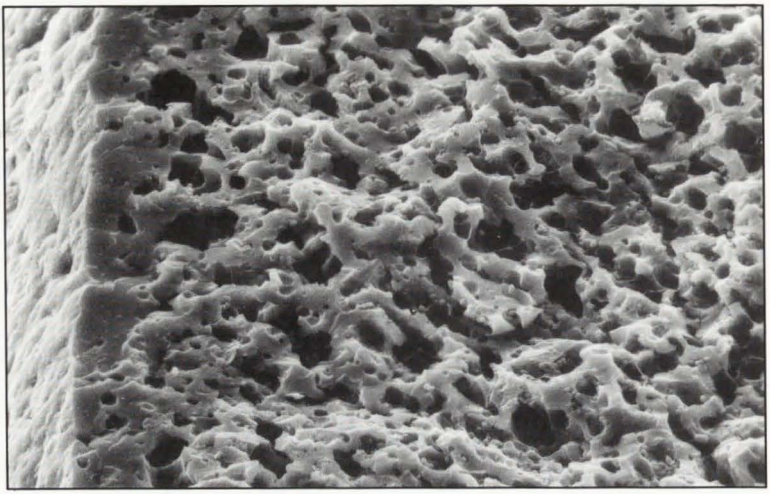
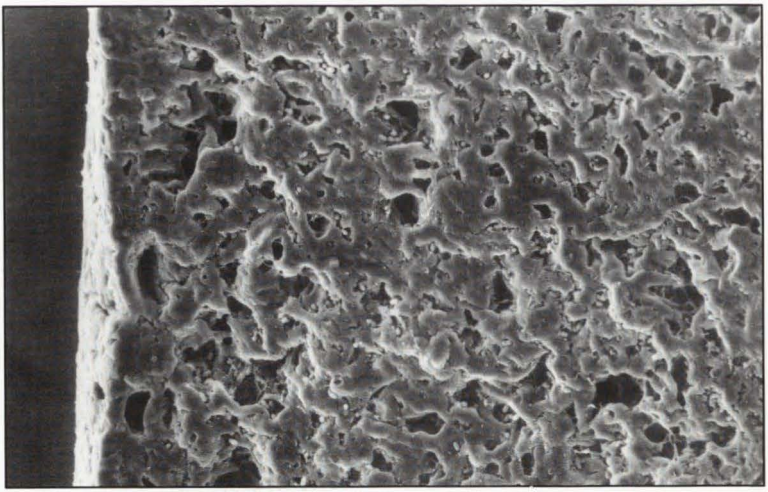


FIGURE 3 Scanning electron micrographs taken prior to dry salting (a) and following dry salting (b), and after brining (c - [following page]).

3-A



3-B



3-C

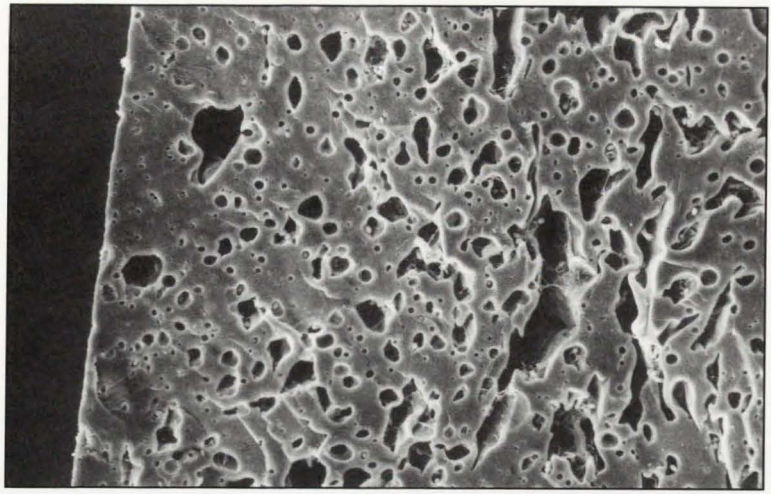
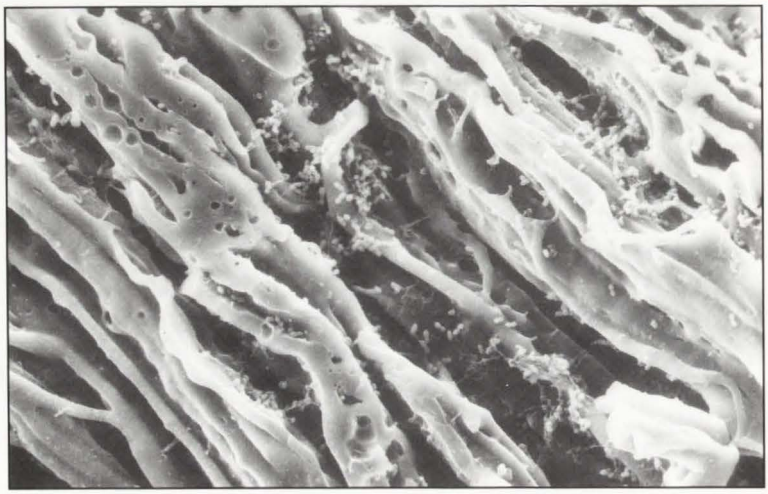


FIGURE 4 Scanning electron micrographs taken of curd after it has been through the stretcher and one hour cooling water bath, longitudinal (a) and cross section (b) views.

4-A



4-B

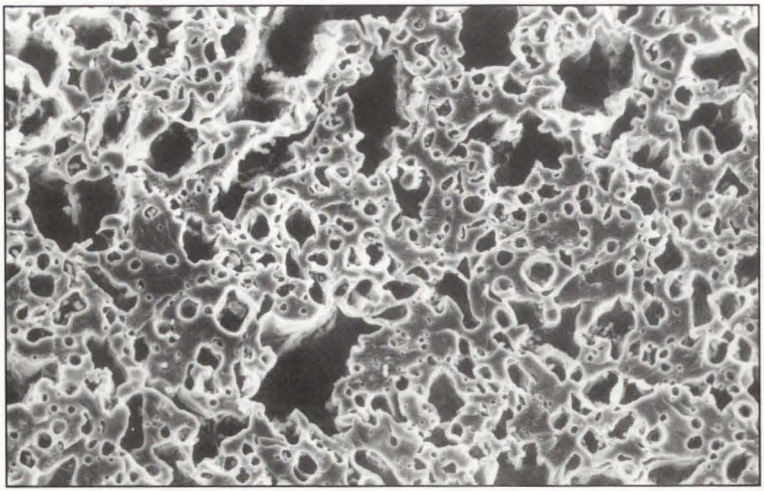
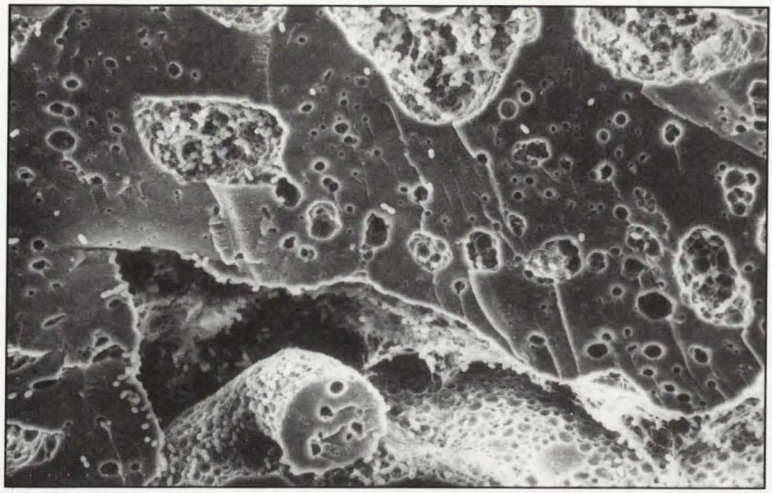


FIGURE 5 Scanning electron micrographs of Mozzarella cheese following one day of storage. Cross section (a) and longitudinal (b) views are shown.

5-A



5-B

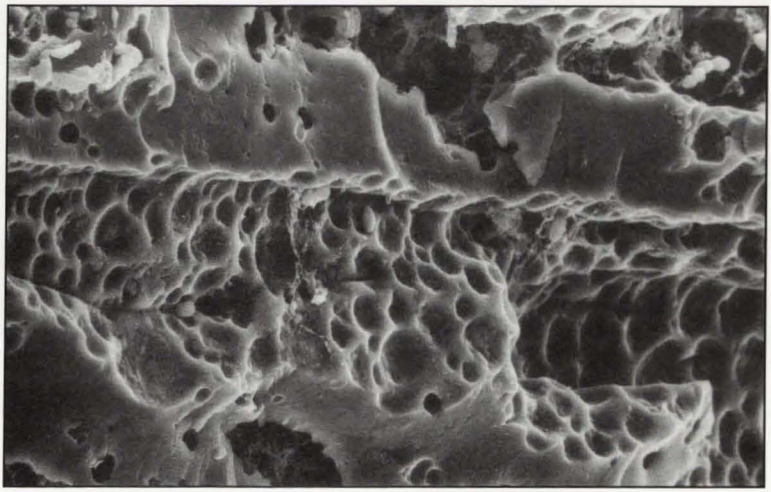
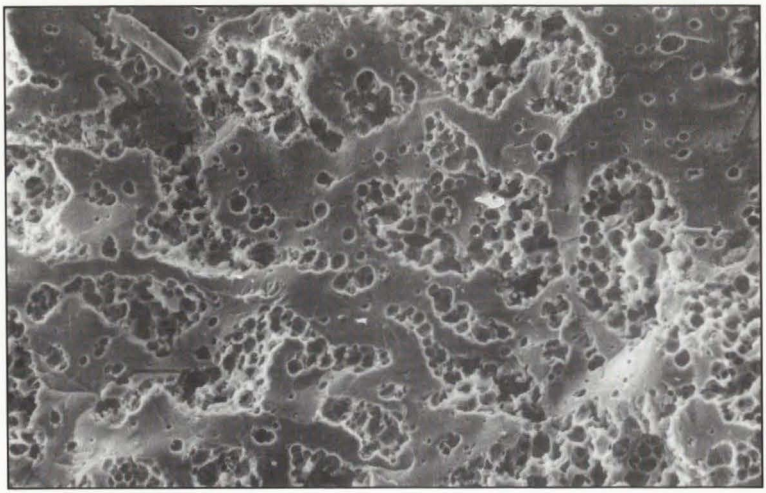
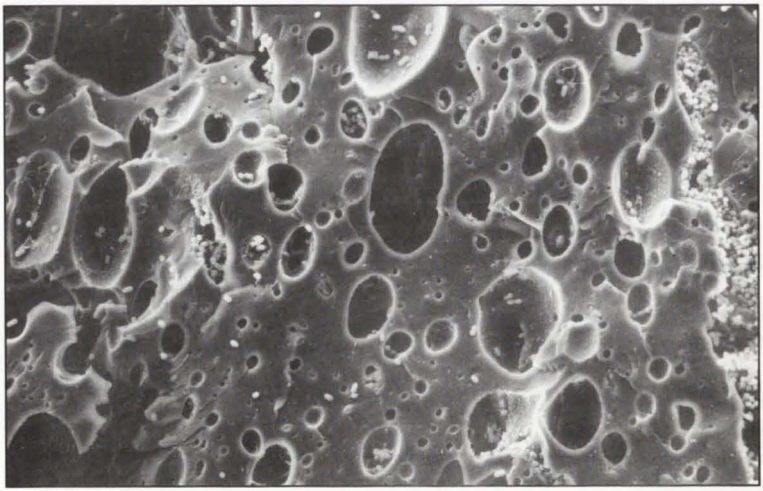


FIGURE 6 Scanning electron micrographs of one day old Mozzarella cheese that has been melted (a) using the tube test and stretched (b) with a helical viscometer.

6-A



6-B



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Plant Renovation - Engineering Design Which Yields A Leaner And Cleaner Processing Facility And Overall Environment

By

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Abstract

One fact many milk processing facilities have in common is that they were built 60 to 100 years ago and have undergone several expansions and additions. Compromises have been made resulting in a less than optimal layout as seen from the process flow, sanitary condition, building construction, and overall environmental viewpoint.

New construction of dairy plants is a capital-demanding decision which often is eliminated because of economical reasons and business hardships due to milk prices, support programs and product prices. Total renovation of an existing facility becomes a more attractive alternative for updating building structures and equipment systems, and for meeting today's and tomorrow's increased demands for larger volumes, mechanization, higher sanitary standards, and more stringent environmental regulations.

Michigan Milk Producers Association (MMPA) has, during the past five years, totally renovated its plant in Ovid, Michigan. This 90 year old typical butter and skim-powder operation has undergone more than a facelift - 80% of the 105,000 sq. ft., three-level plant has been replaced with a lean and clean modern facility housing new flexible equipment and systems. Not a single day of production has been lost during the reconstruction process; new value added food ingredients are being produced; and the price tag has been half of that for a new plant.

This presentation will be based on the MMPA renovation project, its pre-existing conditions, its renovated features and similarities with many cheese plants.

Background and History

Michigan Milk Producers Association is Michigan's largest dairy cooperative, handling approximately 3 billion pounds of milk yearly. This volume is diverted into:

- Class I fluid milk market
- Supply to two Leprino Foods Mozzarella plants
- Manufacturing in MMPA's three processing plants

The largest of MMPA's plants is in Ovid, Michigan. Prior to 1988 this facility was primarily functioning as a balancing plant processing in excess of 700,000,000 pounds of milk yearly into non-fat dry milk (NFDM) and bulk butter - commodities that are very dependent on the CCC purchase program and, therefore, typically low margin products.

The Ovid plant was constructed in 1912 and had, over the years, been modified several times, the latest being in 1980 to handle increasing volumes of milk and different commodity products. The capacity of the plant was up to 2,500,000 pounds of milk daily. However, product

flexibility and processing efficiency were extremely difficult to achieve because the whole process system was linked together as one huge process line in buildings that were very deteriorated and had inefficient space planning.

Future Goals and Objectives

In 1988, MMPA decided to focus on production of value-added proprietary customer blends and milk ingredients for the food and dairy industry to replace its commodity production. One of the goals for the future Ovid plant were to construct a modern facility with maximum plant flexibility and efficiency capable of processing a similar milk volume into high quality products.

State-of-the-art equipment, process controls, technology and an acceptable capital investment were other objectives of high rank.

Build New or Rebuild

In August of 1988, Shambaugh & Son was contracted to conduct an engineering study reviewing the entire Ovid facility as it existed and make suggestions on how to achieve MMPA goals and objectives for the future.

Part One of this four month study documented the pre-existing plant in regards to building conditions, safety standards, equipment, process lay-out, utilities, code violations, compliance with government agency regulations, staffing, product volumes and product loss levels. Part Two included a five-phase plan, with various options, for reconstruction of the facility over a four year period.

During Part Two of the engineering study, the option of building a complete new plant was considered several times. However, the existing facility had too much good equipment and newer additions to walk away from. Also, the estimated price tag of a new plant was $40,000,000 to be invested over 1-1/2 years versus a re-building cost of $23,000,000 over a 4-1/2 year period. This more acceptable capital investment level became a key factor in the decision to rebuild. Other considerations were the need for getting the construction started immediately to eliminate the very unsafe conditions of the existing processing areas.

A major demand during the extensive reconstruction process has been to keep the plant operational at all times to allow for daily uninterrupted processing of the raw milk. This, everyone agreed, would pose the most difficult challenge to the project. It has been a challenge, but it has also provided for quick change-over to new systems, on-the-spot training of operators, and elimination of the traditional costly startup period for a new plant.

Building Design

Structurally the new buildings have a steel frame with a matt foundation. All receiving, process, and product load-out areas are constructed on a single ground floor level with 18' ceiling heights. The process areas have limited access. Milk and CIP transfer lines, utility lines and support equipment are installed on a mechanical mezzanine (10' ceiling height) with rubber membrane (EPDM) roof construction. Building material surfaces were chosen as non-porous. All walls are of insulated metal sandwich panels covered with fiberglass reinforced panels (FRP) on the interior side. The mezzanine floors are constructed of corrugated metal panels with FRP

panels as ceiling in all sanitary areas. All floors in the process areas have a top surface of 3/4" dairy tiles laid in an asphalt membrane on a concrete slab foundation designed for carrying heavy loads at any given point.

This new building design varies greatly from the demolished buildings in accommodating the process flow and sanitation standards. A basement, the two-story process floors, and approximately 15 different roof levels and joints between building additions of the old structure have been replaced by a single level of processing with a service mezzanine above throughout the facility with uniform roof and service elevations.

Sanitary Design and Installation Features

The process areas on the ground floor level are designed to include only the primary processing equipment and piping system of stainless steel. All non-sanitary utility systems are installed in the mezzanine level above with vertical drops to the use points. This provides for easy access to perform preventative maintenance without production interruption and contamination of the clean process area. It also leaves a process area more friendly for being kept clean at all times.

The equipment is arranged with ample access aisle ways and with the piping system supported by floor mounted racks. Electrical conduits, hose stations, interface panels and COP tanks are kept at least 12" off walls and floors to leave easy access for room cleaning and sanitation. Process drain lines and divert lines are piped to drain hubs in order to keep a dry room environment. The air conditioning system utilizes 95% efficient Heppa filters and provides outside air makeup capable of supplying 15 air exchanges per hour and over-pressurizing the process, blending, and silo alley areas and exhaust through the CIP rooms.

All milk receiving, processing, blending, further processing, storage, and load-out operations take place in a totally enclosed system where all product contact surfaces can be cleaned and sanitized in-line. The two central CIP systems, one for the raw product side and one for the pasteurized product side, are each designed as split systems for light and heavy soiled equipment. Both systems are designed as chemical re-use systems and both have a product pre-rinse, recovery, and storage system integrated. Block and bleed valve arrangements are installed at the CIP/water supply points of each cleaning loop to protect CIP solutions from entering the water flush/pre-rinse cycle which is recovered for re-processing.

Cleaning chemicals are received in bulk trucks, stored in silos and distributed to the CIP solution tanks via a day tank system. This eliminates multiple areas in the plant where high strength chemicals must be stored and keeps the environmental safe and clean.

The two central CIP systems have replaced nine single use CIP units, thereby saving the plant considerable costs for chemicals, water and heating energy. Furthermore, the designed product pre-rinse recovery system has permitted the collection of a significant amount of milk solids for re-processing, which before were discharged as waste water.

Computer Integrated Processing Controls

Prior to 1988, the entire plant was operated via pushbuttons and relay switches with manual log keeping of process operation information. Now the equipment automation, process, utility, and control system is built up as a PLC distributed network designed to allow for later incorporation of supervisory, laboratory, and management information and interface functions.

A total of 15 PLC's are installed for the complete automation of the plant. Fourteen operator interface panels are distributed throughout the receiving, processing, and utility areas. These interface panels are for information and operation functions linked to three main control centers containing five computer stations with printers and all required legal chart recorders.

The three control centers are each a separate room with sound protection and climate control and are located adjacent to the main process floor for raw milk storage and processing, pasteurized product blending/storage and evaporator/dryer operations.

Milk volumes, truck load information, milk storage, product recipes, product standardization and CIP cycles are pre-programmed, monitored, and recorded via customized controls software. Information gathering and data logging start at the milk receiving stations and continue through to final product load-out.

This new process control system has automated all start-up and shut-down sequences; it is performing continuous monitoring and fine-tuning of the process in accordance to the selected parameters; and it documents the entire production - all functions that are highly appreciated by operators and management. Process interface is easy, the finished product composition is uniform, and the quality is first grade. Also, working in the control room environment is preferable to the noisy process floor.

Product Flexibility and Processing Efficiency

The transfer from manufacturing of two bulk products (NFDM and butter) in one huge processing line to a variety of products and blended, liquid, or dry food ingredients has demanded an isolation of each process step from the next.

Milk pre-heating, skimming, in-line standardization, and cooling are now done in high efficient plate heat exchangers without interlink to the evaporators.

Additional milk storage capacity is added for both raw milk, pre-processed and final products. Two pasteurizer systems for raw and blended pasteurized products are installed. Some new equipment was purchased, some existing equipment in good condition has been traded for higher capacity components and other equipment or systems have been totally refurbished and updated. The "new" Ovid plant is capable of processing 2,900,000 pounds of milk in 16 hours.

The result of the updated process flow design is an independence between the process lines at start-up, shut-down, CIP, and, eventually, break down periods. Production scheduling, product flexibility, and efficiency have been optimized and, because of breakage of the process line length, product losses have almost been eliminated.

Because of ample raw milk storage capacity, milk receiving is not tied into operating the complete plant. Production of skimmed, standardized, or pasteurized products for liquid bulk sale can now take place without having the milk evaporators running as part of the process line for pre-heating prior to separation. Production planning and scheduling of operators have become manageable and the volume of milk processed per man hour has increased dramatically.

Environmental Improvements

In addition to installing chemical re-use CIP systems and product pre-rinse recovery systems, other environmental problems had to be addressed. These issues were:

- Total chemical usage
- Phosphorous discharge
- Daily effluent volumes
- Water hardness (approximately 29 grains)
- Evaporator condensate discharge (cow water)
- Bulk chemical unloading
- Product spillage - silo overflow and line losses
- Separator sludge discharge.
- Non-contact cooling water discharge

The plant owns 80 acres of land for waste water irrigation. Because effluent volumes consistently peaked at 600,000 gallons per day versus the rated 300,000 gallons per day, the plant faced a decision to build a $3,000,000 treatment facility or reduce the hydraulic load and the phosphorous discharge.

An approval was obtained from the Michigan Department of Public Health (MDPH) and USDA for use of Reverse Osmosis (RO) and Ultraviolet (UV) purified cow water as process water for:

- System startup prior to production
- Water tracing of products at shutdown
- Pre-rinse, chemical and sanitizer makeup water in CIP systems
- Water for hose stations throughout the plant for internal and external rinsing and cleaning of equipment, process systems and milk trucks
- Water for circulation or single pass through plate heat exchanger
- Water supply to boilers

Installation of the purified cow water treatment and distribution system has allowed for in-plant use of 90% of the evaporator condensate produced and, together with the two re-use CIP systems, cut the total plant waste water peak volume to 235,000 gallons, well within the hydraulic capacity of the irrigation field. Expressed in ratio of annual waste water volume to processed milk volume, this represents a drop from 2.77 lb/lb to 1.08 lb/lb.

The availability of soft purified water for use in all cleaning, sanitation and rinse applications as an alternative to hard well water has allowed the plant to substitute the pre-existing 18 different special chemicals with four standard chemicals realizing a savings of approximately $10,000 per month. Furthermore, phosphoric acid has been replaced with a blend of nitric and sulfuric acid which has reduced the phosphorous discharge from 60 ppm to below 8 ppm, mainly representing phosphates from residual milk rinses.

The renovation project has also diverted the single use cooling water from the plant's process drain system to discharge via a dedicated storm water drain system to a nearby creek.

To be in compliance with the EPA standard for receiving and unloading of bulk chemicals, an outside truck unloading retainage pad with curbs and a shutoff drain valve arrangement have been established. Thus, eventually chemicals spilled can be recovered and the pad cleaned through the process drain system prior to diverting back to the storm sewer system for surface water drainage.

Hereby the personnel safety factor of handling concentrated chemicals has been improved and the risk for a chemical contamination of the recipient stream of the plant's storm water system has been minimized.

For collection of the milk separator de-sludge, a system is designed for each machine to automatically pump and water flush this to an outside portable tank. The system is cleaned in place together with CIP of the separator. A pig farmer replaces the 1,000 gallon portable tank daily and uses its content for feed.

The result is less milk solids discharged to the process drain, a lower BOD load with less phosphor to the wastewater irrigation field, and a more sanitary processing floor environment.

Mechanical Utility System

The renovation project has included sizing and new installation of all utility mains and branches for the entire plant. The boiler room and air compressors have remained untouched.

Four reciprocating, 100 ton each, ammonia compressors have been refurbished and reinstalled with a new 235 ton screw compressor. The two existing 200 ton glycol chillers have been refurbished and reconfigured for the new loads. One evaporative condenser has been relocated and a larger new unit installed. All other mechanical utility equipment such as a chilled water system, hot water systems, and cooling towers have been replaced with new equipment of proper size and efficiency.

This extensive redesign and rebuilding of the entire plant's mechanical service system have improved the reliability tremendously, have made maintenance a safe preventive issue, and basically eliminated the costly emergency break down situations which were frequent in the pre-existing plant.

Electrical Installation Improvement

Due to serious problems with code violations and safety of the pre-existing plant's electrical transformers, power distribution and installations, all the electrical service systems have been replaced and upgraded from the main power switch to final points of use.

The "new" plant is serviced by a 3000 KVA indoor transformer and ten distributed motor control centers. Power factor correction features are installed for energy optimization.

A uniform 440/220/110-volt power supply is used plant wide. All electrical and control wiring installation in processing areas is done in PVC- covered rigid conduits with flex connections to use points.

The benefits of the electrical service improvements can be summarized as follows:

- Elimination of safety hazard
- Optimal sanitary conditions (rusty conduits and panels are removed)
- Greatly reduced downtime for maintenance

- Power source is isolated by process area (if one equipment component is taken out of service, the remaining systems can continue to operate)
- One centrally located high voltage power supply versus several
- Plantwide security lighting
- Outdated components are replaced with current.

Keep An Old Plant Running While Installing a Sophisticated New Plant

The successful completion of a complicated renovation project such as MMPA involves detailed planning, coordination and communication. These issues become the key for project success and must be in effect 24 hours of the day, seven days per week.

For plant operators and process and control engineers, specific demands for quick changeover from "old system" to "new system" become daily routines when constructing new production systems while tearing down the old. Specific requirements to the following issues are a must:

- Partnership relation between owner and contractor in all levels of the project organization.
- Excellent coordination between plant operator, food process engineers and control engineers; between pipefitters and electricians; and between engineers and skilled construction workforce.
- Full understanding of old process and control system to keep it running while new is being installed.
- Thorough testing of new systems before switchover from old to new.
- Long hours of start-up once switchover is made.
- Switchover is often done in small sections of overall process area to keep downtime to a minimum.
- Training has to be done "on the fly" as operators go directly from old controls to new controls with little time to learn the new before they are put into operation. This can require considerable time spent on guiding and leading operators in their tasks.

Building a "new" plant in the location where the old one is operating generates numerous concerns relating to sanitary production conditions during the construction period. Temporary partitioning off of production areas from construction areas and working closely with the plant's quality control personnel, production supervision and government agency inspectors have been a necessity. The tight teamwork where all parties have had full understanding of the ongoing activities has resulted in a positive attitude towards accepting the implemented changes and getting the systems working. Successfully it can be reported that not a single day of production has been lost even though more than 30 full time craftsmen and three engineers have been occupied with the MMPA renovation project through the entire 45 construction months.

A Trusting Partnership Relationship between Owner and Contractor

The key words in the business world of the 90's are: TCM (Time Compression Management), IQM (Integrated Quality Management) and TQM (Total Quality Management). However, when establishing a four to five year commercial construction relationship, these words become subconscious. The process of understanding and respecting each individual's strength becomes obvious and the learning process of a trusting relationship is evident.

It is said that you can line up all the engineers in the world - end to end - and still not reach the marketplace; but by creating a True Trusting Partnership and having a positive attitude, we can build out problems - and not build in conflicts. We can, with Trust, Partnership, and Motivation, maximize efficiency and make the impossible possible: Build a new lean and clean plant in the location where we are still operating the old one and create an overall better environment for the benefit of all of us.

State of Research Concerning *Streptococcus salivarius ssp. thermophilus* and *Lactobacillus delbreuckii* Bacteriophage

By

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Introduction

In 1915, F. W. Twort reported a "disease" of micrococcal bacteria caused by a filterable, infectious agent that could reproduce itself. Two years later, unaware of Twort's discovery, F. d'Herelle reported the discovery of a similar "disease" of the enteric bacterium *Shigella*. These reports began the research that has not only explained the nature of these agents as bacterial viruses (bacteriophage or phage), but has played a major role in the development of modern molecular biology. Prior to the discovery of antibiotics in the 1940's, bacteriophages were thought of as potential agents for the control of bacterial diseases of man and have been essential tools for the elucidation of the molecular nature of life (for a review of the history of bacteriophage research, see Duckworth, 1987). At the same time, bacteriophages are the bane of bacterial fermentation industries. It is said that Nature abhors a vacuum and we could add that Nature does not think much of the high populations of isogenic bacteria that we strive for in dairy fermentations either.

The existence of bacteriophage that infect lactic acid bacteria, specifically lactococci, was first reported in 1936 (Whitehead and Cox, 1936). Subsequently, reports of phage attack on all types of starter cultures have been reported (for reviews see Klaenhammer, 1984; Sanders, 1987; Davies and Gasson, 1984).

Bacteriophages of Italian cheese starters have received less attention from the research community than phages of the mesophilic lactococci, and the state of our knowledge of *Lactobacillus delbreuckii* and *Streptococcus thermophilus* phages was reviewed by Dr. M.E. Sanders in her presentation to the Italian Cheese Conference in 1990. Since that time new information has become available on the morphology, host range, and molecular biology of both *S. thermophilus* and *L. delbreuckii* phages. These reports and an analysis of the meaning of this information for the Italian cheese industry are the subject of this presentation.

Streptococcus thermophilus Bacteriophage

It is difficult to document, but the general feeling is that *S. thermophilus* is more susceptible to phage attack than the lactobacilli component of Italian cheese starters. A number of factors contribute to this lack of understanding (reviewed by Sanders, ICS 1990), among them being the difficulty in confirming the presence of lactobacilli phages by traditional agar plate plaque assay.

Rajagopal and Sandine (1989) reported that of six Italian cheese plants reporting slow make procedures, whey samples from five of the plants contained *S. Thermophilus* phage while only one contained *Lactobacillus delbreuckii* phage. Our experience at Marschall Products

supports the belief that there are more phage problems associated with *S. thermophilus* than with the rod component of Italian starter cultures, as well as the belief that thermophilic starters are less susceptible to phage attack than the mesophilic starter lactococci.

Recent reports are beginning to form a cohesive picture of *S. thermophilus* phage populations. As a group, all such phages studied are double-stranded DNA phages of the Bradley Bl morphotype, i.e., they are tailed phages with isometric-shaped capsids or heads. The genomes of these phages range from 30-50 kilobases (Benbadis et al., 1990; Accolas and Spillman, 1979; Neve et al., 1989). Studies by two independent groups indicate that *S. thermophilus* phages, unlike lactococcal phages, belong to a single DNA homology group. The collections upon which these studies were done were recently compared, and the phages in the two collections belong to the same DNA homology group. In addition to DNA homology, phage protein analysis indicates that phages of the single homology group can be further classified into two sub-groups based on the size of the major phage proteins (Benbadis et al., 1990; Neve et al., 1989; Prevots et al., 1989). Unlike the phages characterized above, virulent phages of S. thermophilus isolated in Finland had major phage proteins whose size indicate that the phage may not belong to the single DNA homology group described previously (Kivi et al., 1987). DNA homology studies are needed on these phages to determine whether or not they constitute a second homology group.

If it is true that only one *S. thermophilus* phage DNA homology group exists, perhaps indicating that they all are derived from a single ancestor, it may be more likely that mutations in the host genome which yield greater phage resistance could give the cell resistance to a wide spectrum of phage encountered in the cheese-making environment. In addition, the chances of success for strategies of host strain modification for phage resistance may be enhanced since they could be targeted against phage components that are shared by most if not all *S. thermophilus* phages.

Lactobacillus delbreuckii Bacteriophage

The bacteriophages of *Lactobacillus delbreuckii* ssp. *bulgaricus* and ssp. *lactis* are double-stranded DNA phages of Bradley Bl morphotype. At least two DNA homology types exist with the two types identified to date employing different genome packaging mechanisms. The *cos*-packaging type has a small, isometric capsid and a long, non-contractile tail of approximately 130nm with a 20nm tail fiber (Forsman and Alatossava, 1991). *Cos*-packaging type phage DNA is synthesized in linear concatamers with the packaged phage genome delineated by a specific sequence or *cos* site where terminase protein cuts the DNA prior to the completion of packaging. Each head is filled with a phage genome having identical, homologous, single-stranded ends (Black, 1988).

The *pac*-packaging type phage also has a small, isometric capsid and long, non-contractile tail of approximately 170nm with a tail fiber of 40nm. The genome of a representative isolate (LL-H) of this group was 34 kilobases in size, and five of the seven phage structural protein encoding regions were identified by sub-cloning and expression of sub-cloned DNA in *E. coli* (Trautwetter et al., 1986; Forsman and Alatossava, 1991).

Pac-packaging type phage DNA is synthesized in linear concatamers but differs from the *cos*-type in that there is no specific sequence defining the site at which the phage genome is cut for packaging. The genome is cut in a leading end region, a region that does not code for any phage components, that precedes the phage genome in the concatomeric string. The DNA is then packaged in a processive manner until a full phage head of DNA is packaged. In model

systems such as P22 phage of *Salmonella*, the DNA packaged is greater than 100% of the phage genome; however, the amount of DNA packaged is consistent for each packaging round. When the phage head has been filled, the packaged DNA is cut away from the concatamer in the leading end section of the next genome segment. The resulting packaged DNA, therefore, has redundant termini, i.e., packaged DNA has similar sequences on both ends of the genome, and if enough rounds of packaging are initiated from a particular concatamer, eventually the DNA packaged will be identical to the first packaged genome, i.e., the packaged genomes are circularly permuted (Black, 1988).

Phage LL-H (*pac*-type) was found to be related to a group of *L. delbreuckii* ssp. *bulgaricus* and ssp. *lactis* phages. Members of this group had major structural proteins of identical size and antigenicity as well as shared DNA homology. The *pac*-type and *cos*-type phages do not share homologous DNA, perhaps indicating that they do not share a common ancestor. The sharing of bacteriophages between the subspecies *bulgaricus* and *lactis* is another indication that these organisms belong in the same taxonomic group.

Interestingly, restriction patterns of the genomes of members of the LL-H group were not the same even though the level of DNA homology was high, thus indicating within a given group of phages genome structural change has occurred (Mata et al., 1986). Heteroduplex DNA analysis of LL-H and two closely related phages demonstrated the ways in which deletion and insertion had altered the genome restriction pattern (Forsman and Alatossava, 1991). Similarly, phage host range was found not to correspond to DNA homology patterns in *S. thermophilus* phages, perhaps indicating that genome rearrangement has some effect on phage/host specificity (Benbadis et al., 1990) although restriction patterns were not correlated with host range in this study.

Within the LL-H DNA homology group are both temperate and virulent phages which suggest that temperate phages may be a reservoir for virulent phages in *L. delbreuckii* ssp. (Mata et al., 1986). This does not appear to be true of the mesophilic lactococci or *S. thermophilus* bacteriophage (Jarvis, 1984).

The data on the variability in genomic structure of lactic acid bacteria supplements data already in the literature on genomic variation in phage which are specific for a number of bacterial genera. It appears that this process is important in the overall process of phage evolution (Ackermann and DuBow, 1987).

Lactobacillus helveticus Bacteriophage

All *L. helveticus* phages studied thus far are tailed phages with a contractile sheath belonging to the Bradley Group A. A short (160nm) and a long (260nm) tailed phage type have been identified with short-tailed phages being the most prevalent isolates. Three phage groups were identified, based on host range, from a group of 35 phage isolates (Séchaud et al., 1992).

Séchaud et al. also observed increased phage resistance in lysogenic *L. helveticus* strains. This increase in resistance was characterized by shift in phage sensitivity grouping and super-infection immunity. The phenomenon of super-infection immunity was especially clear in one lysogenic strain that was resistant to all phages tested. When this strain was cured of its prophage, it became sensitive. What was not determined in this particular study was whether the presence of temperate phage in the host genome protected the host only from phage in the same phage DNA homology group or whether the protection was wider in scope.

The phenomenon of lysogen-related phage resistance clouds the issue of whether or not we should have lysogenic bacteria in starter cultures. On the one hand, lysogenic bacteria can be the source of virulent phage; but, on the other, if the lysogen is cured of its temperate phage, it may be sensitive to a wider range of bacteriophage.

"New" Bacteriophage

It is a common experience that new strains in a plant have a "grace" or "honeymoon" period where they are relatively free of phage attack and it seems that "all of the sudden" or "over night" the strain begins to have phage problems. This is especially puzzling since plant whey samples are usually screened to insure that the plant is free of phage which attacks the new strain before it is introduced. Most often, however, this phage screening is done with the relatively insensitive BCP-milk assay which may not detect low levels of lytic phage in the plant.

Instead of a relatively quick emergence of phage which attacks a given strain, it is more likely that the "over night" phenomenon is the product of the "grace" period wherein phage types existing in the plant, propagated on other starter cultures or perhaps by organisms in the milk supply, challenge the defenses of the new strain. The point where we notice the effect of this challenge and response period as increased phage sensitivity is the "all of the sudden" event.

The scenario presented here also could explain our experience that new strains tend to develop phage problems at about the same rate in geographically distant locations and is consistent with our current understanding of the origins of lytic phage in dairy fermentations.

It is clear from the studies discussed above that phages of dairy starter cultures are undergoing change at the genomic level. The mechanisms responsible for these changes in starter culture phages are those of bacteriophage evolution, i.e., point mutation, recombination, genome rearrangement (Ackermann and DuBow, 1987).

Current knowledge suggests, at least for the mesophilic lactococci and *S. thermophilus*, that the source of "new" phage in a cheese plant are phages that already exist in that environment through the process of phage evolution. Couple this with the fact that modern cheese making, i.e., high through-put, tight schedules, is well designed to produce high phage populations if proper sanitation is not practiced, and we have a perfect environment for the development of "new" phage for strains introduced into the cheese plant.

By keeping phage populations in the cheese plant low, two things are accomplished: (1) Starter cultures in current use in the plant can be used without significant slow-downs for extended periods, and (2) in the event that a new strain must be integrated into the starter program, low populations of phage in the environment reduce the chances of rapid development of "new" phages for the introduced strain.

Summary

Presentations to the Italian Cheese Conference in recent years (Klaenhammer, 1989; Sanders, 1990) have stressed the potential of molecular biology techniques in the fight against bacteriophage in dairy fermentations. These hopeful messages must be tempered, however, with the realization that the state of research on bacteriophage and bacteriophage resistance in Italian starter cultures is inadequate to allow for such improvements of starter strains today.

Although the number of groups engaged in bacteriophage and bacteriophage resistance research in the thermophilic lactic acid bacteria remains small, a coherent view of the

characteristics of thermophile bacteriophages is emerging and this will aid in the proper management of Italian starter cultures as well as build a base for any future efforts to enhance phage resistance via strain modification.

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Cheese Performance And Blending; What Your Customers Are Asking

By

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I'd like to start today talking about cheese performance. What it means, what your customers are asking about it, why it's important to them, and what you can do to take advantage of what you already know about your products. First, let's define performance.

When we talk about performance, we refer to the way a particular type or variety of cheese responds to handling during preparation. Performance can also be broken out into two specific areas; cold or hot. Cold performance refers to the way a cheese responds to mechanical manipulation and the most important aspects for considerations include:

- slicing, by hand or on a machine
- slice separation
- grating
- shredding
- cubing

Cold performance would also apply to how a cheese is best handled for cutting and wrapping at retail.

Hot performance refers to the way a cheese responds to the application of heat. It would typically include:

- baking
- broiling
- sauce applications
- microwavability
- steamtable holding

Now, what are your customers asking about your product's performance? Basically all the things we just mentioned. The most common questions I receive about cheese from retailers and food service operators are in some way related to performance. And here's something else to consider. The questions they don't ask are probably even more dangerous to you than the ones they do ask. Let me give you some examples. I'll pull them right out of real situations.

A retailer has a slicing cheese section and Mozzarella is included in the selection. This particular Mozzarella is a whole milk Mozzarella because that's what stocked in the bulk cheese section for cut and wrap. The slicing cheese section is in a deli service case with an open back and the Mozzarella is stacked on top of the pile, the warmest spot. Everytime this retailer tries to slice the Mozzarella for a customer, it sticks to the slicer and just simply doesn't slice well. After going through this exercise in futility about a dozen times and having to clean the slicer everytime it's used for Mozzarella, the retailer decides there is a problem and typically does one

of two things. He either takes Mozzarella out of the slicing section or replaces it with another brand. Also, the customer who took home the sliced Mozzarella and couldn't get the slices apart probably won't return for more.

You and I may know that the whole milk Mozzarella should have been well chilled for slicing or that a part skim Mozzarella would have sliced better and offered better slice separation. But this retailer didn't know since there was no information on the package or in the box. Also, I don't remember ever seeing information from any cheese manufacturer about recommended slice thickness.

That's just one example of cold performance problems at retail. Now let's consider food service. There is a chef at a big and prestigious resort who had recently decided to start using freshly grated Parmesan on his menu. Since he was going to the trouble of grating it fresh, he wasn't just using it, he was featuring it in a number of recipes at four separate restaurants. Freshly grated Parmesan made a big difference to the quality of the taste of the dishes. They were very popular and he was using a lot of cheese. The problem that came up was that he was literally burning up his robot coupe grating cheeses. As a matter of fact, he bent the drive shafts on three machines in two months. The chef proceeded to call the manufacturer directly to see if they could recommend a machine that was well suited to grating. They told him, "We don't know anything about equipment; you need to switch to a pre-grated Parmesan." Management decided they couldn't afford to continue burning up machines, so fresh Parmesan came off the menu. Instead of using over 300 pound of Parmesan a week, that figure went down to about 30 pounds a week. This may not have been the manufacturer's fault, but they should have treated it like it was their problem.

When retailers and operators position cheese as something special and promote it accordingly, it's a real shame to end up taking that option away from them.

These are only two examples and I could go on almost indefinitely with problems related to lack of communication about product performance.

Let's move on and talk about hot performance. We already said that hot performance refers to the way a cheese responds to the application of heat. It would typically include:

- baking
- broiling
- sauce applications
- microwavability
- steamtable holding

It's important to keep in mind that cheeses perform differently depending on the source of heat. For example, baking or broiling with electric and gas will yield very different results. Also, you need to consider whether ovens are regular or convection.

In the case of hot performance, we'll start by using food service operations as an example. There is a major misconception that all chefs know how to handle cheese. The CIA, or Culinary Institute of America, is reknowned for its curriculum and many chefs who enter food service in this country take their training at the CIA. Here is the only part of their formal curriculum that mentions cheese. Sixteen words. "It can be sliced for sandwiches, cubed for party platters, or shredded and added to omelets." If you assume that chefs know how to handle your products properly and how they perform, in most cases you'll be making the wrong assumption. And in the end it's your products that will suffer.

We conduct seminars on a regular basis for food service wholesalers, distributors, and their customers who are food service operators and chefs. Once again, the most common questions are related to product performance. The questions aren't complicated either. Usually they just want some basic guidelines on how a specific cheeses melts or browns or they want tips for incorporating them into sauces. They also consistently ask about storing cheese properly. It may be second nature to you that your cheese shouldn't be frozen, but if you want them to know that, you've got to tell them. One aspect of hot performance that is probably most applicable to those of you here today is pizza. There are over 53,000 pizzerias in operation today accounting for over 10 percent of all restaurants. Presently over 60 percent of all retail stores have some sort of fresh pizza program. Pizza captures over 25 billion dollars a year in sales. Pizza is big business. Some pizza operations are large and very familiar with cheese performance as it relates to pizza, but the vast majority of operations are not as familiar with the way cheeses perform or how to test them. The lion's share of questions at seminars and conferences are regarding crust and cheese. Usually their product testing starts at square one without any basic guidelines to go on. Many operators don't know that whole milk Mozzarella melts and flows best to cover a pizza, or that part skim Mozzarella has better cold performance attributes of slicing or shredding, and browns quicker.

Just yesterday, I was at the FMI/NAWGA supermarket food service conference where I was moderating a panel on "Pizza Programs That Pan Out." As is usually the case, much of the discussion revolved around two components; crust and cheese. I'd like to share with you some of the comments made about cheese from several of the panelists.

The first panelist is a consultant for pizza operations that range in size from single unit operations to a chain with hundreds of units. He has also written a pizza manual entitled "The Pizza Managers Guide To Ingredient Purchasing And Preparation." In his talk, he told the retailers who were present that the most important ingredient to consider in their pizza operation was cheese . Here are his recommendations on selecting a cheese.

First, pick a manufacturer who will provide general guidelines for the way his products perform, and who will detail basic specifications for their products. In short, find a manufacturer who will communicate with you.

Second, when you find one, stop skimping on pennies buying bulk or commodity products. Use a branded product and specify it throughout your operation. That means that if you need guidance on product testing or have questions, you have someone to call.

Third, when you have a problem with performance, try to solve it by communicating with your manufacturer.

Another panelist was a top retailer with 120 stores that had pizza programs and he had virtually the same advice regarding cheese. Keep in mind that the retailers in attendance typically had from 50-100 stores in their operations. Also, the Mozzarella they use in their pizza programs usually ends up as the Mozzarella in the cheese case too.

Another retailer on the panel brought his in-store pizza manual with him, and it was most interesting. This retailer had three distinctly different types of pizzas in his program and had done thorough testing on his products. After he had developed his programs and tested them, he proceeded to go back and take pictures of what the pizzas should look like when done properly and then take pictures of bad pizzas. He took the time to overwork dough, use too much yeast, too little yeast, overcook pizzas, and undercook pizzas. He then photographed them so that employees could identify problems and troubleshoot when problems came up. He did the same sort of documentation and testing for the cheeses on his pizza.

I was talking to him after the session was over and he told me something that I found very interesting. He sourced all his cheese from one particular manufacturer because the manufacturer asked for pictures of his test procedures. He said that he knew if they were interested enough to want to know more about how their products were being used, they would be a more trusted resource if problems did arise. Imagine that. Just because they wanted to know how their products were being used, they added more perceived value to their products.

I cannot emphasize enough the importance of knowing how your products perform and communicating that information to your customer. Here are some things that you might consider doing to accomplish that.

Prepare guidelines for the way your products perform or their specific attributes and include them in your packaging. Information that you consider basic is exactly what your customers want - information on handling, preparation, and proper storage. If one melts quicker, list it. If one slices better or browns faster, let them know.

Familiarize yourself with the way your customers are using your products. Consider this. They are actually doing product testing for you. Their successes and failures will help you will glean valuable information.

As you find the different ways customers are using your products, document them. If, for example, one customer is using a certain type of oven that's popular in the industry, document cooking time, temperatures, and results. When you find someone using a different oven, document it the same way and compare the results. Keep this information on file, and believe me, it will come in useful. Problems with hot performance are typically affected by the type of oven being used. Examples are top heat or bottom heat, how far from the heat source, cooking times, and temperatures. You don't need to build a test kitchen to find out how your customers are presently using your products.

Put yourself in your customer's shoes. Think about ways that make it possible for them to use your products. If you are selling a hard grating cheese to a food service operator or chef, ask them what equipment they use and ask if it works well. Once again, this sort of information is easy to get. Ask your salesman, broker, distributor, or wholesaler to help in obtaining it.

It's not something you can do overnight, but it's necessary for the future of your products. Learn everything you can about how your products are being used and you will be a greater resource to your customers.

Another topic related to performance that I'd like to touch on briefly is cheese blending. When we talked about pizza, one thing I didn't mention was cheese blending. Over 90 percent of all pizzas produced today use a cheese blend of some sort. Because of the number of requests we've had to address performance and blending, we developed a brochure called "The Pizza Blend Primer," and it has received tremendous response. We have copies of this in the back of the room if you would like to pick one up on the way out.

Business is changing everyday and I'd like to read a quote from this week's Cheese Market News, entitled "Innovation, Adaptability Critical To Survive". The quote is from Lee Gentine, President of Sargento of Wisconsin.

"More than ever before, cheese marketers must get involved with their customers to survive. It's not good enough to simply make a good piece of cheese these days. More and more customers are interested in developing solid relationships with their suppliers." says Gentine. "They have more expectation of what they want and that frequently leads to the formation of alliances."

Another quote from the same article comes from Paul Christ, Vice President of Dairy Foods at Land O Lakes. He says, "These alliances are more long term and less flexible than in years past. Tolerances are more narrow because each customer wants the cheese he buys to perform in a specific way."

With new technology and make procedures, virtually every one of you out there today is making or has the ability to make cheese that meets most every performance requirement imaginable. I don't hear retailers or food service operators asking for impossible things. They just want to know how to use the products that are out there. I believe that it is imperative for cheese manufacturers to find out more about their customers and how they are using their products. I also believe that those who do so will reap the rewards. Thank you very much for the opportunity to speak with you today, and if there are any questions, I'd be happy to try and answer them for you.

Browning in Stirred-Cured, Directly-Salted Parmesan Cheese Linked to α-Dicarbonyl Production by Wild Lactobacillus Contaminants

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Abstract

Non-enzymic browning defects in cheeses have been considered to be caused largely by residual reducing sugars reacting with certain amino acids. Recent research has shown that many lactobacillus produce α-dicarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) which also provide carbonyl groups to react with free amino acids that can lead to the production of brown pigments. Quantitative measurements of α-dicarbonyls in stirred-curd, directly-salted Parmesan cheese exhibiting browning defects showed that methylglyoxal was most likely involved in the development of the browning defects in this cheese. While low to modest levels of methylglyoxal are produced by many lactic acid bacteria, some wild strains of lactobacillus (non-starter lactic acid bacteria) produce high levels of methylglyoxal. High concentrations of methylglyoxal were found in directly-salted Parmesan cheese, and thus were associated with the development of brown discolorations during aging. Therefore, control of the production of methylglyoxal by restriction of contaminating non starter lactic acid bacteria during cheesemaking should assist in suppression of browning in stirred-curd, directly-salted Parmesan cheese.

Introduction

Various forms and degrees of discoloration occur in cheese, and a variety of factors have been associated with the development of these defects. However, discussions of cheese discolorations are limited here to either browning or pinking. It is sometimes difficult to clearly differentiate between brown and pink defects or discolorations in cheese because the two colors overlap from a spectral standpoint. Further, sometimes both brown and pink discolorations may occur simultaneously in cheese resulting in brownish-pink colors. When this occurs, it appears that at least two different chemical mechanisms contribute to the color formation, one of which is the Maillard reaction.

Browning or the development of brown pigments in foods frequently results from the formation of melainin pigments derived from the Maillard reaction which initially involves reactions between carbonyl and amino compounds. In process American cheese where elevated temperatures are encountered during cooking and subsequent cooling periods, browning has been attributed to residual galactose reacting with amino acids (Bley et al., 1984). Similarly, excessive browning of Mozzarella cheese during heating has been found to be associated with residual galactose (Johnson and Olson, 1985). While any reducing sugar (i.e., lactose, glucose, and galactose) could provide the requisite carbonyl group for reaction with an amino compound,

galactose is most often implicated in the reaction when sugars are considered to be involved in the development of browning defects. When certain strains of galactose-non-fermenting lactic acid bacteria, such as *Lactobacillus delbruekii* ssp. *bulgaricus* are employed as starters, residual lactose in cheese is hydrolyzed to glucose and galactose, but only glucose is readily metabolized and removed from the cheese. As a result, corresponding residual galactose not utilized by the lactic culture accumulates, and participates in subsequent carbonyl-amino browning reactions. Browning caused by residual galactose can be prevented by screening and selecting starter cultures for the ability to readily utilize galactose. When browning problems are encounted in cheese, residual galactose always should be suspected, and analysis for reducing sugars should be performed.

Pink or reddish discolorations in cheese have also been frequently described in the literature (Barnicoat, 1937; Breed and Pederson, 1938; Park et al., 1967; Shannon et al., 1968; 1969; 1977; Shannon and Olson, 1969). Pinking in Cheddar cheese colored with annatto occurs frequently (W. Wendorff, personal communication), and it apparently occurs only in cheese containing water-soluble annatto. The chemical basis of annatto colored cheese pinking is unknown, but surface pinking occurs as a result of high-intensity lighting. Internal pinking in Cheddar cheese appears to relate to whey proteins and the sulfhydryl content of the cheese. Pinking in Swiss cheese has been associated with the use of certain strains of *Propionibacterium* (Park et al., 1967), and certain strains of lactobacilli have been implicated in a similar pink discoloration which occurs in Italian cheese varieties (Shannon et al., 1968; 1969; Shannon and Olson, 1969).

Pink discoloration in Italian varieties can occur as a uniform band beneath the surface of the cheese, as an irregular band following any cracks in the cheese, or as a uniform discoloration through the cheese (Shannon et al., 1968). The pink pigment has been associated with the fat free, nondialyzable fraction of cheese (Shannon et al., 1968). Since the pink discoloration was associated with certain strains of lactobacilli, such strains were examined for traits which might reveal the mechanism of formation of the pink pigments in cheese. Strains of *Lactobacillus delbruekii* ssp. *bulgaricus* and *Lactobacillus helveticus* associated with the pink discoloration of Italian cheese produced more highly oxidized conditions in cheese than strains not associated with the defect (Shannon et al., 1969). Development of pink discoloration was accelerated by higher curing temperatures and appeared to be enhanced by penetration of oxygen into cheese, but not by absorption of sodium chloride by the cheese.

In studies on the mechanism of the pink discoloration in Italian cheese, Shannon et al. (1977) found that addition of tyrosine to cheese intensified the pink discoloration of cheese made with a strain of *L. delbruekii* ssp. *bulgaricus* that consistently produced the defect. Strains of *salivarius* ssp. *thermophilus* which did not produce the pink defect did not yield pink cheese when tyrosine was added to the cheese. While the chemistry of the pink discoloration has not been resolved, rapid screening tests have been developed to predict the tendency of cultures to produce the defect (Shannon and Olson, 1969). Growth of cutures in either an autoclaved milk-calcium carbonate or milk-phosphate medium for five to ten days at 37 C reveal cultures that always are associated with the pink defect by development of a dark brown color in the whey of the milk calcium carbonate medium or a pink band of discoloration in the milk-phosphate medium. No discoloration is observed in the media with cultures that do not produce the pink defect in cheese.

Studies on Browning in Directly-Salted Parmesan Cheese

Stirred-curd, directly-salted Parmesan cheese has replaced traditional brine-salted Parmesan cheese for many applications where economy in manufacture is an important consideration. However, according to industry sources, stirred-curd, directly-salted Parmesan cheese frequently develops a brown pigmentation within four to six months of curing. The browning in stirred-curd, directly-salted Parmesan is different from that caused by residual galactose because freshly manufactured cheeses contain virtually no reducing sugars. Further, the browning does not appear to be related to the earlier-reported pinking because cultures employed in the manufacture of the cheese give negative results when tested (Shannon and Olson, 1969) for the tendency to cause the pink defect.

Because the development of brown pigments in foods is so widely associated with the Maillard reaction, sources of carbonyls other than reducing sugars were examined. We became involved in studies on the browning defect as a result of investigations on the production of α-dicarbonyls by lactic acid bacteria in relation to the production of cheese flavors (McDonald and Lindsay, in review a). During these studies, a method for the quantitative analysis of α-dicarbonyls in cultures and cheese was developed (McDonald and Lindsay, in review b), and it was used to examine the role of the α-dicarbonyls in browning of stirred-curd, directly-salted Parmesan cheese.

Commercial samples of directly-salted Parmesan cheese from four to ten months of age were obtained, and analysis showed that glyoxal and diacetyl concentrations did not vary significantly among the samples. However, younger samples of cheese (<4 mo) contained higher concentrations (to 1.2 ppm) of methylglyoxal than more aged samples (>8 mo; <0.3 ppm). The general color of the younger cheeses was off-white whereas more aged samples varied from light brown to dark brown. Notably, samples of cheese exhibiting browning also possessed flavors that were described as brothy, burnt and caramel, all of which are typically associated with Maillard browning. These observations supported the hypothesis that methylglyoxal was involved in the formation of the brown discoloration because the disappearance of this α-dicarbonyl compound paralleled the development of more intense brown colors and caramel-like flavors in cheese.

Model system studies were also conducted which involved combinations of glyoxal, methylglyoxal, or diacetyl alone or in combination with amino acids, including tyrosine. Methylglyoxal reacted readily with tyrosine yielding red-brown precipitates while glyoxal and diacetyl were much less reactive. Additional model systems were prepared where a medium-aged uncolored Cheddar cheese was used as a base for incorporation of the α-dicarbonyl compounds. While all of the compounds formed some brownish pigmentation, methylglyoxal gave much more intense brownish pigments in the cheese base. Therefore, these trials showed that the α-dicarbonyls gave brown pigments when contacted with suitable amino compounds.

Samples of unripened (ca 1 mo) commercial brine-salted and direct salted Parmesan cheese were analyzed and compared for concentrations of α-dicarbonyls, and much higher concentrations of methylglyoxal were consistently found in the direct-salted cheese samples (to 6 ppm) compared to brine-salted samples (to 0.7 ppm). Thus, the potential for development of brown pigments was found to be much greater in the direct-salted Parmesan cheeses even though the defect was not yet apparent.

Analysis of samples of curd and cheese obtained consecutively during the various stages of manufacture of stirred-curd, directly-salted Parmesan cheese provided some key information. The concentration of methylglyoxal rose rapidly between salting (ca 1.5 ppm) and after pressing

(to 3.7 ppm) indicating that the conditions for production of methylglyoxal were very favorable during this period of time. Since the introduction of salt was a major variable at this point, trials were carried out to determine the effects of salt upon the starter culture (*L. helveticus*) used in the manufacture of the cheese. This culture produced only a very modest level of α-dicarbonyls, and in MRS nutrient broth it yielded only about 0.2 ppm of methylglyoxal. Further, in the presence of six percent sodium chloride in a modified MRS nutrient broth, the production of methylglyoxal was not noticeably altered, and the production of glyoxal or diacetyl was also low and unaltered by the presence of the salt. Thus, a connection between higher salt concentrations provided by direct-salting procedures and methylglyoxal production was not established, and evidence for involvement of the starter culture was not obtained.

Earlier studies on the production of α-dicarbonyl compounds by lactic acid bacteria had revealed that most produced these compounds, but many wild strains of *Lactobacillus* sp. produced high levels (to 8 ppm) of methylglyoxal (McDonald and Lindsay, in review a). Apparently, low to modest levels of the α-dicarbonyls contribute to cheese flavors through Maillard-related reactions, but when excessive concentrartions are produced, brothy off-flavors and brown pigments result.

The procedure of direct-salting of the Parmesan curd would provide an opportunity for introduction of the wild lactic culture that is inherently present in every cheese factory. As a result of introduction of highly competitive wild lactic acid bacteria at the point of salting, proliferation would occur in the freshly-pressed curd, and could account for the rapid formation of methylglyoxal observed during pressing. Such a series of events is highly compatible with the observations and results obtained in this study.

In summary, measurements of concentrations of methylglyoxal have established a relationship between methylglyoxal concentrations and the development of browning in stirred-curd, directly-salted Parmesan cheese. Since the *L. helveticus* starter cultures produced only modest concentrations of methylglyoxal, it was unlikely that such levels of this compound were sufficient to contribute to browning. However, since many wild lactobacilli produce high concentrations of methylglyoxal, contamination of curd during the salting stage of manufacture of stirred curd Parmesan appeared to be related to the production of elevated concentrations of methylglyoxal observed after pressing and subsequently during the early stages of ripening. Thus, while other factors affecting the production of α-dicarbonyls may be discovered subsequently, the studies strongly supported the hypothesis that contamination of the curd with wild lactic acid bacteria was at least in part responsible for the development of browning in stirred-curd, directly-salted Parmesan cheese.

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1993-1

Automatic Mozzarella Processing System

By

Keith Ray
Manager of Engineering
Stainless Steel Fabricating, Inc.
Columbus, WI

Abstract

Our interviews with cheese makers, owners, managers, and top management of mozzarella processing companies across the United States resulted in our quest for accurate data from which an automatic Mozzarella processing system could be designed.

Definition

An "automatic Mozzarella processing system" is one capable of handling curd to product ready for packaging.

The Challenges

1. Cheese Composition Variables
   - pre-salting
   - solids
   - butterfat
2. Final Product Variables
   - size and shape
   - weight control
   - appearance
   - dimensional stability
3. Cooling
4. Production Speeds
5. Product Quality
6. USDA Requirements
7. Cost
8. Space

The Solutions

1. Data Gathering
2. Modular Concept Design
3. Field Testing
4. Data Gathering

Data Gathering

Our discussion today is limited to data gathering which continues to be an ongoing effort. As is often the case, machine design and testing appears to be far less of a challenge than gathering sufficiently accurate data from which design criteria can be established.

Cooling

And of all the challenges facing us in system design, by far the most demanding is that of cooling the Mozzarella to the kind of target core temperatures necessary. As a result, we are limiting our presentation to cooling and to all those variables associated with that process.

During this discussion, we have to make the assumption that all other variables remain constant so we can first begin to solve this most challenging riddle.

The Cooling Challenges

Cooling, in turn, is dependent upon an impressive list of variables experienced every day in the Mozzarella cheese making process:

- solids
- butterfat
- salting techniques
- production speed

And if we may keep these previous variables constant for the purpose of this discussion, we can center our attention on the cooling methodology itself and its primary criteria:

- cooling media
- cooling technique
- system costs

Disregarding hybrid solutions because of the issue of reasonable cost while accepting sweet water as the media of choice left us with uncovering cooling techniques.

To examine the relative success of the various "automatic" techniques now used to produce Mozzarella, we went into the field and gathered cooling data on the various machines.

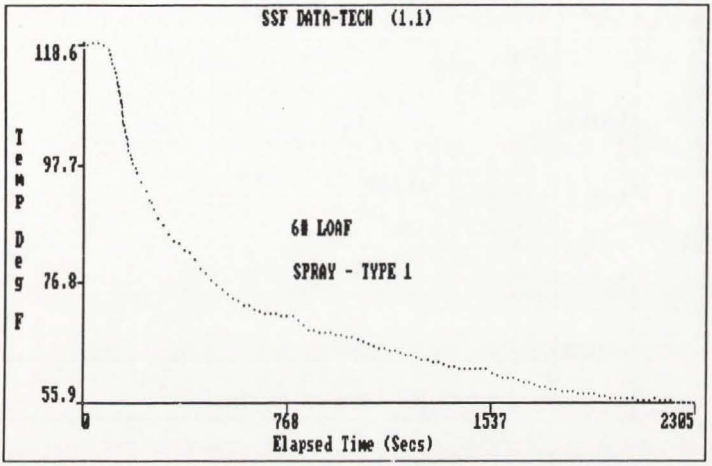
Field Data Gathering

For our purposes, we have defined the various manufacturing systems according to how they cool the product:

- spray
- immersion
- transfer

The following curves represent the data gathered from our investigation

Slide 1 qqq

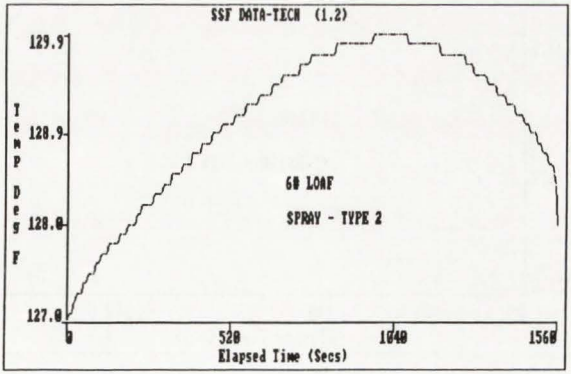


Cooling Technique: SPRAY - TYPE I

Graph demonstrates skin temperature cooling as heat is transferred to the colder, stainless steel mold. Within 5 minutes, the product is cool enough to be discharged without losing its shape.

Reducing the core temperature to the 46° F. using the same technique, however, would require more than 26 hours!

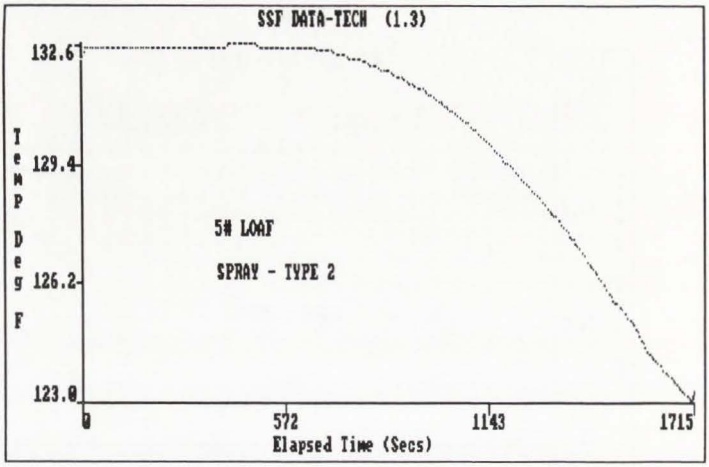
Slide 2



Cooling Technique: SPRAY - TYPE 2

With this technique, the core of the loaf cools about 2° F. in 26 minutes. Notice that 15 minutes pass before cooling even begins.

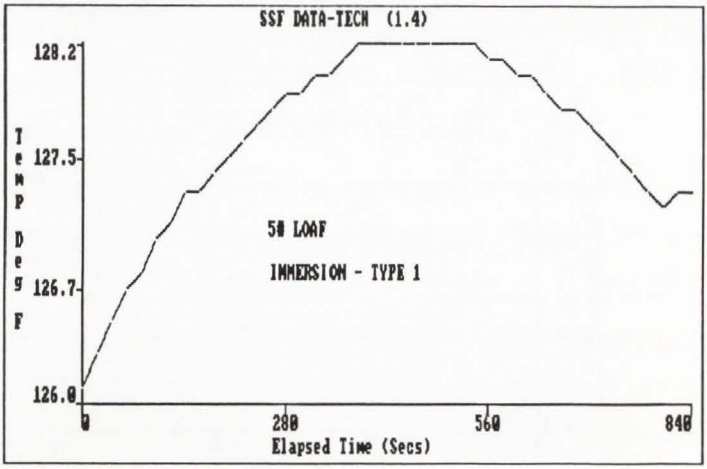
Slide 3



Cooling Technique: SPRAY - TYPE 2

This is a gradient in the loaf approximately 3/4" from the surface, indicating cooling is inversely proportional to the distance from the skin surface.

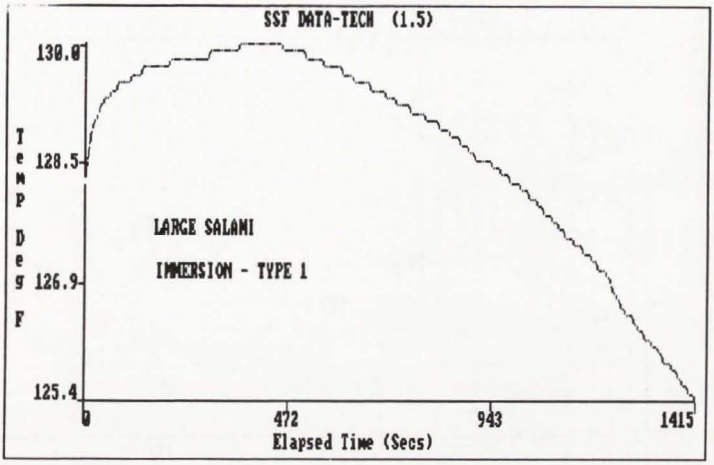
Slide 4



Cooling Technique: IMMERSION - TYPE 1

The "flat" portion of the graph demonstrates this product did not cool any measurable amount until it was discharged from the machine into the molder.

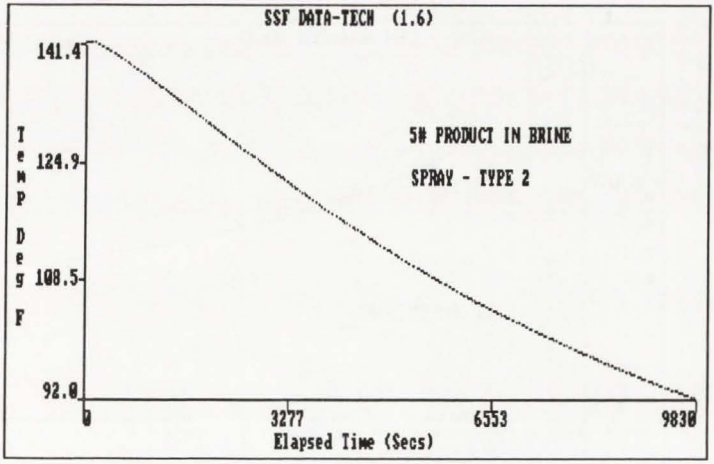
Slide 5



Cooling Technique: IMMERSION - TYPE I

Once again the phenomenon of a "flat" on the graph demonstrates a lack of cooling of this product until discharge into the brine. It seems apparent that immersion techniques allow the onset of a thermal barrier around the mold.

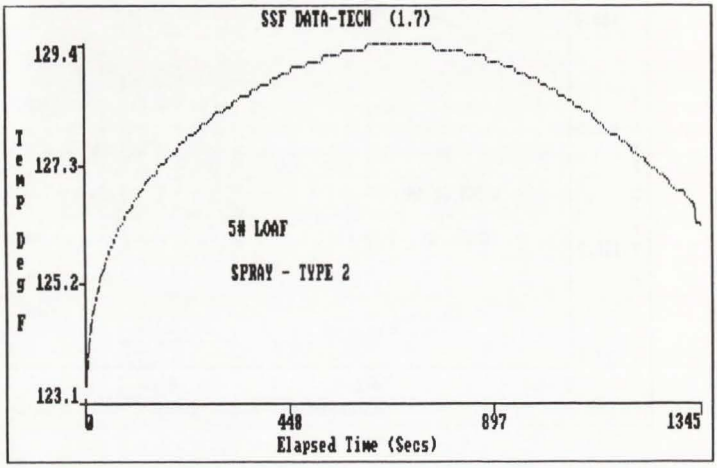
Slide 6



Cooling Technique: 5# LOAF IN BRINE

Classical cooling curve for a homogeneous substance.

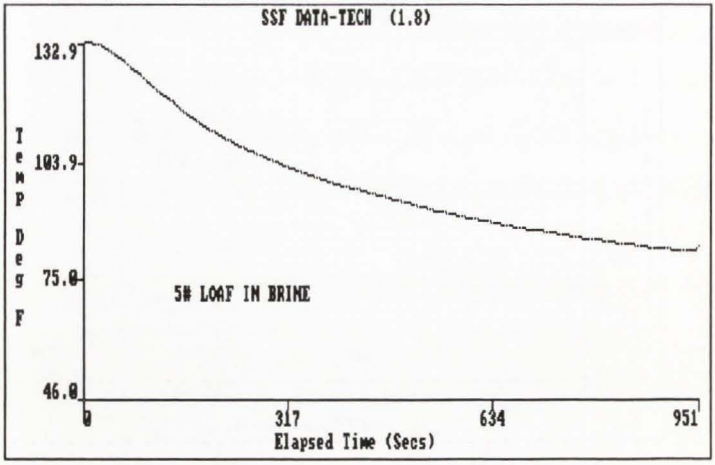
Slide 7



Cooling Technique: SPRAY - TYPE 2

4° F. cooling took place within the product's core after about 11 minutes of exposure to the spray system.

Slide 8



Cooling Technique: 5# Loaf after discharge into brine.

Asymptotically approaching 46° F. brine temperature with time.

Conclusion

The data demands a new look at the phenomenon of cooling Mozzarella with respect to the techniques used and the media employed.

It appears obvious that while immersion does little to reduce core temperature, the several types of spray cooling systems on the market need additional attention if "packageable" product is the goal.

Our design team has been working steadily on successfully on a unique, commercial solution to this challenge. We welcome your input.

How Starter Culture Metabolism Affects Properties of Mozzarella Cheese

By

Robert W. Hutkins
University of Nebraska-Lincoln

Abstract

The function of lactic starter cultures in cheesemaking is quite simple - the culture should ferment the sugars in milk and produce lactic acid, such that a desirable pH is reached within a suitable time. In the manufacture of Mozzarella cheese, however, there are additional requirements that Mozzarella cultures must satisfy.

These additional requirements may not easily be met because many of the functional properties of Mozzarella cheese are influenced by the metabolic behavior of the cultures used to make the cheese. The propensity of Mozzarella cheese to brown during high temperature pizza manufacture is particularly affected by culture metabolism, because *Streptococcus thermophilus* (one of the organisms used as a Mozzarella culture) does not ferment the galactose portion of galactose. The former is instead released into the cheese where it can react to form brown pigments during pizza baking. Although this "galactose-releasing" phenotype is typical for most strains of *S. thermophilus*, we have recently isolated several strains which ferment lactose completely. These strains were used to make Mozzarella cheese which contained significantly less galactose than conventional cultures (0.15% vs. 0.63%). Cheese made using these cultures browned significantly less that control cheese, even when cooked at nearly 600° F, based on objective color analysis using a Hunter colorimeter. Pizza made with low-browning cheese also browned significantly less, according to sensory analysis. Our results demonstrate that low-browning Mozzarella cheese can be made using *S. thermophilus* cultures that more efficiently ferment the galactose portion of lactose.

Introduction

The primary role of lactic acid bacteria in cheesemaking is to ferment lactose to lactic acid. The lactic acid then lowers the curd pH to a level appropriate for the given cheese. Although the metabolic pathways chosen by starter bacteria may vary, depending, for example, on the specific bacterial species, how the lactose is actually fermented or how the target pH is ultimately reached is generally of little concern to the cheesemaker. Of course, cultures should also be resistant to bacteriophages and should not produce bitter or other off-flavors; however, as long as the fermentation occurs in a timely, consistent, and predictable fashion, most cheesemakers will be satisfied, and the culture will not be expected to do much else. For most dairy fermentations, such as those which occur in Cheddar cheese manufacture, *how* sugars are actually metabolized by the starter bacteria has relatively little influence on the outcome of the finished product.

In the case of Mozzarella cheese, however, starter culture metabolism has a profound effect on the final functional and physical properties of the cheese. Although the starter culture must lower the curd pH to 5.2 so that the cheese will stretch, how the pH actually gets to 5.2 and the specific metabolic

properties of the starter culture dramatically affect the quality of Mozzarella cheese. This is because there are additional requirements that Mozzarella cultures must satisfy. For example, Mozzarella cultures must produce cheese having specific stretching, melting, and browning properties (3). Moreover, these requirements are not easily met because of the inherent physiological properties and metabolic behavior of the strains used as Mozzarella starters. Also, the thermophilic rods and cocci used in the manufacture of Mozzarella are physiologically and genetically distinct from their mesophilic counterparts, thus, manipulation or improvement of thermophiles requires a greater understanding of the specific steps and reactions involved in metabolism. In this presentation, I will review current knowledge of sugar metabolism in thermophilic lactic acid bacteria. I will also describe how various metabolic activities affect the properties of Mozzarella cheese and how improved starter cultures can be used to improve the quality of Mozzarella cheese.

Nutritional Requirements

The dietary needs of thermophilic lactic acid bacteria are not unlike that of the mesophiles - for the most part, they simply need a fermentable carbohydrate and a ready supply of pre-formed amino acids. In the milk environment, the fermentable carbohydrate is readily supplied as lactose. At 5%, lactose actually exists in excess of that which is needed. This in an important point, because, as will be discussed in more detail below, *Streptococcus thermophilus*, has the luxury of wasting half of the available energy (i.e., lactose) as it grows in milk. Although the amino acid requirement can seemingly be satisfied by 2.5% casein, the latter is not easily hydrolyzed by all thermophilic cultures; *S. thermophilus* is particularly deficient in this respect and requires the more proteolytic *Lactobacillus helveticus* to generate an adequate supply of amino acids. The action of dairy thermophilic bacteria on milk casein, therefore, is another activity that affects the outcome of the fermentation and the properties of the finished Mozzarella cheese.

General Features of Sugar Metabolism

Carbohydrate metabolism by lactic acid bacteria involves two separate events. First, the sugar, i.e., lactose, must be captured and transported into the cell. The accumulation of lactose (and most other nutrients for that matter) is a process that requires energy. In fact, as much or more than a fourth of the energy a starter culture cell gains from metabolizing lactose must be spent in order to obtain more lactose. Thus, transport is an important first step in metabolism, and more will be said later about the details of lactose transport in thermophilic lactic acid bacteria. The second metabolic event involves a series of enzymatic reactions commencing with the hydrolysis of lactose and ending with the production of lactic acid. Although all lactic acid bacteria possess the means of producing lactic acid from lactose, many of the thermophilic bacteria do so in a manner quite unlike other lactic acid bacteria, as will be discussed below. It is worth mentioning here, however, that one consequence of this unusual metabolic behavior is that the galactose moiety of lactose is, for the most part, unfermented, and as Mozzarella manufacturers are aware, the unfermented galactose accumulates in the cheese where it can later cause browning problems.

Lactose Transport and Metabolism by *S. thermophilus*

S. thermophilus transports lactose via a membrane-bound transport system, conveniently called LacS (6). This simple process results in lactose being carried across the cell membrane and into the cytoplasm of the cell without any change in form (Figure 1). This process requires energy or a driving

force. Ordinarily, the driving force is simply a hydrogen ion (or proton) gradient, the formation of which requires energy in the form of ATP. This allows the cell to couple uptake of one solute, lactose, with another solute, a proton. The intracellular lactose is then split by the enzyme β-galactosidase to form glucose and galactose. The glucose is then readily metabolized or fermented; the cell makes for itself ATP, and lactic acid is made as an end-product. However, most strains of *S. thermophilus* as well as many strains of *Lactobacillus* lack the ability to ferment the galactose portion of lactose and are phenotypically galactose-negative (Gal^-). Why *S. thermophilus* behaves in this way and what happens to the galactose are questions to which we have devoted much research attention.

As stated above, the first step in lactose metabolism, namely transport, requires energy (i.e., ATP). If *S. thermophilus* has to spend energy to transport lactose, it would seem wasteful for it to ferment only half of the available sugar (the glucose) and dispose of the other half (the galactose). Indeed, *S. thermophilus* has learned how to dispose of the galactose in an efficient and novel manner. Instead of simply excreting galactose into the medium (i.e., milk or curd), *S. thermophilus* couples the "downhill" efflux of galactose out of the cell with the "uphill" uptake of lactose into the cell (1). In other words, *S. thermophilus* exchanges intracellular galactose for extracellular lactose (Figure 2). The cell is then spared the energy it would normally spend to take up lactose. This "revolving door" type of process provides an energetic advantage for the organism, with galactose efflux driving lactose uptake (1).

There is another reason why most strains of *S. thermophilus* do not ferment galactose. The metabolic pathway used by most bacteria that ferment galactose is the Leloir pathway, and the first and rate-limiting enzyme of this pathway is galactokinase. Although most *S. thermophilus* strains can produce this enzyme at very low levels (even Gal^- strains), under ordinary growth conditions, the enzyme (or more specifically, the gene coding for this enzyme) is usually turned off, especially when lactose is present. In milk, then, synthesis of galactokinase is repressed and galactose cannot be metabolized. It is important to mention that even strains which can ferment free galactose fail to ferment galactose when lactose is available. If, however, repression is lifted or the cell can be induced to make more enzyme even in the presence of lactose, galactose-fermentation can occur. Toward this end, we recently isolated, cloned, and sequenced the gene coding for galactokinase in *S. thermophilus*. This genetic information provides us with the opportunity to amplify expression of this enzyme in proven cheesemaking strains which may allow us ultimately to convert these strains into galactose-fermenters. It should also be noted that some of the thermophilic lactobacilli used as starter cultures are physiologically similar to *S. thermophilus*, with respect to lactose and galactose metabolism, and that they may similarly modified .

Practical Implications

From a microbiological point of view, the peculiar manner in which *S. thermophilus* and other thermophilic lactic acid bacteria ferment lactose and galactose is scientifically quite interesting, since few other bacteria behave in this fashion. However, from an applied viewpoint, the behavior of thermophilic cultures in milk has profound effects. The galactose which accumulates in Mozzarella cheese is a particular problem when the cheese is exposed to high temperatures as would occur during pizza baking. Under these conditions, the galactose reacts with free amino acids in the cheese to form brown pigments via the well-known Maillard browning reaction (2). Although residual lactose may also participate in browning reactions, galactose is generally more reactive than lactose. Browning of Mozzarella cheese is also enhanced as the temperature increases; as marketing considerations force pizza makers to produce a cooked pizza in a matter of minutes, oven temperatures rise and even cheese containing as little as 0.1% galactose may brown excessively. Mozzarella manufacturers are, therefore, under considerable pressure to reduce the galactose content in cheese in order to produce cheese having low browning potential.

As mentioned above, the other factor which contributes to browning is the amount of free amino acids present in the cheese. Amino acids are produced from protein hydrolysis, with the milk, the rennet and starter and non-starter bacteria serving as potential sources of proteolytic enzymes. Although the mixing-stretching step exposes the curd to water temperatures of 80° C (180° F), and the curd itself reaches as high as 60° C (140° F), not all enzyme is destroyed. Residual proteinases may then continue to degrade casein, resulting in accumulation of amino acids. Also, the starter bacteria may survive the stretching step and continue to grow during storage and hydrolyze protein. It is believed that the greatest source of residual proteinases are from the coagulant and the starter culture. Therefore, by selecting non-proteolytic starter strains, casein hydrolysis is reduced and the browning potential is minimized. However, as reviewed by Kindstedt (3) and Oberg (5) the use of such strains also affects other important functional properties including stretching and melting. Similarly, adjustment of the rod-to-coccus ratio is another common way to control browning and other functional properties of Mozzarella cheese since the rods are generally more proteolytic than the cocci.

Perhaps, the best way to control browning of cooked Mozzarella cheese without affecting other functional properties would be to use culture strains that did not release galactose into the cheese curd. Unfortunately, there are few such strains which exist. As mentioned previously, it may soon be possible to metabolically engineer existing strains of *S. thermophilus* so that they utilize, instead of release, galactose. Another approach, however, is to isolate suitable strains, having the galactose-fermenting, non-releasing phenotype from natural sources. We have recently isolated several strains of lactic acid bacteria having this phenotype, and used these cocci, along with galactose-fermenting *Lactobacillus helveticus*, as starter cultures for the manufacture of Mozzarella cheese.

The cheese was made in our dairy processing plant (40 pounds cheese per vat) according to procedures described by Kosikowski (4), and was stretched in a Supreme 640 mixer. All cheeses had identical composition, except that the cheeses made with the galactose non-releasing cocci contained significantly less galactose than control cheese made with a culture which released galactose (Figure 3). More importantly, the former cheese browned significantly less when heated to over 300° C (585° F), based on Hunterlab Colorimetric analyses (Table 1). Other cheese properties, including melting and oiling off, were not affected by the culture used. Because all culture strains, including the control, were non-proteolytic, we concluded that the galactose-fermenting phenotype, and not the proteolytic activity, was responsible for the non-browning performance of the cooked cheese. When this cheese was used in the manufacture of pizza and was exposed to high cooking temperature (as above), similar differences in browning were observed.

Conclusions

Increased production demands and rigid customer specifications for Mozzarella cheese have forced cheesemakers to produce cheese having very specific functional properties. Research has shown that several of these properties, in particular browning, are affected by starter culture metabolism. By understanding the actual biochemical processes and physiological activities of the culture bacteria, it may be possible to manipulate existing strains or to isolate new strains from nature having desirable cheesemaking properties.

Acknowledgments

I thank the National Dairy Promotion and Research Board for their support of this work.

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Table 1. Values of the L* (light-to-dark) index of Mozzarella cheese for each treatment on days 5 and 28.

Temp (time)	Treatments (Day 5)				
C (min)	KK-1	KK-2	KK-3	KK-4	KK-5
232 (2.00)	63.8	63.7	65.9	63.6[a]	66.3[a]
260 (1.25)	68.1	69.3	69.2	67.5	69.5
288 (1.00)	69.1	69.0	69.1	67.4	69.7
307 (1.00)	66.8[a]	67.6[b]	69.6[ac]	64.5[bcd]	68.4[d]
307 (1.25)	64.0	62.7[a]	64.8	62.7[b]	66.1[ab]

[abcdefg]Values in same row with same letter differ significantly ($P<.05$).

Temp (time)	Treatments (Day 28)				
C (min)	KK-1	KK-2	KK-3	KK-4	KK-5
232 (2.00)	63.7a	63.1	63.7[b]	60.9[abc]	64.1[c]
260 (1.25)	65.7	65.0	65.3	63.9	66.2
288 (1.00)	64.8	64.3	65.1	63.8[a]	66.6[a]
307 (1.00)	64.4[a]	63.5	64.5[b]	61.6[abc]	65.4[c]
307 (1.25)	62.3[ad]	59.2[abc]	63.1[be]	57.5[def]	63.3[cf]
307 (1.50)	61.9[ae]	57.2[abcd]	61.4[bf]	52.3[cefg]	61.1[dg]

[abcdefg]Values in same row with same letter differ significantly ($P<.05$).

Figure 1

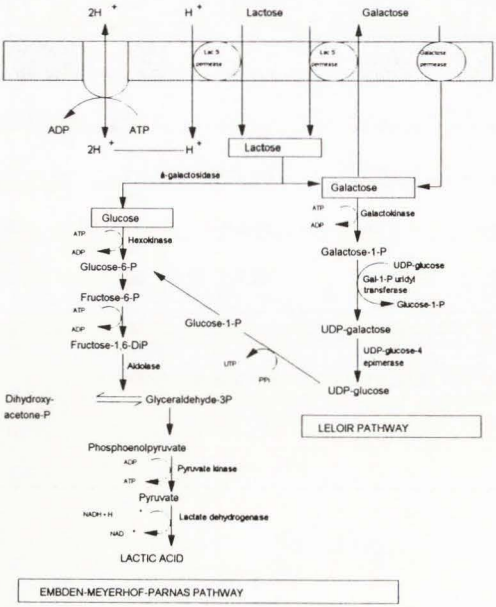


Figure 2

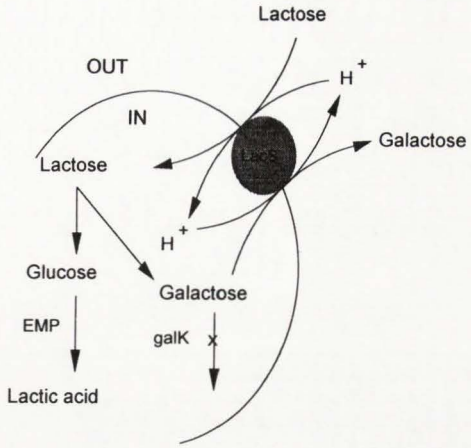
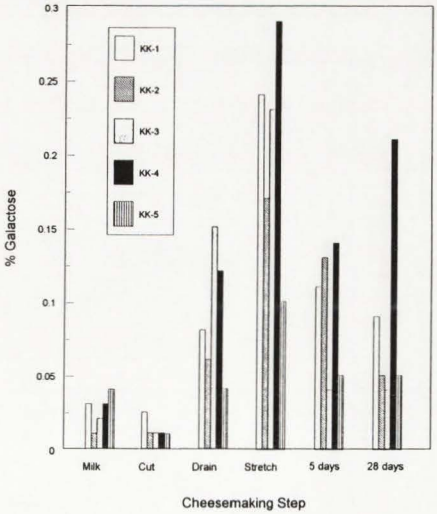


Figure 3



1993-3

Specialty Cheese - A World of Opportunity and Potential

By

James Path
Cheese Outreach Specialist
Center for Dairy Research
University of Wisconsin -Madison
Madison, WI

Topics

How do you define a specialty cheese?
A look at varieties of specialty cheeses from around the world.
Impact on the Wisconsin Dairy Industry.
The development of the Specialty Cheese program at the Center for Dairy Research.

Introduction

I would like to say at the beginning of this presentation that since my specialty cheese program is Wisconsin based and funded by the Wisconsin Milk Marketing Board, most of my remarks will refer to Wisconsin references.

What is a Specialty Cheese?

One of the hardest things to do is to define the term "specialty" cheese. I have spent hours in meetings where people have tried unsuccessfully to reach a definition of specialty cheese.

So what makes an "ordinary" cheese "special?" Some elements which are commonly found in cheeses classified by marketers as specialty cheese are:

1. Perception of added value
2. Normally low volume (less then 50 million pounds per year)
3. Labor intensive
4. Location of production
5. Ethnic background
6. A special manufacturing process
7. Special bacterial cultures
8. Special packaging
9. Unique flavor
10. Unique shape

Let's take a look at a few!

Location - What about a cheese made in France, in a Convent, by Nuns?

Special Processing, Special Packaging - Or is this processed cheese, manufactured for the Orient, cut into strips, with thin layers of fish placed on it, then packaged in a gas flush tray. Is it special? I would think so.

Unique Flavor, Special Packaging - Or a cream cheese flavored with orange and manufactured in Germany?

Special Packaging - Or a Cheese which looks like a smoked fish?

Location, Shape - Or a cheese like Gouda Balls, originally a farmstead cheese, which is now mass produced in Holland, but sold as a specialty cheese in America and many other countries?

These is a wide diversity of cheeses from mass produced Gouda to the Convent cheese which is produced by the Sisters after Mass, from processed to natural. But I think that you would agree that they are all special.

In fact, I have reached the conclusion that all cheeses can be special in their own unique way. From a practical stand point, however, when you have a specialty cheese program, you must set limits on your definition or you will outstretch your available resources.

For our purposes, at the Center For Dairy Research, we have decided our primary concentration is to encourage specialty cheeses which may include the elements mentioned previously, but should include four important characteristics:

1. Must have a value added concept (previously mentioned)
2. Are not currently produced in the State of Wisconsin
3. Natural cheeses (vs. processed)
4. Promotes the expansion of cheese varieties

How may cheeses fall under this definition? Well, if I have a "religion to preach," it is the huge variety of cheeses that are produced worldwide, which fall under our CDR definition. They offer tremendous opportunities for our Wisconsin manufacturers.

This sales booklet illustrates over 300 varieties of cheese which are currently being sold in the U.S.A. I would like to emphasize "**currently being sold in the U.S.A.**" Over 250 of these varieties are imports and all of the cheeses sell for from 50 cents to 4 dollars over the Green Bay market price for cheddar cheese.

Lets take a look at some of them: Madrigal (a sweet Swiss type), Mimolette and Raclette, Semisoft Drakkar, White mold St. Andre and Tome Du Rouergue. On the left - Cantal, Morbier (note the vein of ash in the center of the cheese) , Blue D'Auvergne and Saint Nectaire. On the right - Doux De Montagne, Chaumes, Gourmandise and Croustin, Semisoft cheeses wrapped in chestnut leaves or rolled in pepper or other spices and English cheeses soaked in Elderberry Wine (Winsor Red) or with Hazelnuts and raisins (Nutwood).

Surprisingly, importers I have contacted have been very encouraging when I explain our CDR program. They have indicated to me that because of the drop in the value of the dollar, many imported cheeses are being priced out of the reach of the American consumer. The concern is that this upscale market may be entirely lost. American versions of these cheeses could replace imported products, at prices still in the upscale level, but below the escalating imported prices.

More cheeses are: fresh Mozzarella, originally made from water buffalo milk, a recent import from Italy. Taleggio, also from Italy. In fact Italy offers a tremendous bouquet of cheeses. This book, "DOC Cheeses of Italy" published by the Ministry of Agriculture and Forestry, describes about fifty

Italian cheese varieties of cows, ewes, goats and water buffalo milk origin. Considering that many of the people in this audience manufacture for this ethnic market, these cheeses may offer some additional possibilities.

From Spain the bullet shaped S. Simon, the mushroom-shaped Cebreiro and the pointed Tetilla - all cows milk cheeses. The book "Quesos Espanoles" by Simone Ortega lists over 120 Spanish cheese varieties of cows, ewes, and goats milk origin,

But one of the most decorative Spanish cheeses is a sheeps milk cheese called Manchego. The beautiful basket weave design on the outside of the cheese was originally imprinted by woven baskets or cord wrap in which it was pressed. This tradition is carried on in the design of the modern plastic hoops.

At this point you might ask, "Jim, why in heavens name are you talking about a sheeps milk cheese; and I think you have also slipped in some goats cheeses and even water buffalo cheese?" I would only remind you that cheeses such as Blue, Feta, and even Mozzarella have their origins in non-cows milk. It only took a brave cheesemaker and maybe an enterprising marketer to convert these cheeses to cows milk. The conversion might not have been a perfect translation, but the end results were high caliber cheeses in their own right. It has had a huge impact on usage of cows milk dairy production. I would guess that in the State of Wisconsin alone there is more Mozzarella cheese of cows milk origin produced than there is water buffalo Mozzarella cheese produced world wide.

Tired of one cheese? People are fascinated with contrasting textures and flavors. The English combine layered cheeses to get different tastes and striking eye appeal. The Duchess combines layers of mellow Wensleydale with tangy Shropshire, which is blended with cream cheese. It is similar to one of my favorites, Huntsman, a Double Gloucester-Blue Stilton combination. Or, if you don't like layered, what about a swirl with a soft full-fat cheese rolled in a Leicester cheese.

Some groups of cheeses that started as ethnic specialties have grown into major commodity cheeses. In the U.S.A., Italian type cheeses have grown from specialty ethnic cheeses to a point where the production of Italian types of cheese exceeds that of Cheddar cheese. (1)

There still are many ethnic and racial groups who may provide growing markets. The two fastest growing ethnic cuisines are Italian and Mexican, both of which rely on cheese for many recipes.

Total Hispanic population is projected to grow in the United States from the current level of 19 million to 30 million by the year 2010. (2) 1992 figures place Hispanic cheese consumption at a 33 million pound market with a growth rate of 14% per year. (3)

With the North American Free Trade Agreement about to be ratified, this may open even more new cheese markets in the Hispanic area.

Potential ethnic markets are also ethnic groups with lactose intolerance for milk. Since most of the lactose is utilized in the cheese-making process, cheese becomes the perfect milk substitute for ethnic groups of people with lactose intolerance. The Asian rim is a potential market. Did you know that the country of China produces more cheese than either the countries of Switzerland or New Zealand? (4)

Earlier I showed you a package of Orange Flavored Cream Cheese. If you turn the package over, you will find that the package is destined to an Asian country. Another cheese exported to Japan is the nutty flavored Norvegia from Norway.

I might add that New Zealand has been particularly successful in shipping cheese to Asia. The quantity of cheese it exports to Japan now exceeds either exports to U.S.A. or home consumption.

Market size of the Japanese natural cheese segment surpassed the processed cheese segment in 1989 and continued at an average annual growth rate of 9% per year.

While dessert cheeses are prevalent in Europe, dessert cheeses are relatively undeveloped in the United States. How about the popular white mold triple cream from France, Saint Andre or its cousin L'Explorateur?

Will we see a designer cheese? The artistry of this Fol Epi from France is an attempt to develop upscale markets.

This Paneer cheese, of Asian origin, has been positioned as 100% vegetarian, another new trend in eating habits.

By the year 2030, nearly a quarter of the U.S. population will be at least 65 years old. That's almost double the 1990 rate. (5) This could open the door for stronger-flavored cheeses. Perhaps shelf-ripened cheeses like Chaumes or a Tomme De Savoie will make a come back. Or maybe a Stilton with its irregular blue veins but placed in a collector crock.

This is a fraction, I repeat, a fraction of the cheeses that are currently being produced every day, world wide, to a willing consumer group.

Can specialty cheese production really have an impact on the Wisconsin Dairy industry? Let's look at three current issues: (1.) milk usage, (2.) declining number of cheese factories and (3.) improved pricing of cheese products.

1. Impact on Milk Usage

Under most definitions, specialty cheese varieties have total annual production volumes of less then 50 million pounds of cheese per year. A median level (halfway between 0 and 50 million pounds production) of 24 Million pounds of cheese per year would represent 240 million pounds of milk per year. Since Wisconsin produces about 24 billion pounds of milk per year (1991 figures), one specialty cheese variety would represent about 1 percent of the total state milk volume. (6)

Cheese newly introduced in the last 10 years such as String (70 million pounds U.S. production) and Feta (28 million pounds U.S. production) have already had an impact. (7)

Now I may be a dreamer, but I'm also a realist. **I want to caution that it will take from three to eight years for new varieties to develop. Some may never develop!** But even two or three new successful varieties would have an impact on the Wisconsin Dairy Industry.

2. Impact on Cheese Factories

We currently have only about 170 cheese factories left in the State of Wisconsin. **Approximately half of the cheese factories in Wisconsin fall into a small volume category.** They produce less then five million pounds of cheese per year per plant, with an average production per plant of 1.8 million pounds of cheese per year. (8) A successful new specialty cheese at the median level of 24 million pounds of cheese per year would equal 6 cheese factories utilizing 100,000 pounds of milk per day or an average production of 4 million pounds of cheese per year. Even very small levels of interest in these varieties could help these operations.

3. Pricing

Specialized products are normally labor intensive, require more care and sell at higher prices. Not only are prices higher for these products, but in many cases the moisture is also higher, resulting in better yields. This should result in higher returns.

A cheese factory which processes 500,000 pounds of milk a day manufactures about 50,000 pounds of cheese a day. If they have a one-cent-per-pound margin, they net five hundred dollars per day.

A cheese factory which processes 50,000 pounds of milk a day manufactures only 5,000 pounds of cheese a day. But if they have a 10 cent per pound margin, it also nets five hundred dollars per day.

Diversification of varieties also could help to stabilize prices. If you are producing five types of cheese and the market in one cheese becomes saturated, the price drop has a 1/5th impact on the mix

price you can pay for milk. If you produce only one type of cheese, you feel the full impact of the market change. (Note - specialty cheeses should be priced independently of the Green Bay Cheddar cheese market.)

The objectives of our program at the Center for Dairy Research are three fold:

1. Implementation of a program that will result in the development of specialty cheese technology that will be available to Wisconsin cheese manufacturers.
2. Provide the necessary training and technical support to cheesemakers adopting these specialty cheese technologies.
3. Provide support to Wisconsin cheesemakers in addressing technical questions regarding cheesemaking.

Specialty Cheese Program - Things Happening !

Applications Research
- Originally visited over 100 cheese plants
- Currently working with individual plants
- Quality Assurance Program

Specialty Cheese Seminar Series
- Packaging Seminar
- Danish Cheese Seminar
- Wisconsin Cheese Artisan Certification

Other
- Video series with Swiss Technical School
- Cheesemaker Exchange Program

I also would like to add that although my remarks dealt only with the CDR research area of the specialty program, there is also a marketing facet to the program and that will be addressed by WMMB in their portion of this seminar.

Acknowledgments

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1993-4

A Method for Manufacturing Reduced Fat Mozzarella Cheese

By

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Introduction

Reduced fat and nonfat dairy products are filling an important sales area in the dairy industry, making dairy products available to people who would not normally buy them. However, reducing or eliminating milkfat in cheese can result in physical and flavor changes which are often detrimental. Manufacture of reduced fat Mozzarella cheese has been carried out by some companies, but only with partial success. Recently the USDA has been developing a reduced fat Mozzarella cheese (with 9% fat) for use in the school lunch program. On September 8th, the U.S. Secretary of Agriculture demonstrated the USDA's renewed emphasis on nutrition by launching a long-term initiative ("Fresh Start") to improve school meal programs across the United States (The Cheese Reporter, 10 Sept. 1993). As part of "Fresh Start", reduced-fat Cheddar cheese will be available on a test basis in some schools.

CHEESE AND NUTRITIONAL GUIDELINES

School Lunch Program

According to Ellen Haas, the Assistant Secretary of Agriculture for Food and Consumer Services, "school-age children get too many of their calories from fat and do not eat enough fruits and vegetables." A cry has also been raised by the consumer organization Public Voice for Food and Health Policy that "school lunch programs had become a virtual dumping ground for high-fat cheese and butter" and that "school lunches do not meet Dietary Guideline recommendations that no more than 30 percent of calories come from fat" (The Cheese Reporter, 10 Sept. 1993).

The National School Lunch Program serves about 25 million lunches a day and operates in nearly 95 percent of the nation's schools. USDA has purchased almost 60 million pounds of cheese (including 17 million pounds of Mozzarella) for use in the school lunch program during the past 12 months. That is only about 1% of annual production but if you are selling cheese to USDA it is significant, and almost all of that cheese was purchased at market prices. With fast-food restaurants opening up outlets in school cafeterias, Pizza Hut has over 4,500 high school outlets, this percentage seems set to increase. The only problem is that because of this trend, some so called nutritional "experts" are accusing schools of jeopardizing kids' health.

Cheese Research at Utah State University

Research on reduced fat Mozzarella cheese has been underway at Utah State University for the past two years. During that time we have been refining the methods used to study the microstructure of cheese. Out of that study we have made a five to ten-fold increase in resolution obtainable using scanning electron microscopy, so that we can distinguish components that are as little as three nanometers apart (McManus *et al* , 1993)

This year at the Western Center for Dairy Protein Research and Technology (which comprises researchers from Utah State University, Oregon State University, Brigham Young University, and the University of Idaho) we have initiated a research strategy into low fat cheeses. Our aim is to create a multi-faceted research team focused on solving the current problems faced by manufacturers of low fat cheeses, i.e. poor flavor and texture. Included in this is a joint three year project with researchers at the University of Wisconsin–Madison and the University of Minnesota, and we are actively looking for other people both in industry and academia who would like to be a part of such a low fat cheese research team.

At the Western Center we have realized that researchers need to band together in solving such problems rather than competing with each other. Especially, we need to have coordinated industry and academic research institutions working together for the overall good of our cheese future.

Reduced Fat versus Low Fat

There are companies marketing Mozzarella cheese with a lower fat content than part skim Mozzarella, but less than 30% of the milkfat is typically removed and this is far from meeting the dietary guidelines on fat consumption. And right or wrong, those guidelines are going to be used by a lot of people in making food choices.

With the new FDA regulations on food labeling, we need to look carefully at what we call products in which we have lowered the fat content. If we reduce fat content by 25% we can call it reduced fat. If we lower the fat content by 50% we can call it low fat provided the product is within the nutritional guidelines of no more than 30% calories from fat. As you well know, there are four categories of "Mozzarella cheese" in the U.S.A: Mozzarella, Low Moisture Mozzarella, Part Skim Mozzarella, and Low Moisture Part Skim Mozzarella. They differ in fat content and moisture content (USDA, 1980). Mozzarella cheese must have at least 45% fat on dry basis (FDB) and a moisture content in the range of 52 to 60%. Mostly we produce cheese in the pizza cheese category, i.e. Low Moisture Part Skim Mozzarella with a fat content of 30 to 45% FDB and a moisture content of 45 to 52%.

So, assuming most manufacturers are aiming to make their cheese with at least 50% moisture, low moisture part skim Mozzarella can have from 22% fat down to as little as 15% fat. The amount of fat you put into your cheese will probably continue to depend on the value of butterfat. If you can sell it at cheese prices, rather than butter prices, then it would be foolish from an economic viewpoint to make low moisture part skim Mozzarella at anything less than the maximum allowable fat content. However, to maintain the spirit of the labeling laws, a reduced fat Mozzarella would probably need to have no more than 11% fat (a 25% reduction from 15%) even though the comparison cheeses would typically contain 20% fat or more. At a 50% reduction of fat, a Mozzarella cheese with 7% fat could be classified as a "Low Fat" food (Table 1). This is a distinct advantage over Cheddar cheese which at a 50% lower fat content is still well above the nutritional guidelines as shown below. To have only 30% of calories come from fat, Mozzarella cheese needs to be made with no more than approximately 7% fat.

Table 1. A comparison between Cheddar and Mozzarella cheeses on the effect of reducing fat content on the proportion of calories from fat.

Cheese	Fat	$Calories_{FAT}$
Cheddar	32%	73%
Red.Fat Cheddar	15%	47%
Part Skim Mozz	20%	65%
Red. Fat Mozz	9%	35%
Low Fat Mozz	7%	30%

Removing Fat From Cheese

As the fat is removed from Mozzarella cheese, however, the desirable physical properties of the cheese, which play an important role in its consumer acceptability, are lost. And commercially available non-fat Mozzarella cheese is the extreme example of this and has none of the melt and stretch properties expected of Mozzarella cheese. It is very tough and has poor melt and stretch properties and its structure (Figure 1) bears little resemblance to regular Mozzarella cheese (Figure 2).

The most important characteristics of Mozzarella cheese are moderate toughness, adequate stringiness, proper melt, desired cook color and gloss, and how well it shreds and slices. These physical properties vary greatly based on cheese age, pH, moisture level, salt content, and the starter cultures used. Also, increasing moisture content, as a means of making lowfat Mozzarella cheese will significantly affect the physical properties.

If fat is removed the protein content increases and it is thought that this makes the cheese too tough to melt and stretch properly. It has been suggested that stretching properties may be related to higher concentrations of intact casein and large peptides (Creamer, 1976) and that differences in the proteolytic properties of thermophilic starter cultures can significantly modify physical properties.

In addition to using modified cultures, physical parameters of the cheese such as calcium levels and cheese pH can be used to control melt properties of cheese (Keller *et al*, 1974; Kiely *et al*, 1992). Previous work at Utah State University with direct acid Mozzarella cheese made with chymosin, bovine pepsin, porcine pepsin, or *Mucor miehei* protease has also shown that melt and stretch are affected by the type of enzyme used (Oberg *et al*, 1992).

UTAH STATE UNIVERSITY REDUCED FAT MOZZARELLA CHEESE

Modifying the Make Procedure

During the past 18 months, we have been studying the relationship between fat and protein in Mozzarella cheese in an effort to understand how stretch and melt properties can modified. To do this we have been studying the structural changes that occur in cheese curd as it is made into Mozzarella as a means to understand how best to make a low fat Mozzarella (Oberg *et al*, 1993a).

We have produced cheese using milk with casein-to-fat ratios of 1.2 (for part skim Mozzarella), 1.6, 2.0 and 2.4 for reduced fat Mozzarella (Merrill *et al*, 1993). There were many different process variations that we used in an attempt to produce a cheese that retained the desired level of moisture as we reduced the amount of fat in the cheese, but not all of them were successful. Variations that were

unsuccessful included draining the whey at a high pH, reducing cook temperature to 96°F, and adding wash water to the curd.

Eventually a procedure was developed for which we were able to make a cheese with 10% fat and 47% moisture (although we would prefer to have it around 50-53% moisture so that the concentration of protein in the water phase remains constant). No doubt there are a number of ways in which the same cheese could be made but this manufacturing procedure worked well for us. The area of greatest concern was to retain as much moisture in the cheese curd as possible because this appeared to be the limiting factor in avoiding production of a cheese with a very tough and rubbery body.

An elevated pasteurization temperature (174°F for 29 s) was used to retain a small amount of denatured whey proteins which have a better water holding capacity than renneted caseins. The milk was pre-acidified to pH 6.0 with lactic acid before adding rennet so that we could reduce the make time by not requiring as much acid production by the starter cultures (it also reduced the clotting time to 10 minutes with the rennet concentration we were using). When you are trying to maintain moisture content as high as possible, if acid production is too slow then the curd will have to be held longer in the vat, or on the drain table, and more moisture than desired will be lost.

Cheesemakers want consistent performance from their starter cultures because any time they have erratic starter performance they will be making cheese that is highly variable in composition and functionality. We overcome some of this variability by accomplishing a lot of the acidification by direct addition of acid. We diluted lactic acid 1:2 with water and added it to our small 7 liter vats. For commercial production the acid could be gradually added to milk as the milk is pumped into the vat.

The curd was cut with 3\4" knives so as to give the curd particles a smaller surface to volume ratio and trap more moisture in the curd by slowing whey expulsion. Cook temperature was lowered to 100°F, again so that we would reduce whey expulsion but this could be varied depending on the cultures being used.

While the curd was still in the whey, we reduced the amount of stirring of the vat so we could minimize curd shattering, and because we were working with only small quantities of curd we hand cheddared the cheese but turned the curd less frequently than traditionally used. If you can keep the cheese cooler you will have slower acid production and thus slower whey expulsion. For a commercial situation this translates into doing all that you can to reduce the extent of mechanical agitation that promotes expulsion of whey from the curd. And although many of you may simply make a stirred curd cheese rather than cheddaring, the same general principles apply.

Modifying Starter Cultures

This work is continuing at the Western Dairy Center and we are currently working on manufacturing cheese that falls below the nutritional guidelines of 30% calories from fat. Included in this is further work on starter cultures, especially those that are highly proteolytic and would help accelerate protein breakdown to get better melting properties. For our original work on reduced fat Mozzarella we used single strains of *Lactobacillus helveticus* and *Streptococcus thermophilus*. More recently we have been using *Lactobacillus casei* ssp. *casei* as either an adjunct or as the rod portion of the starter culture mix. We have found that our reduced fat cheese made with partial or total replacement of *L. helveticus* with *L. casei* ssp. *casei* had more melt and less cook color than comparable cheese made with *L. helveticus* and *S. thermophilus* (Oberg *et al.* 1993b). Overall the physical properties of our reduced fat Mozzarella compared favorably with low moisture, part skim Mozzarella except that there was a color change of the cheese as the fat was removed.

Physical Properties

The same changes in properties of the reduced fat cheese occurred during storage as occurs in regular Mozzarella although at a slower rate. Fresh Mozzarella melts poorly, has a tough granular consistency and is too elastic which makes it unsuitable for use as a pizza ingredient. Then during 2 weeks of refrigerated storage considerable proteolysis occurs; Farkye *et al.* (1991) reported a decrease of intact α_{s1}-casein by 25% and intact β-casein by 40%, and the cheese texture mellows to a more moderately elastic state. We observed this as a decrease in stretch as measured using helical viscometry (Figure 3). At Day 1, the reduced fat cheese was twice as tough as the control but by Day 7 they were comparable. In contrast, the reduced fat cheese actually melted slightly more at Day 1 but it took 28 days to reach the meltability that the control cheese reached by Day 7 (Figure 4).

This difference in the rate at which meltability changes during storage explains why our statistical analysis showed a significant interaction between fat content of the cheese and storage time even though overall it did not show a significant effect of fat content on meltability Cook color also increased over time as would be expected as more free amino groups are released from proteolysis (Figure 5). The slight differences (however not statistically significant) at Day 1 may simply have been caused by slight differences in the make procedure between the control and reduced fat cheeses.

MICROSTRUCTURE OF LOWFAT MOZZARELLA

Forming the Protein Network

When milk coagulates, the casein micelles aggregate into chains that eventually all link together into a mesh-like structure that encompasses the fat globules. At the time the curd is cut, there is an open network of chains and clusters of individual micelles of varying sizes (Figure 6). There are many crosslinks between the chains forming numerous "cages". Large spaces also exist in the network where the fat globules are present and act to interrupt the network. The rigidity imparted by the protein network depends primarily upon the size of the cells and the thickness of the chains forming them. The sizes of the cells will be controlled by the size of fat globules while the thickness of the chains will be controlled by the distance between the fat globules. The less fat there is, the greater the distance between fat globules and therefore the greater the space in which the proteins can move, and the thicker the protein strands can become.

Because we acidified the milk to pH 6.0 before renneting the casein structure in the reduced fat curd is more open than is usually observed in cheese curd at the time of cutting. When the pH is lowered the casein micelles undergo a much more rapid coagulation although at this stage the individual para-casein micelles can still be observed at high magnification using scanning electron microscopy. We used a 10 minute set time when making the reduced fat cheese; when the same amount of rennet was added to non-acidified milk we had to wait 50 minutes before the curd was firm enough to cut. In spite of this longer set time, there was more micelle fusion that had taken place in the acidified milk at the time of cutting than normally occurs in cheesemaking.

Shrinkage of the Curd Matrix

As whey is expelled after the curd is cut, then the mesh-like structure shrinks around the fat globules (Figure 7). The protein network becomes more compact and micelles fuse together with many of the chains forming into thicker strands. The cheesemaker observes this as the curd becoming more firm.

Although this firmness or rigidity is principally controlled by the rigidity of the casein network (i.e. the rigidity of the "cages") the fat still plays a significant role. The presence of fat within the cages modifies and limits the extent of deformation, adding rigidity to the structure. At the same time the water acts as a low viscosity lubricant between fat and casein. Provided there is a sufficient quantity of it, the water occupies all the space between the fat and the casein. It is the combination of all these effects which gives rise to the rheological properties of the final cheese.

Forming Protein Fibers

Initially, the protein network is seen as chains extending throughout a continuous serum phase in the curd (see Figure 6 and 7). However, as more serum is lost as whey, the hydrated protein becomes the continuous phase that encloses pockets and pools of serum and fat droplets (Oberg, *et al.* 1993a). When a reduced fat cheese is made, there is simply insufficient fat to keep the protein strands well separated.

As shown at last year's seminar, when Mozzarella curd is mechanically stretched the orientation of the protein produces a very dramatic looking structure (Figure 2). However, as I mentioned, for experimental work such as I have described today, we use hand stretching which does not give us the same extent of fiber orientation (Figure 8). Formation of protein fibres was observed for both our control and reduced fat cheese with columns of serum and fat separating them.

Conclusion

By using an elevated pasteurization temperature, pre-acidification of milk, 3\4" cutting knives, 100°F cook temperature, and less mechanical agitation we produced a reduced fat Mozzarella cheese with 10% fat and 47% moisture. The cheese produced using this Utah State University method had melting characteristics comparable to those of a part-skim low moisture Mozzarella cheese with 20% fat.

At Day 1 after manufacture, the reduced fat Mozzarella cheese was twice as tough as regular part skim Mozzarella, but after 7 days of storage it had the same stretch characteristics. By 7 days, it melted slightly less than the regular Mozzarella but was still in an adequate range. By 28 days, it exhibited the same extent of melting.

Acknowledgments

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Figure 1. Internal structure of a retail purchased non-fat Mozzarella cheese.

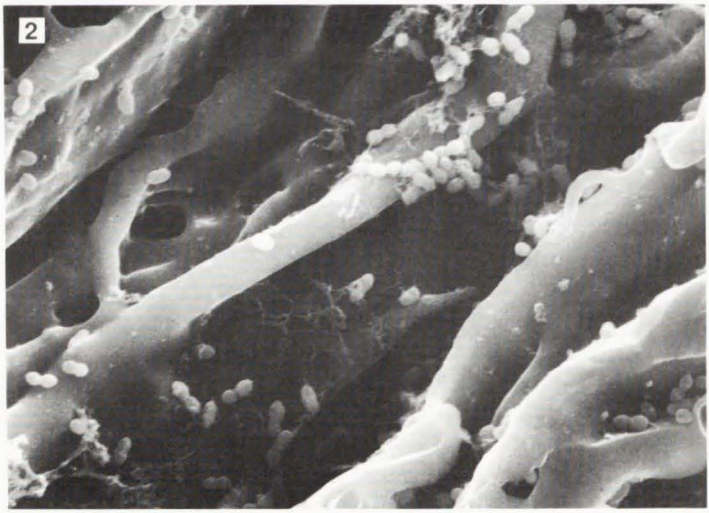


Figure 2. Internal structure of a low moisture part skim Mozzarella cheese immediately after mechanical stretching.

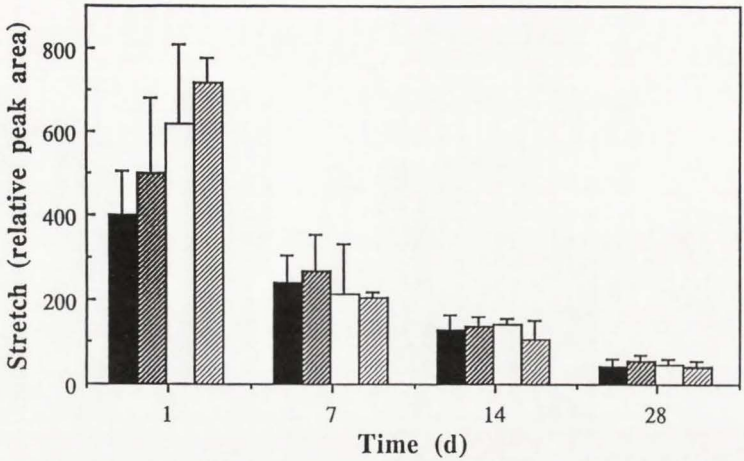


Figure 3. Changes in stretchability, during storage time, of Mozzarella cheese made from milk of various casein to fat ratios.

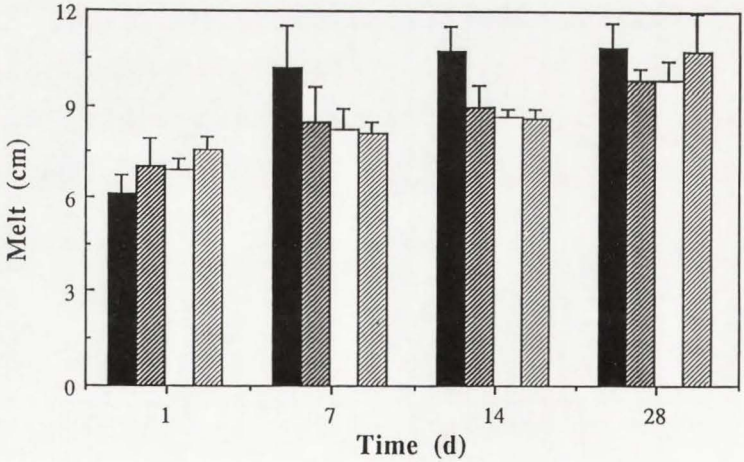


Figure 4. Changes in meltability, during storage time, of Mozzarella cheese made from milk of various casein to fat ratios.

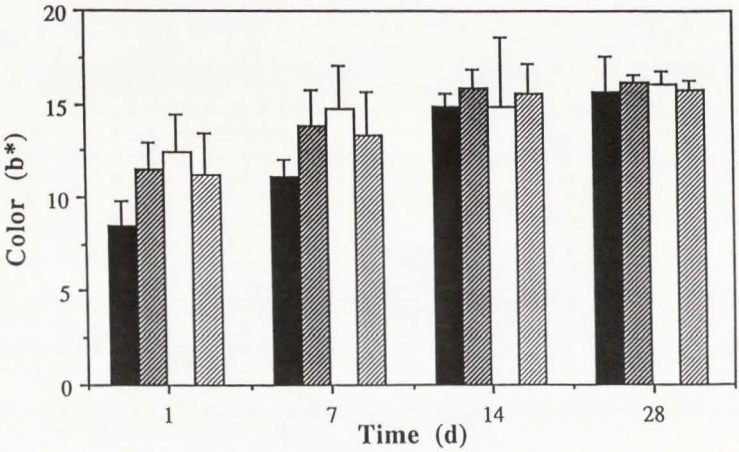


Figure 5. Changes in cook color, during storage time, of Mozzarella cheese made from milk of various casein to fat ratios.

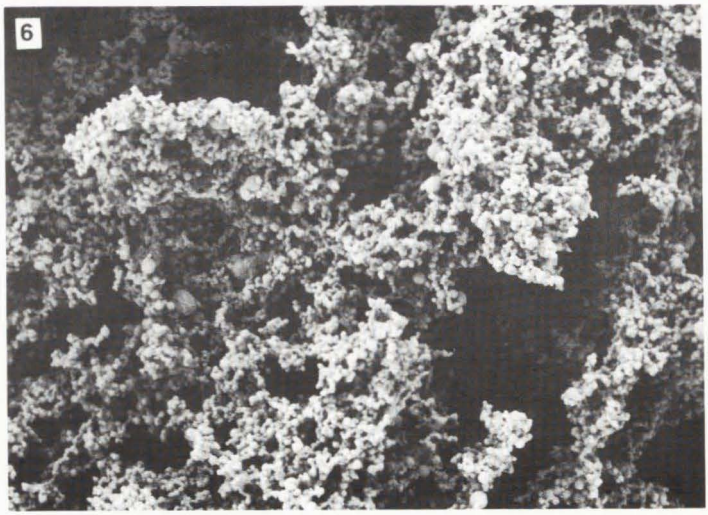


Figure 6. Internal structure of cheese curd at the time of cutting.

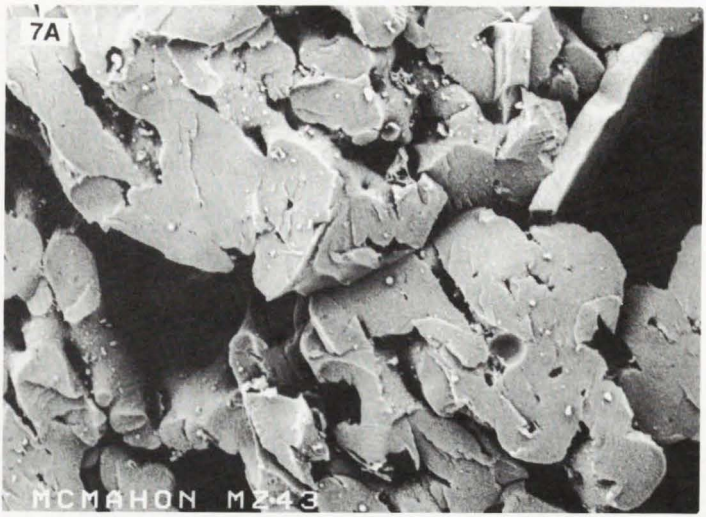
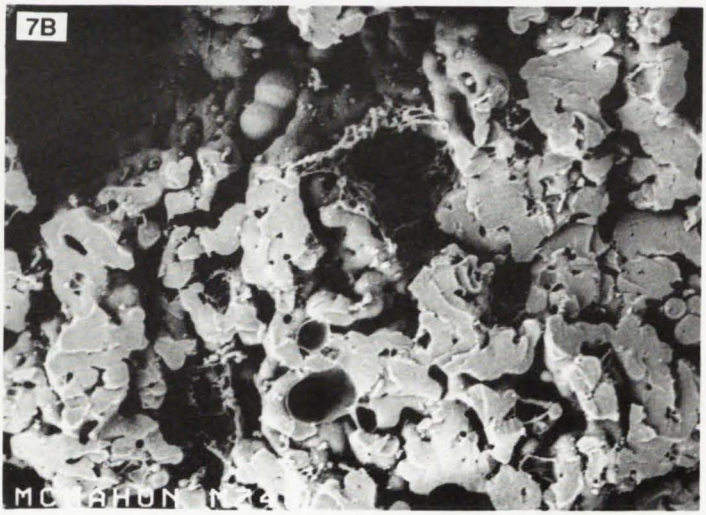


Figure 7. Internal structure of (A) reduced fat Mozzarella cheese curd and



(B) part skim Mozzarella cheese curd prior to draining the whey.

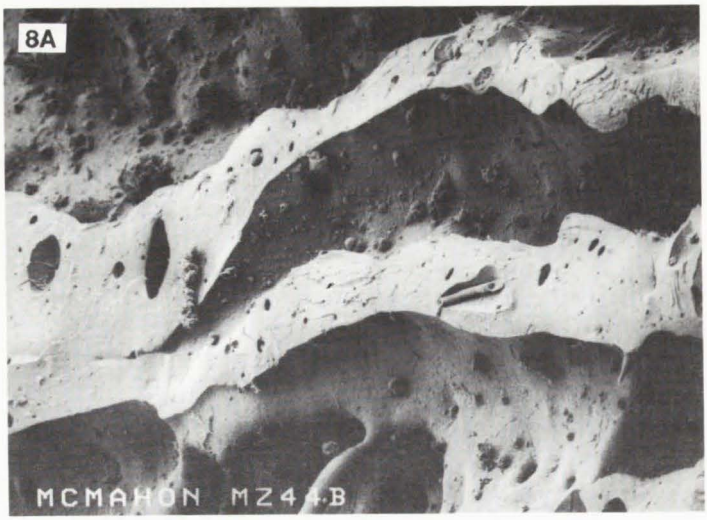
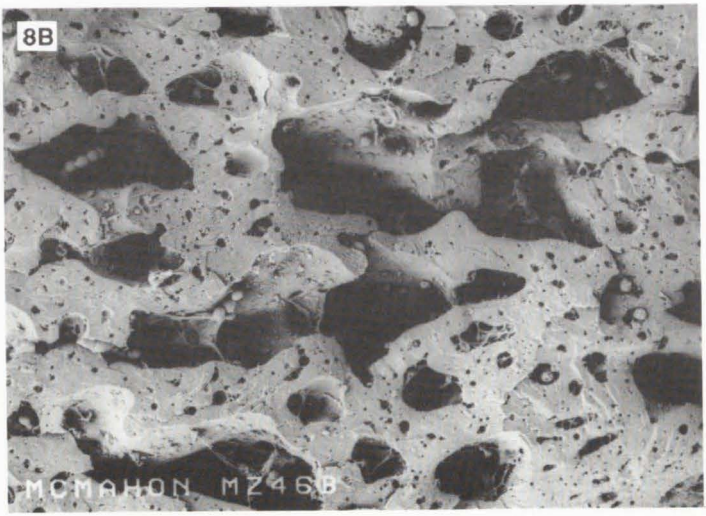


Figure 8. Internal structure of (A) reduced fat Mozzarella cheese curd and



(B) part skim Mozzarella cheese curd after hand stretching.

1993-5

Features and Benefits of Using Custom Starter Programs

By

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To better understand where the Italian cheese industry is with starter programs today, it is valuable to know where we have been. Most of the emphasis back in the Sixties and Seventies was placed on developing Italian cheese standards and standardizing cheesemaking procedures. In 1966, Dr. George W. Reinbold reported at the Marschall Italian Cheese Seminar that selected single-strain starter cultures, both rod and coccus, should be introduced for Italian cheesemaking. The effect of these single-strain coccus and rod cultures on cheese body, flavor, and texture should be assessed. He also did not recommend the use of whey starters.

The Marschall Italian starter program at that time was LB and ST cultures grown in NFDM at 11% solids producing a starter with a one-to-one coccus-to-rod ratio. The LB culture, a single strain of *L. helveticus* and the ST cultures, single strains of *S. thermophilus* were both available in liquid frozen bulk sets. There were about four or five ST strains to choose from. The existence of phage for cocci and rods was known but not readily accepted in the field.

In 1975, Verle W. Christensen reported at the Marschall Italian Cheese Seminar about the preparation and use of CR 150 gallon bulk sets grown in Thermostar at 10% solids, producing a coccus-to-rod ratio of one-to-one. This gave the cheesemaker a dependable acid producing system, because Thermostar provided phage protection in the starter tank and a selection of CR cultures was available to provide a phage stable rotation This starter system was "state of the art" for that time, allowing the cheesemaker to make cheese meeting the standards.

It was in the 1980's that much automation equipment was incorporated into Italian cheese production creating specialized needs for starter programs. At the same time, markets developed for cheeses that function in very specific and different ways. Some examples of these cheese functions would be degrees of browning on a pizza, degrees of stretch on a pizza, tenderness to the bite on a cooled pizza, white Parmesan, tight knit hard styles, and correct melts for deep fat frying Mozzarella sticks, to name a few.

In 1985, Marschall introduced a line of defined single strain coccus and rod cultures, with known flavor development, cheese body breakdown, salt tolerances, temperature tolerance and preference and moisture retention. These cultures can be grown in bulk starter systems using Thermolac media at 7.4% solids or Thermogold and Thermostar II at 7% solids. The cultures can be grown in various combinations and ratios to meet a wide variety of production needs. Specific growth patterns can be repeated to grow these cultures in consistent strain balances and coccus-to-rod ratios with the use of our automatic starter system controller, the CT2000SS.

Of the medias mentioned above, Thermogold would provide the most phage protection in the starter tank, while Thermostar II is an internally buffered media used primarily for rod growth. A typical bulk culture scheme includes one pair of rods and three to four pair of cocci. When just rods are grown in Thermostar II, the usual is to grow a pair or triplet of rod strains and introduce the cocci liquid frozen

or freeze-dried direct to the vat, varying the amounts and ripening times of the cocci and rods to set the ratios for the different cheese styles.

In 1990, Marschall introduced a line of direct-to-the-vat Flavo Bac cultures to be used as starter adjuncts to influence flavors, melting characteristics, tender cheese to bite on a cooled pizza, light/white colored cheese, low browning and cheeses with tight body.

We at Marschall believe we can provide the cheesemaker with the culture systems required to be successful in today's Italian cheese market, allowing him to maximize moisture and fat usage by standardizing with the addition of condensed or reconstituted dry skim milk solids, utilizing highly automated equipment and still maintaining cheese characteristics consistent to their company's marketing tradition.

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1993-6

Impact of Whey pH at Draw on Composition, Proteolysis, and Functional Properties of Mozzarella Cheese

By

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Abstract

Cultured low moisture, part skim Mozzarella cheeses were produced (400 lbs/vat) using the same milk, starter, and coagulant, but with two different whey pH at draw (6.40 and 6.15). Cheese was made by the milled-curd "no-brine" method with a 106°F (41°C) cook temperature, 5.25 milling pH, and 135°F (57°C) stretching temperature. Cheese making was repeated 3 times on 3 different days. With decreasing draw pH from 6.40 to 6.15, calcium content in the cheese decreased (from .83 to .75%), and cheese moisture increased (from 45.7 to 46.3%). However, there were no significant differences in protein, fat, and salt content. During storage, soluble protein contents, meltability, and free oil increased, and $_{S}$-casein and apparent viscosity decreased for all cheeses. There were significant influences on the interaction of draw pH with storage time on soluble nitrogen content. Thus, proteolysis during refrigerated storage was influenced by differences in draw pH. On average, TPA hardness, TPA springiness, and apparent viscosity were lower with lower draw pH during the storage (the lower the draw pH, the softer the cheese).

Introduction

Changes in manufacturing variables can affect chemical composition and functional properties of Mozzarella cheese. Previous studies have shown that variations in draw pH can affect cheese texture by changing the retention of mineral and coagulant enzyme (Lawrence et al., 1983; 1987; Holmes et al., 1977). Texture is important in Cheddar because cheese with poor texture would also develop uncharacteristic flavor (Lawrence et al, 1983). For Mozzarella, texture of unmelted cheese is important because of its influence on shredding property of the cheese.

Mozzarella cheese undergoes proteolytic changes during refrigerated storage. These proteolytic changes affect the functional properties such as melting and stretching characteristics. If the retention of coagulant enzyme in the curd changes by varying the draw pH, then the proteolytic changes during storage could be affected, and the cheese functionality upon baking may also be affected.

Draw pH is, therefore, one of the manufacturing variables that can affect the quality of Mozzarella cheese. The objective of this experiment was to determine the effect of whey pH at draw on cheese composition, proteolysis, and functional properties of Mozzarella.

Cheesemaking

To produce low moisture part skim Mozzarella cheese with two different draw pH, a milled-curd no-brine method (Yun et al., 1993) was used. Cheesemaking was replicated on 3 different days as a randomized complete block design. The flow diagram of the cheese making method is shown in Figure 1.

Raw skim milk and raw cream were standardized to 2.3% fat and pasteurized. To the milk (400 lbs/vat), direct-to-vat frozen starter cultures, Streptococcus salivarius subsp. thermophilus (Marschall product Thermococcus C120) and Lactobacillus delbruekii subsp. bulgaricus (Marschall product Thermorod R160) were added.

Milk was ripened for 60 minutes at 96°F (36°C), and fermentation produced chymosin was added. Following a 30 minutes set, the milk coagulum was cut with a 1.2 cm wire knife and allowed to heal for 5 min. Next, the curds were stirred gently without heat for 10 minutes, followed by heating from 96°F (36°C) to 106°F (41°C) over 15 minutes with continuous agitation.

The agitation was continued and temperature maintained until the whey pH reached the desired draw pH (i.e., 6.15 or 6.40). Then whey was drained and curd was piled in the center of the vat. Curd slabs were turned (cheddared) every 15 minutes until the curd reached a milling pH of 5.25. Then the cheese curd was milled, salted, and stretched. Stretching of the curd was done in salt solution at 135°F (57°C) using a pilot scale Mozzarella mixer (model 640; Stainless Steel Fabricating Co., Columbus, WI). The cheese was then cooled, vacuum packaged, and stored at 4°C until the analysis.

Analyses and Tests

Changes in titratable acidity of milk and whey (Richardson, 1985) and pH of milk, whey, and curd were monitored during cheese making. A Xerolyt electrode (Ingold Electrode) and Accumet pH meter (Fisher Scientific) were used for pH measurements. The pH of whey and curd were measured at 100°F (38°C) after calibrating the pH meter with reference solutions for pH 4 and pH 7 at 100°F (38°C) (Yun et al., 1993a)

Fat content of milk, whey, and cheese were determined using Babcock tests (Yun et al., 1993a). All nitrogen determinations were performed by Kjeldahl. Percentages of nitrogen from the analyses of total nitrogen were multiplied by 6.38 to give milk protein equivalents (Yun et al., 1993a). Cheese moisture was determined gravimetrically by drying 2 g of ground cheese at 100°C in a forced air oven for 24 h (Richardson, 1985). Calcium concentration in cheese was determined by complexometric titration (Kindstedt and Kosikowski, 1985).

All tests during storage were performed after 3, 8, 15, 21, 29, and 50 d of refrigerated storage at 4°C. To monitor proteolysis, the amounts of nitrogen soluble in pH 4.6 acetate buffer and in 12% TCA were determined. The soluble nitrogen values were expressed as a percentage of total nitrogen content of cheese. The amount of intact α_s-casein was determined by SDS-PAGE. More detailed references for these analyses are found in the previous report (Yun et al., 1993a)

Texture Profile Analysis (Bourne, 1978) of Mozzarella cheese was done using the Instron Universal Testing Machine. Cheese meltability was measured by a modified Schreiber test (Kosikowski, 1982). Apparent viscosity of melted Mozzarella cheese was measured by helical viscometry (Kindstedt and Kiely, 1992). Free oil of Mozzarella cheese was measured using the centrifugation method (Kindstedt and Rippe, 1990). Detailed explanations of the testing methods for functional properties have been reported previously (Kindstedt and Kiely, 1992; Kindstedt and Rippe, 1990; Yun et al., 1993b).

Results and Discussion

The cheese making time was not affected by the changes in draw pH. The duration from coagulant addition to draw was longer with lower draw pH (i.e., 65 and 95 minutes for draw pH 6.40 and 6.15, respectively). However, the time from draw to mill was shorter with lower draw pH (i.e., 67 and 31 minutes for draw pH 6.40 and 6.15, respectively). Thus, the total make times from coagulant addition to mill for both draw pH were similar (i.e., 132 and 126 minutes for draw pH 6.40 and 6.15, respectively).

As shown in Table 1, with decreasing draw pH from 6.40 to 6.15, calcium content in the cheese decreased (from .83 to .75%). This is consistent with previous studies relating the mineral retention in the curd with draw pH. The calcium contents in both cheeses are within the usual range for the low moisture part skim category (USDA, 1976)

The cheese moisture was slightly higher (from 45.7 to 46.3%) in the cheese with lower draw pH. The slightly shorter cheese making time for the cheese made with lower draw pH may be the reason for the slightly higher moisture. Draw pH did not have a significant impact on protein, fat, fat on a dry basis, and salt content of the cheese (Table 1). Moisture and fat on a dry basis for both cheeses are within the legal range for the cheese (CFR, 1991).

The results of pH 4.6 soluble nitrogen showed that, overall, lower draw pH seem to cause more proteolysis during storage (Figure 2). There were interactions of draw pH with storage time on both types of soluble nitrogen contents: the rate of increase in proteolysis during storage occurred faster with lower draw pH (Figure 2 and 3).

We did not determine the amount of coagulant retained in the curd or cheese. Thus, we cannot attribute this higher proteolysis solely to the higher coagulant retention in the curd at lower draw pH. However, more proteolysis, especially by pH 4.6 soluble nitrogen, seems to indicate that lower draw pH may have caused higher coagulant retention, which then produced more proteolysis. This postulation may be even more valid considering that pH 4.6 soluble nitrogen is affected more by coagulant than by starter culture enzymes which is another cause of proteolytic changes in cheese (Chu et al., 1993).

With increased storage time, both types of soluble protein increased (Figures 2 and 3), and α_s-casein decreased for all cheeses (Figure 4). These proteolytic changes are expected (Yun et al., 1993a) and will affect texture and functional characteristics of the cheese during storage (Yun et al., 1993b).

On average, TPA hardness (the overall resistance of cheese to compression) appeared to be lower with lower draw pH (Figure 5) probably because of less calcium in the cheese. Although less obvious, the TPA springiness (the height of cheese bounced back between compressions) also appeared to be lower with lower draw pH (Figure 6), again probably because of less calcium in the cheese. The reduced amount of calcium in the cheese and the more active proteolysis may have contributed to a weaker network structure in the cheese with lower draw pH.

Changes in functional properties were normal for the low moisture part skim Mozzarella (Yun et al., 1993b). During storage for both cheeses, the meltability increased (Figure 7), free oil increased (Figure 8), and apparent viscosity (Figure 9) decreased. On average, apparent viscosity (resistance of melted cheese against the shear at high temperature) tended to be lower with lower draw pH. The reason for this would be similar as the reason for the weaker structure in the cheese with lower draw pH: more active proteolysis and less calcium are probably the reason for the reduced apparent viscosity.

Overall, the effect of draw pH (between 6.40 and 6.15) on texture and functional properties was smaller than the effect of storage (during 50 days at 4°C). However, there were some differences caused by the changes in draw pH. These differences combined with the influence of other parameters in the cheese-making procedure may be useful in providing cheeses that meet the varied functionality criteria of individual customers. The cheese with lower draw pH would give slightly softer cheese with slightly less stretch and more melt characteristics.

Conclusions

Lowering draw pH (6.40 to 6.15) reduced calcium content in the cheese (from .83 to .75%) and increased cheese moisture (from 45.7 to 46.3%). However, draw pH did not affect protein, fat, and salt content of the cheese.

Soluble protein contents, meltability, and free oil increased, and $_S$-casein and apparent viscosity decreased for all cheeses during storage. On average, TPA hardness, TPA springiness, and apparent viscosity were lower with lower draw pH throughout the storage.

Thus, variation in the draw pH will cause variations in texture and functional characteristics. Selecting appropriate whey draw pH and then consistently controlling to that point will produce cheese with more consistent functional characteristics.

Acknowledgments

We thank Robert Rasmussen, Scott Hawkes, Maureen Chapman, Wen-I Tsai, and George Houghton for their technical assistance. Financial support was provided by Northeast Dairy Foods Research Center.

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CHEMICAL COMPOSITION OF FRESH CHEESE

	DRAW pH 6.40	DRAW pH 6.15	P VALUE
CHEESE pH	5.16	5.12	.24
% H_2O	45.73	46.28	.05*
% FAT	20.79	20.71	.52
% FDB[1]	38.32	38.55	.50
% PROTEIN	28.03	27.59	.38
% SALT	1.50	1.59	.50
% SALT/H_2O	2.96	3.16	.59
% CALCIUM	.83	.75	.04*

FDB[1]: fat on a dry basis

Table 1.

"MILLED-CURD NO-BRINE" MOZZARELLA CHEESE MAKING
WITH 2 DIFFERENT DRAW pH

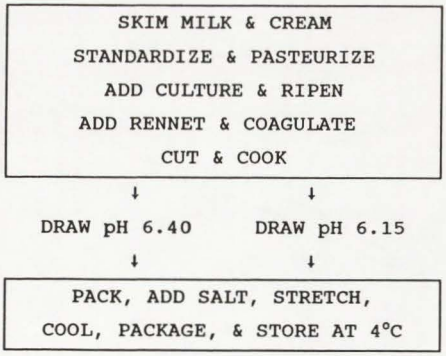
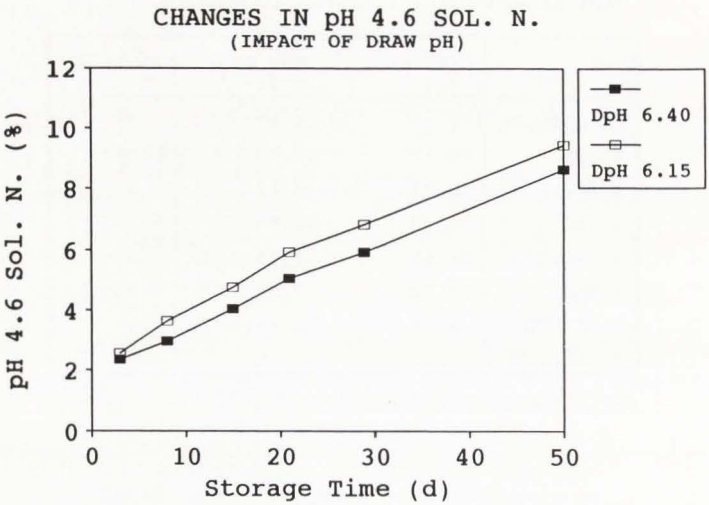


Figure 1.



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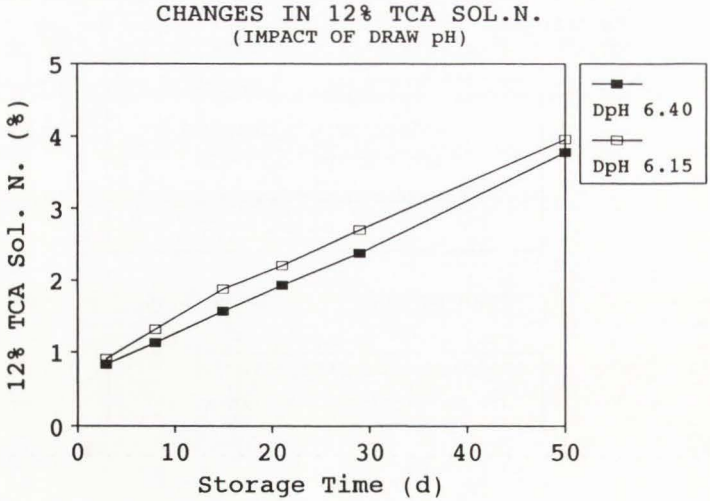

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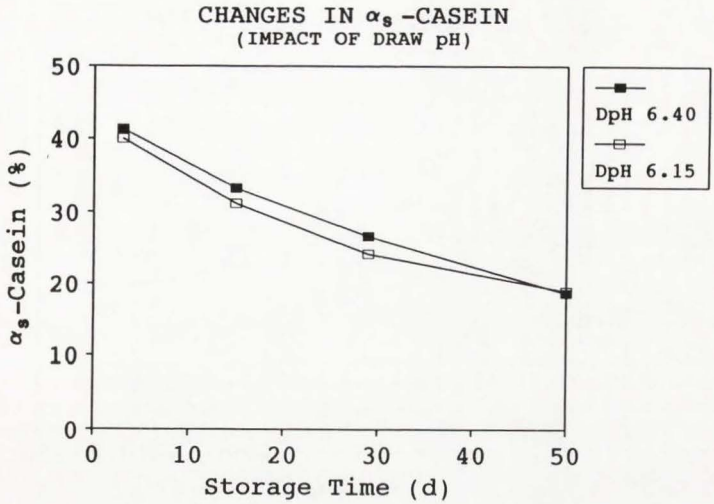

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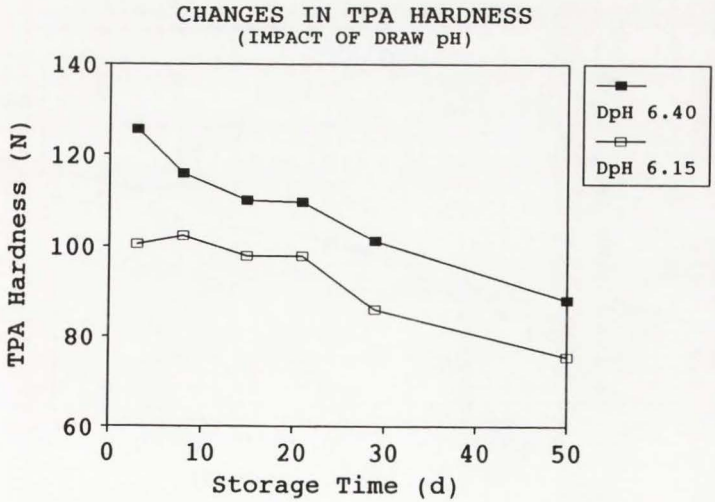

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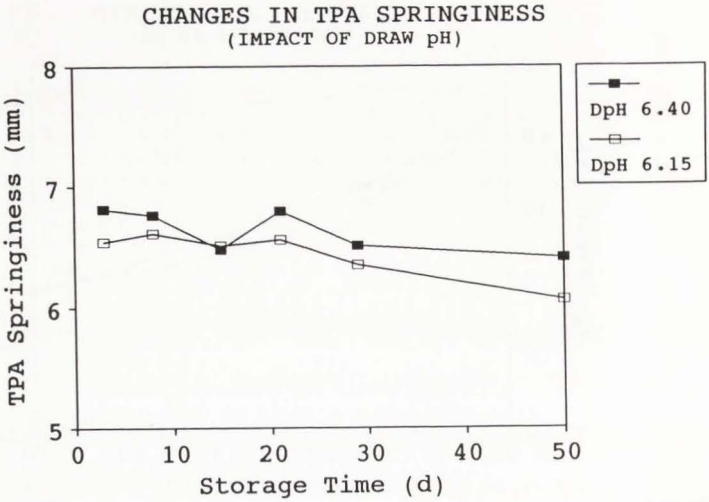

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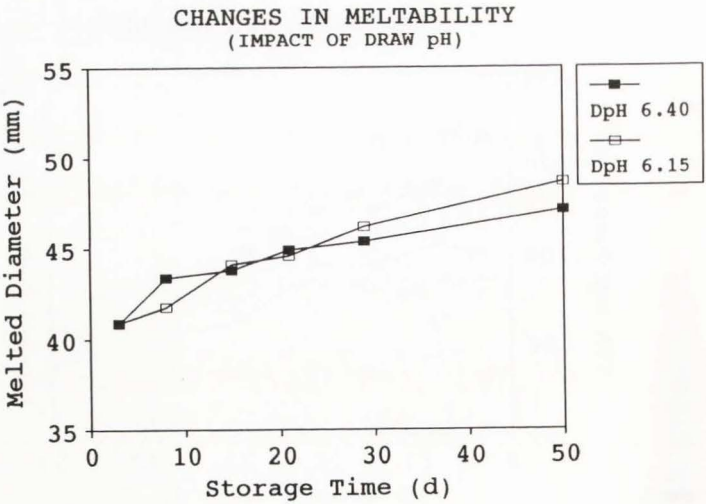
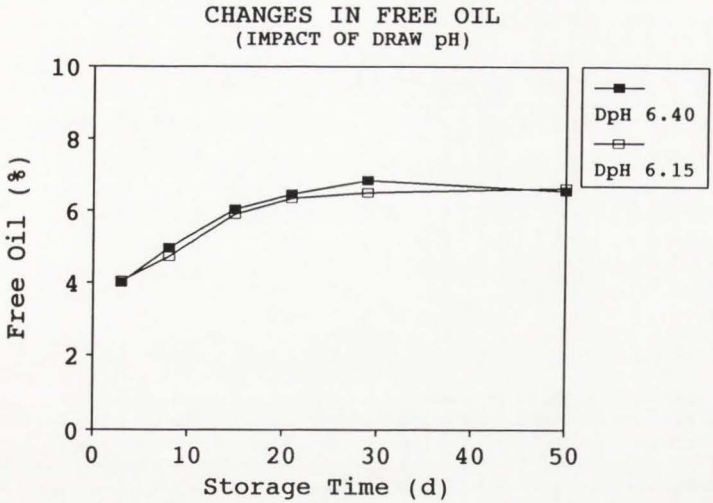


Figure 7.



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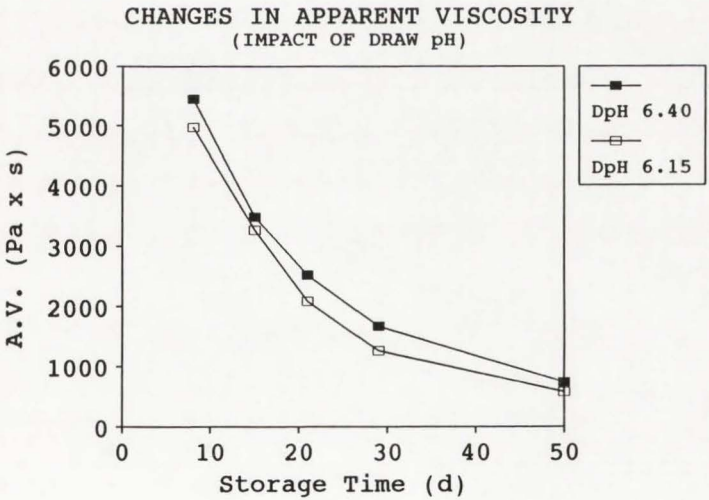

Figure 9.

1993-7

Impact of Coagulant Level on Compostion, Proteolysis, and Functional Characteristics of Mozzarella Cheese

By

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Introduction

It is well known that the coagulant used in cheese making has a dual role in most cheeses. The primary function is to coagulate the cheese milk, thereby producing a curd which is subsequently transformed into the final cheese. In addition, a small proportion of the coagulant is carried over into the cheese. This residual coagulant remains proteolytically active in most aged cheeses and plays an important role in the development of texture and flavor during aging.

In Mozzarella cheese, the impact of coagulant on proteolytic and functional changes during aging will depend on three factors: 1.) the ability of the coagulant to withstand inactivation at the high curd temperatures attained during cooking (i.e., before draining) and during stretching; 2.) the type of coagulant and 3.) the amount of coagulant that is carried over from the milk to the final cheese.

Is Residual Coagulant Inactivated During Cheesemaking?

In the past there was controversy as to whether residual coagulant in Mozzarella cheese remains active or whether it is inactivated by the high temperatures attained during cooking and stretching (1). Recent investigations have shown that the former is likely in most cases. For example, at the 1991 Marschall Italian Cheese Seminar we presented data comparing Mozzarella cheeses made with 3 different commercial coagulants (fermentation-produced chymosin, Mucor miehei protease, and Endothia parasitica protease). The experimental cheeses were made using a 41°C (106°F) cooking temperature. The curds were stretched in 57°C (135°F) water, which resulted a curd temperature of 55°C (131°F) at the exit of the cooker-stretcher, which is typical of commercial practice. The study provided strong evidence that all 3 coagulants remained proteolytically active during aging and thus withstood the 41°C (106°F) cooking temperature and the 57°C (135°F) stretching temperature. Moreover, it was shown that coagulant type had a large impact on proteolytic and functional changes during aging.

More recently, the same 3 coagulants were evaluated for activity loss following various heat treatments in sodium phosphate buffer (0.1 M) at pH 5.2, and in a Mozzarella cheese analogue (2). Coagulant activity was determined using the ortho-phthaldialdehyde proteolysis test. Exposure times and temperatures were chosen to approximate the thermal conditions in commercial cooker-stretchers. Figures 1-3 show activity losses for each of the 3 coagulants (fermentation-produced chymosin, Mucor miehei protease (heat labile), and Endothia parasitica protease) when exposed in pH 5.2 buffer to temperatures ranging from 50° to 75°C (122° to 167°F) for up to 10 minutes. Exposure to 50°C (122°F) for up to 10 minutes had virtually no effect on any of the three coagulants. At 55°C (131°F), Endothia parasitica protease showed partial inactivation, whereas Mucor miehei protease and chymosin were

unaffected. At 60°C (140°F) Mucor miehei protease and chymosin showed partial inactivation, whereas activity of Endothia parasitica protease decreased to below the level of detection after 10 minutes. At 75°C (167°F), activities of all 3 coagulants were reduced to undetectable levels. It is evident that thermal stabilities of the 3 coagulants differed in the following order: fermentation-produced chymosin > Mucor miehei protease > Endothia parasitica protease. Similar patterns of inactivation were obtained when the coagulants were heat-treated in a Mozzarella cheese analogue (data not shown). The experiments confirmed that all 3 coagulants can withstand stretching temperatures in the range of 50° to 55°C (122° to 131°F), with Mucor miehei protease and chymosin able to withstand somewhat higher temperatures. However, stretching at very high curd temperatures (>75°C (167°F)) will inactivate all 3 coagulants.

What Determines the Amount of Residual Coagulant in Mozzarella Cheese?

Holmes et. al. (3) showed that the retention of calf rennet in Cheddar cheese curd was highly dependent on the pH of the whey at draining. Lower pH values at draining resulted in greater retention of calf rennet within the cheese curd. However, draining pH did not affect the retention of coagulants derived from microbial sources (Mucor pusillus var. Lindt, Mucor miehei).

It is reasonable to expect that the amount of coagulant added to the cheesemilk will also influence the level of residual coagulant in the final cheese. This has important practical implications because it is well known in the industry that the amount of coagulant used to set the vat can be reduced considerably below normal recommended levels without adversely affecting the cheese making process. Micketts and Olson (4) successfully manufactured directly acidified Mozzarella using up to 75% less calf rennet than the accepted norm. Consequently, some manufacturers of Mozzarella cheese may use less coagulant in order to economize. However, it is unclear whether using less coagulant influences residual coagulant level in the final cheese, proteolysis by the coagulant during aging, and development of functional characteristics such as melt and shred. Therefore, the objective of our study was to determine the impact of reducing the level of chymosin by up to 40% of normal usage on composition, proteolysis and functional characteristics of cultured Mozzarella cheese.

MATERIALS AND METHODS

Cheesemaking

Three 185 kg vats of cultured low moisture, part-skim Mozzarella cheese were made at Cornell University on the same day using the same milk and starter, but with three different levels of double strength fermentation-produced chymosin: .1, .08, and .06ml/kg milk, representing 100, 80, and 60% of normal usage. Cheeses were made using the milled curd "no-brine" cheesemaking method with a 6.40 draining pH, 41°C cooking temperature, 5.25 milling pH, and 57°C stretching temperature, as described previously (5), with the following modification. The milled curds were briefly washed in water before dry salting to elevate the moisture content of the final cheese. Cheesemaking was replicated on three different days as a 3 X 3 Latin square design. Cheese samples were vacuum packaged and stored at 4°C (40°F) until analysis. Samples of each cheese were also sent on ice by overnight express mail to the University of Vermont for analysis.

Cheese Composition

The initial chemical composition of the cheese was determined. Cheese samples were ground in a blender to obtain a particle size of about 2 to 3 mm. Ground samples were packed in 50 ml plastic

snap-lid vials, without headspace, to minimize moisture loss from cheese during storage at 4°C (up to 2-d prior to analysis). Cheese moisture was determined gravimetrically, in quadruplicate by drying 2 g of cheese at 100°C in a forced air oven (Model OV-490-2, Blue M, Blue Island, IL) for 24 h. Salt content of cheese was determined by the Volhard method (6) and fat content by Babcock (6). Total nitrogen was measured by the Kjeldahl method and converted to protein using a factor of 6.38 (7). Calcium was determined by complexometric titration with EDTA (8). Cheese pH was measured using a pH electrode (Xerolyt, model HA405, Ingold Electrodes, Inc., Wilmington, MA) and an Accument pH meter (Model 915, Fisher Scientific, Springfield, NJ).

Proteolysis

Both pH 4.6 acetate buffer soluble nitrogen and 12% TCA soluble nitrogen content of cheese were measured at 3, 8, 15, 21, 29, and 50 days of storage at 4°C (40°F). All soluble nitrogen values are expressed as a percentage of the total nitrogen content of the cheese. SDS-PAGE (9) was used to monitor α_s and β-casein breakdown during refrigerated storage. The results are expressed as the amount of remaining intact α_s-casein and β-casein at various times of refrigerated storage.

Unmelted Cheese Texture

Cheese samples were analyzed for unmelted texture by Inston Texture Profile Analysis (TPA) (10) at 3, 8, 15, 21, 29, and 50 days of storage at 4°C (40°F).

Cheese Melting Characteristics

Changes in meltability (modified Schreiber test), apparent viscosity (by helical viscometry (11)), and free oil formation (modified Babcock test (12)) were measured at 3, 8, 15, 21, 29, and 50 days of storage at 4°C (40°F). Values for apparent viscosity on day 3 were not included in the statistical analyses because the measurements were generally off-scale and could only be estimated.

Statistical Analysis

Data were analyzed for statistical significance by analysis of variance using the SAS Statistical Software Package. Treatment effects less than the .05 level of probability were deemed significant.

Results And Discussion

Cheesemaking

Varying the chymosin level did not result in any obvious differences in the cheese making process. All vats were cut at 30 minutes after addition of the coagulant regardless of chymosin level. Curd firmness at cutting was not measured in this study. Average fat and protein contents in the milk and whey for each treatment are shown in Table 1. Concentrations of fat and protein in the whey were virtually identical for all 3 chymosin levels. The resulting cheese curds stretched normally with no obvious differences among the 3 treatments.

Cheese Composition

The chemical compositions of cheeses made with different levels of chymosin are compared in Table 2. There were no detectable differences (P>.05) in the moisture, fat, total protein, salt, pH, and calcium contents due to differences in coagulant level. Coagulant level did influence fat on a dry basis (FDB) (P<.05), with highest values occurring when chymosin usage was reduced to 80%. The reason for this difference in FDB is unclear. Micketts and Olson (4) reported that fat recovery in directly acidified Mozzarella cheese increased when calf rennet was reduced by up to 75%. Chymosin level had no effect on whey fat levels in this study (Table 1). Further work is needed to evaluate the impact of coagulant level on solids recovery and cheese yield.

Proteolysis

Changes in pH 4.6 acetate soluble nitrogen and 12% TCA soluble nitrogen are shown in figures 4 and 5. As shown by numerous researchers, both types of soluble nitrogen increased significantly with time of refrigerated storage. However, the rate of increase differed significantly (P<.05) with coagulant level. Both forms of soluble nitrogen showed the slowest rate of increase in cheeses made with 60% chymosin level. Average pH 4.6 soluble nitrogen values were similar for 100% and 80% chymosin cheeses throughout the 50 d storage period, whereas average 12% TCA soluble nitrogen increased more rapidly in 80% chymosin cheeses than in 100% chymosin cheeses.

The amount of residual α_s and β-casein during storage are shown in Figures 6 and 7, respectively. Residual α_s-casein decreased with time for all treatments. Coagulant level did not affect changes in residual α_s-casein (P>.05). The amount of residual β-casein was constant with time, indicating that little if any proteolysis of β-casein occurred during 50 days of storage.

Overall, the impact of reducing chymosin level by up to 40% on proteolysis was initially small but increased with storage time. Thus, the practical impact of using reduced levels of chymosin will probably be greatest for Mozzarella cheese that is held in refrigerated storage for extended periods, such as retail cheese which may require a refrigerated shelf life of several months.

It should also be noted that the pH of the whey at draining in this study (i.e., 6.4) was somewhat higher than the levels normally used in commercial practice. As noted earlier, whey pH at draining influences retention of chymosin by the cheese curd, with greater retention occurring at lower draining pH. Therefore, the results of this study are specific for the draining conditions used in these experiments. It is not known whether the same results would be obtained if cheese making were conducted using a lower pH at draining .

Unmelted Cheese Texture

Changes in TPA hardness (i.e., the force in Newtons required to compress the cheese sample by 50%) are shown in Figure 8. Hardness of all cheeses decreased significantly (P<.05) during refrigerated storage, indicating a progressive softening of the cheese body. There were no detectable differences (P>.05) in TPA hardness due to the differences in coagulant level.

Changes in TPA springiness (i.e., the rebound height of the cheese sample after being compressed by 50%) are shown in Figure 9. Cheeses became less springy with storage time (P<.05); there were no detectable differences (P>.05) in TPA springiness due to the differences in coagulant level.

Cheese Melting Characteristics

Changes in meltability, as assessed by a modified Schreiber test, are compared in Figure 10. This test measures the increase in diameter of the cheese sample as it melts and spreads during heating. Meltability of all cheeses increased ($P<.05$) with storage time, but there were no significant differences ($P>.05$) in meltability due to coagulant level.

Changes in apparent viscosity (by helical viscometry) are shown in Figure 11. A high apparent viscosity value typically indicates a tough, elastic melt, whereas a low apparent viscosity value indicates a softer and more fluid melt. Apparent viscosity decreased significantly ($P<.05$) with time of storage for all cheeses, but there were no detectable differences ($P>.05$) in apparent viscosity due to coagulant level.

Figure 12 shows changes in free oil formation, a measure of oiling off, during refrigerated storage. Free oil increased significantly ($P<.05$) with time. In addition, coagulant level had a significant effect ($P<.05$) on free oil, with 60% chymosin cheese showing the lowest levels of free oil throughout storage. However, the differences in free oil due to coagulant level, though statistically significant, were small and probably of limited practical importance.

Conclusions

1. Reducing the level of chymosin by up to 40% had no obvious effect on the cheese making process, no effect on fat and protein losses to the whey, and virtually no effect on general cheese composition.

2. Reducing the level of chymosin resulted in a slower rate of proteolysis as measured by the formation of pH 4.6 acetate soluble nitrogen and 12% TCA soluble nitrogen.

3. Within the ability of our analytical methods to distinguish textural differences, reducing the level of chymosin did not influence unmelted cheese texture during refrigerated storage for 50 days.

4. Reducing the level of chymosin did not influence meltability or apparent viscosity during refrigerated storage for 50 days, but did result in lower free oil. However, reductions in free oil due to chymosin level were small and probably of limited practical significance.

5. Overall, reducing the level of chymosin by up to 40% had a limited impact on cheese characteristics over 50 days of refrigerated storage. However, it is expected that the impact would be greater if the storage period were extended substantially beyond 50 days.

6. This study was conducted using a relatively high draining pH (whey pH = 6.4). It is not known whether the same results would be obtained using a lower draining pH.

Acknowledgments

The work of Sandra McConnell on thermal inactivation of coagulants is appreciated. Also are appreciated are the contributions of Bob Rasmussen, George Houghton, Wen-I Tsai, and Maureen Chapman.

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TABLE 1. Levels of Fat and Total Protein in the Cheese Milk and the Whey From Mozzarella Cheeses Made with Three Different Levels of Fermentation Produced Chymosin (average of 3 trials).

	CoagulantLevel		
	100%	80%	60%
Cheese Milk			
% Fat	2.23	2.25	2.25
% Total Protein	3.14	3.14	3.14
Whey			
% Fat	.21	.21	.21
% Total Protein	.92	.91	.91

TABLE 2. Chemical Composition of Mozzarella Cheeses Made with Three Different Levels of Fermentation Produced Chymosin (average of 3 trials).

	CoagulantLevel		
	100%	80%	60%
% Moisture	45.67	45.93	45.58
% Fat	21.58	21.96	21.46
% FDB	39.73	40.61	39.43
% Salt	1.54	1.52	1.57
pH	5.22	5.22	5.24
% Calcium	.769	.767	.793

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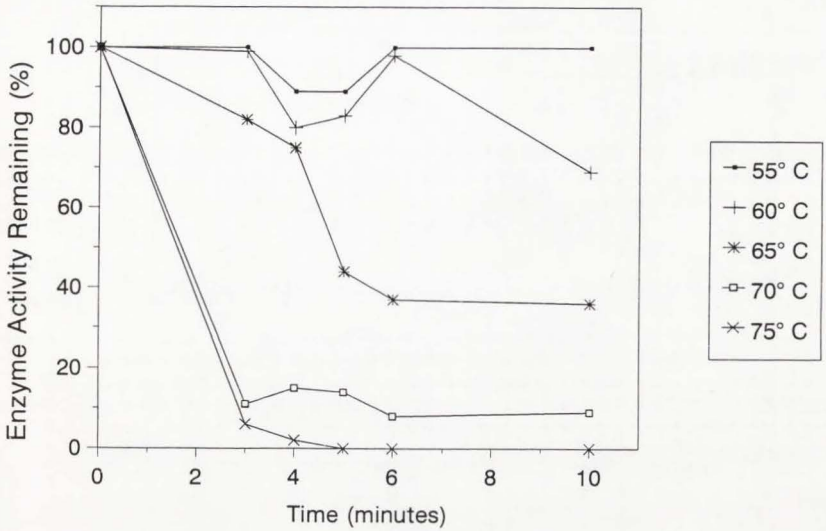
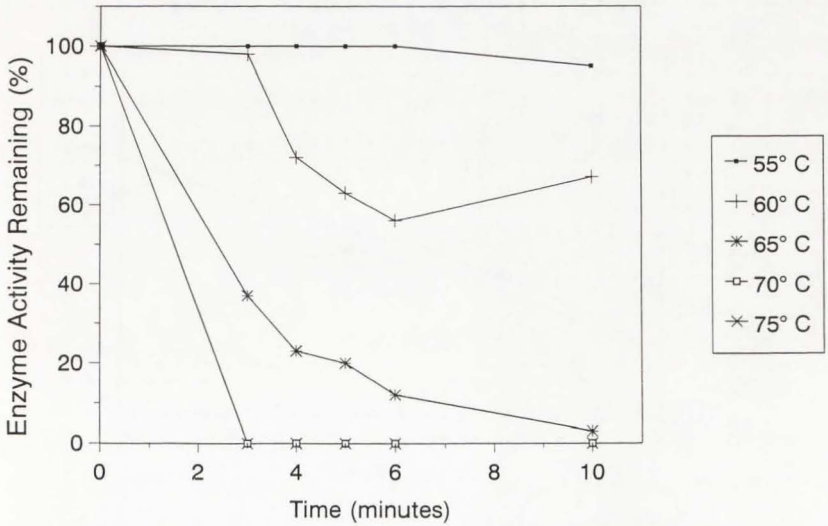
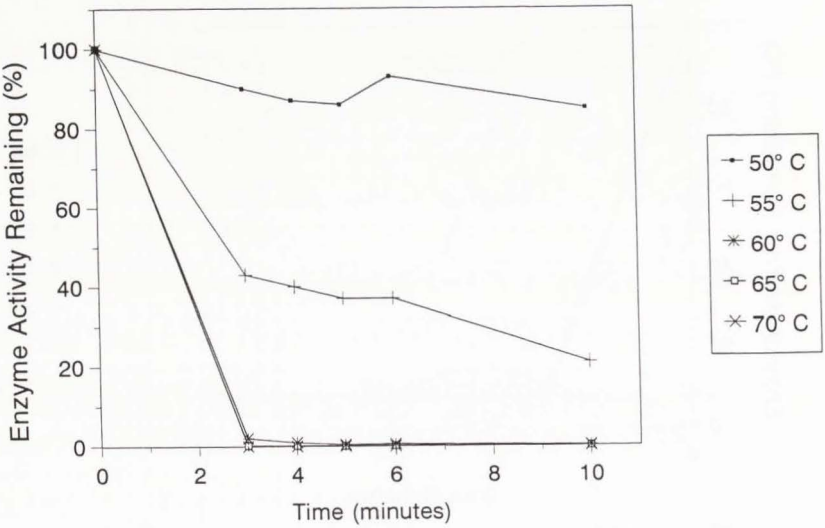
Thermal Inactivation of Fermentation Produced Chymosin

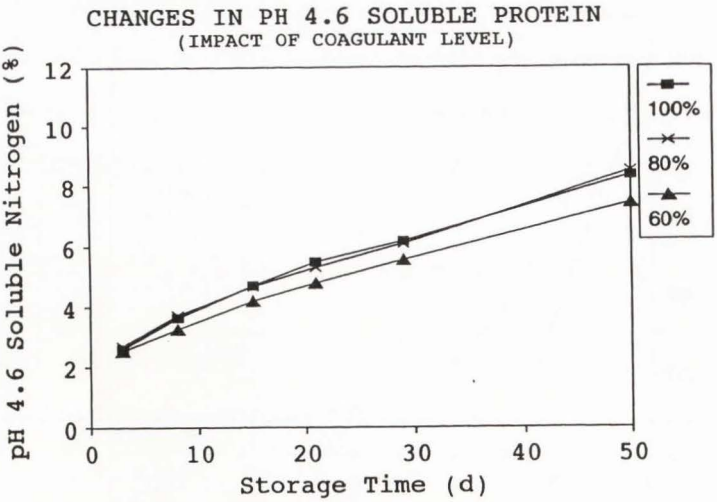


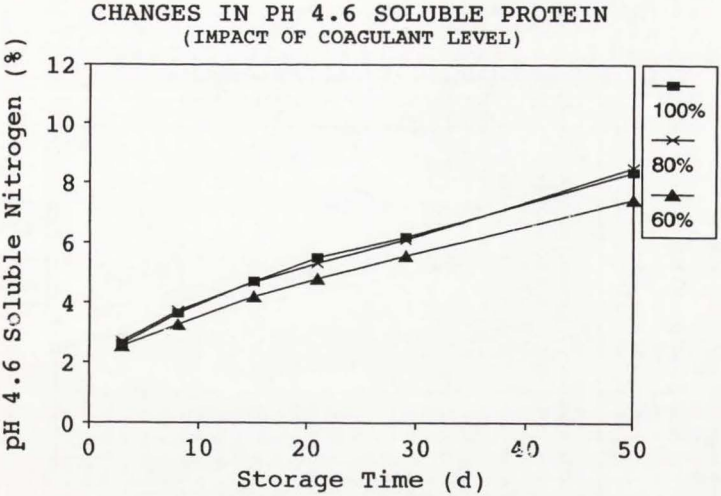
Thermal Inactivation of *Mucor miehei* Protease

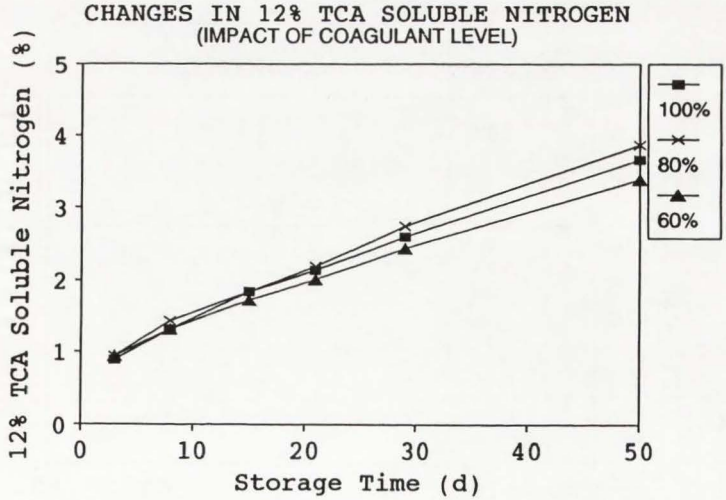


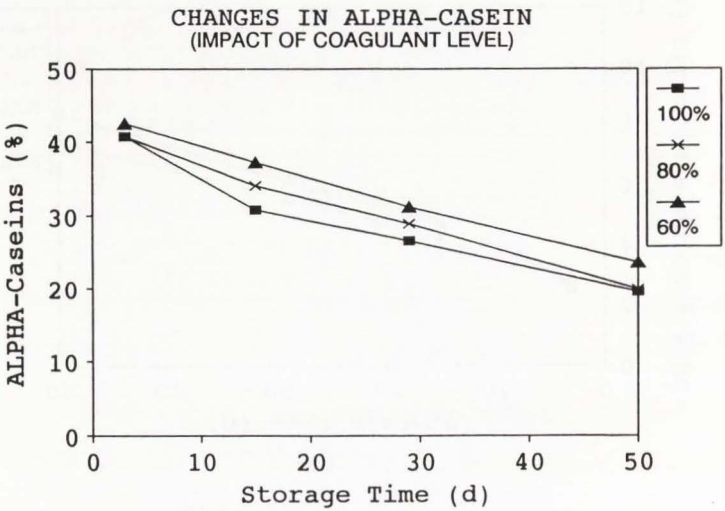
Thermal Inactivation of *Endothia parasitica* Protease

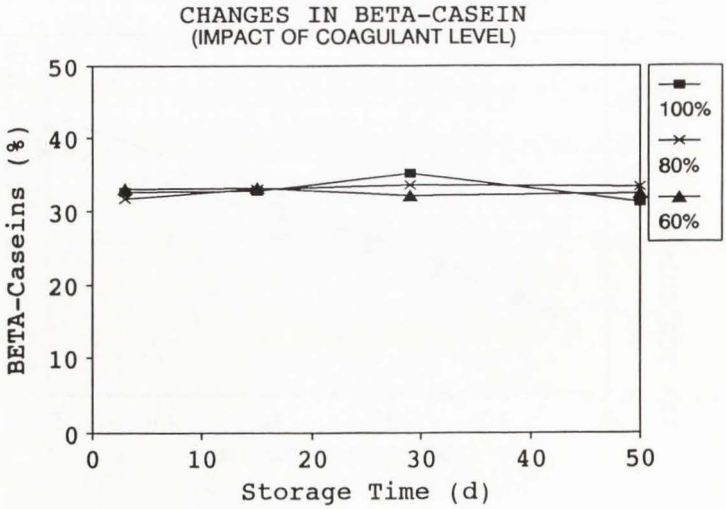


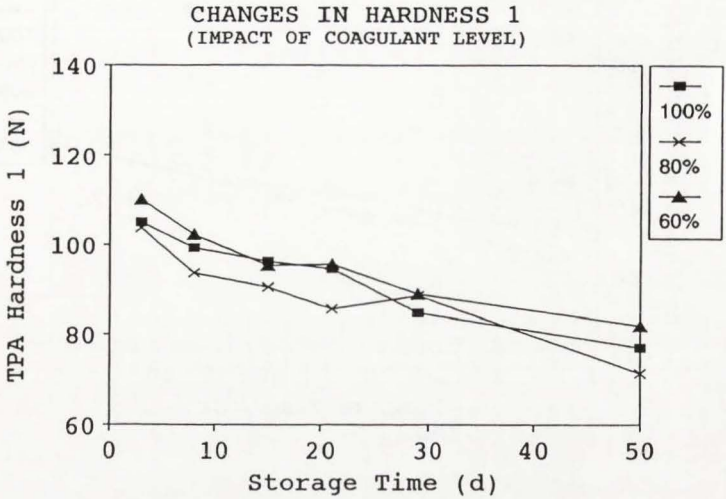


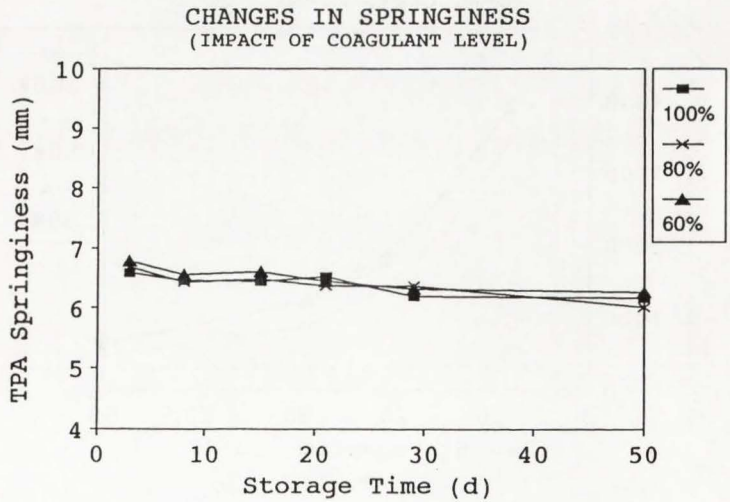

CHANGES IN PH 4.6 SOLUBLE PROTEIN
(IMPACT OF COAGULANT LEVEL)
pH 4.6 Soluble Nitrogen (%)
12
10
8
6
4
2
0
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%


CHANGES IN 12% TCA SOLUBLE NITROGEN
(IMPACT OF COAGULANT LEVEL)
12% TCA Soluble Nitrogen (%)
5
4
3
2
1
0
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%


CHANGES IN ALPHA-CASEIN
(IMPACT OF COAGULANT LEVEL)
ALPHA-Caseins (%)
Storage Time (d)
100%
80%
60%
0
10
20
30
40
50


CHANGES IN BETA-CASEIN
(IMPACT OF COAGULANT LEVEL)
BETA-Caseins (%)
Storage Time (d)
100%
80%
60%
0
10
20
30
40
50


CHANGES IN HARDNESS 1
(IMPACT OF COAGULANT LEVEL)
TPA Hardness 1 (N)
140
120
100
80
60
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%


CHANGES IN SPRINGINESS
(IMPACT OF COAGULANT LEVEL)
TPA Springiness (mm)
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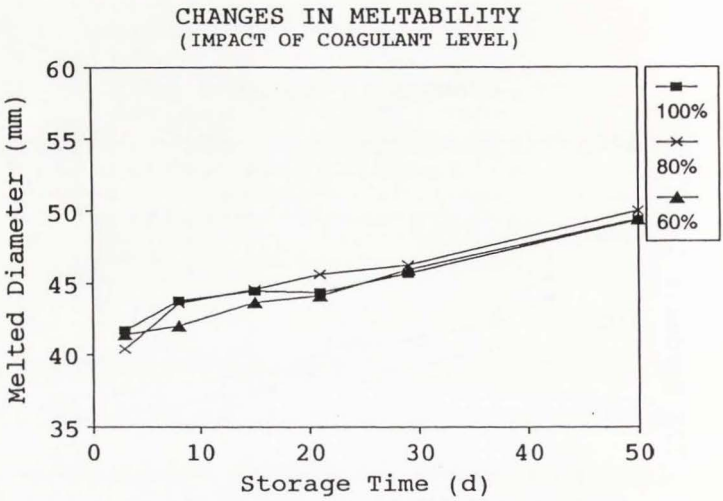
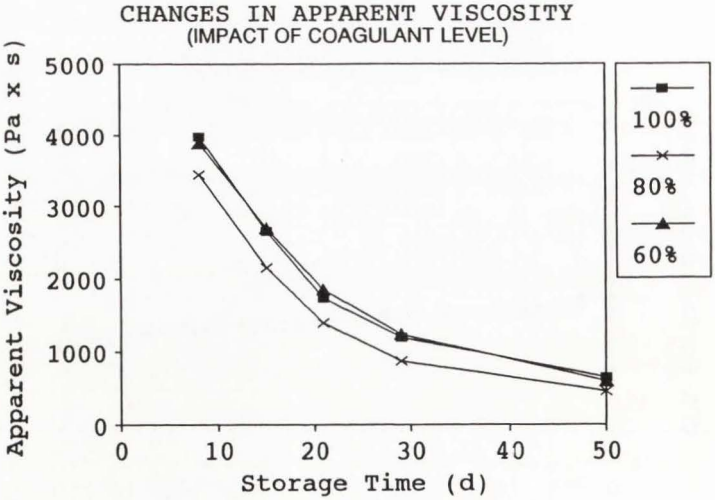
9
8
7
6
5
4
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%


CHANGES IN MELTABILITY
(IMPACT OF COAGULANT LEVEL)
Melted Diameter (mm)
60
55
50
45
40
35
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%


CHANGES IN APPARENT VISCOSITY
(IMPACT OF COAGULANT LEVEL)
Apparent Viscosity (Pa x s)
5000
4000
3000
2000
1000
0
0
10
20
30
40
50
Storage Time (d)
100%
80%
60%

1993-8

Contributions of Coagulant, Starter, and Milk Enzymes to Proteolysis and Browning in Mozzarella Cheese

By

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Abstract

Four types of Mozzarella cheese (i.e., control, rennet-free, starter-free, and rennet-free, starter-free) were made from the same batch of milk. The cheesemaking was replicated four times each using different batches of milk (total of 16 different cheeses). Changes in proteolysis during 51 d of storage at 4°C were measured by pH 4.6 soluble nitrogen, 12% TCA soluble nitrogen, and SDS-PAGE. Active rennet retained in the cheese produced medium to large molecular weight peptides from casein during refrigerated storage. Starter proteases and peptidases significantly affected production of low molecular weight peptides. It was necessary to have both coagulant and starter cultures present to obtain the extent and depth of proteolysis that was observed in control cheese. Endogenous milk proteases contributed little, if any, to the proteolysis of Mozzarella cheese during refrigerated storage. Coagulant had some effect on "b" value (yellow/blue) of baked Mozzarella cheese. However, low molecular weight proteolytic products produced by starter enzymes had the most significant impact on overall browning of baked Mozzarella cheese.

Introduction

Functional properties of Mozzarella cheese are important in pizza and other prepared foods. Functional properties of Mozzarella cheese include shredability, meltability, stretchability, oiling-off, and browning characteristics (1, 13). To reach the optimum functional properties of Mozzarella cheese, Mozzarella is usually aged for a short period of time (15 to 35 d) before use. During this short period of aging, proteolysis of casein continues, cheese texture changes, and optimum functional properties are developed (13).

Sources of Proteases in Cheese

Proteolytic enzymes in cheese can come from the milk, nonstarter bacteria, starter and the coagulant. Each of these sources of proteases may or may not contribute to proteolysis during cheese aging. In addition, the type of proteases or peptidases contributed by each source may have different specificities and produce different types of end products from milk proteins. Proteolysis will influence functional properties of the cheeses. Too much proteolysis may produce a cheese that will not shred very well and may have too much liquid characteristic when melted on the surface of a pizza. Ryan (22) indicated that improper ratio of rod-to-coccus, especially excess rods, might cause too much casein breakdown and soft body defects. In contrast, a cheese with too little proteolysis may not melt well and produce a pizza that is too chewy and tough.

Proteolysis and Browning

Browning is an important characteristic of Mozzarella cheese during pizza making. Some pizza makers like the cheese to remain white, while others like light brown blisters of uniform size and distribution on pizza. Nonenzymatic browning (the Maillard reaction), that occurs during heating of Mozzarella cheese, involves a complex set of reactions. Nonenzymatic browning occurs in foods which contain reducing sugars and free amino groups usually derived from proteolysis products of proteins (6).

Browning may be influenced by the lactose or galactose content of Mozzarella cheese (12) which can be affected by several factors including the starter cultures. Most strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* are unable to ferment galactose, while strains of *Lactobacillus helveticus* are able to use galactose. Oberg et al. (17, 18) detected no difference in cook color of Mozzarella cheese when using different milk-clotting enzymes. However, the cook color did change with storage time for all cheeses. They also found that Mozzarella cheese made using starter cultures increased in brown cook color with time of refrigerated storage, but Mozzarella made by direct acid addition showed little brown cook color and no significant increase of color with time. Olson et al. (20) stated that the rate of cooling in Italian cheese had an impact on the development of brown color during cooking. Cheese cooled rapidly (i.e., 24 h) developed lighter color than the cheese cooled slowly (i.e., 50 h).

No one has conducted a study to determine the individual effect of coagulant, starter, and endogenous milk enzymes on the changes in proteolysis and browning characteristics of Mozzarella cheese during refrigerated storage. This type of study has been conducted for proteolytic changes in Cheddar (19) and Gouda (25, 26) cheese. Thus, our objective was to determine the individual effect of coagulant, starter, and milk enzymes on browning and the extent, depth, and characteristics of proteolysis in Mozzarella cheese during storage at 4°C.

Materials and Methods

Experimental Design

From the same batch of skim milk and cream, four types of low-moisture, part-skim Mozzarella cheese were made, i.e., control, rennet-free (RF), starter-free (SF), and rennet-free starter-free (RFSF) cheeses. Milk was processed and pasteurized on the first day of the cheesemaking week. Control and RF cheeses that contain starter cultures were made on one day, and SF and RFSF cheeses that contain no starter cultures were made on the other day in order to avoid contamination of SF cheeses with starter bacteria. Cheesemaking was replicated in four different weeks with separate batches of skim milk and cream each week. Therefore, a total of sixteen Mozzarella cheeses were made in the experiment.

Cheesemaking

Control, RF, SF, and RFSF Mozzarella cheeses were made as described by Chu et al. (8). The RF cheese was produced by removal of about 30 to 40% of the calcium from the milk by ion exchange prior to addition of rennet. This allowed the rennet to cleave -casein without clotting the milk. Next the rennetted milk was pasteurized to inactivate the rennet and then calcium was added back to the milk to achieve a coagulation. The SF cheese was made by using a combination of lactic acid and glucono– –lactone as a replacement for the lactic acid that would have been produced by the culture. The RFSF cheese was produced using a combination of these two techniques. The control cheese was produced using both starter culture and rennet. Additional precautions were taken to protect all cheeses from contamination with nonstarter bacteria during cheesemaking.

Chemical Analyses

Moisture content of cheese was determined gravimetrically by drying 2 g of ground cheese at 100°C in a forced-air oven (model OV-490A-2; Blue M, Blue Island, IL) for 24 h (21). Fat content of milk (2) and cheese (21) were determined by Babcock test. Salt content of cheese was determined by the Volhard method (21). Cheese pH was determined by immersing a Xerolyt electrode (model HA405; Ingold Electrode, Willmington, MA) directly into ground cheese (25° C.)

Titratable acidity of cheese was determined (2) by adding 10 g of cheese to 95 ml of 60°C distilled water. The mixture was blended for 30 s, and filtered (Whatman #1). Twenty five ml of the filtrate were titrated with .1 N NaOH, and the acid content of the cheese was calculated as the percentage of lactic acid.

Total nitrogen (TN) content of skim milk and cheese was determined by Kjeldahl (2). Noncasein nitrogen (NCN) in skim milk was determined by the International Dairy Federation method (11). All nitrogen components were multiplied by 6.38 to give the appropriate "protein" equivalents; CP = 6.38 x TN, casein = 6.38 x (TN - NCN).

Calcium concentration in milk and whey samples was determined using an atomic absorption spectrometer. Calcium concentration in cheese was determined by complexometric titration (14).

Proteolysis During Refrigerated Storage

The extent and depth of proteolysis were monitored by measuring nitrogen soluble in pH 4.6 acetate buffer and 12% TCA, respectively, for all the cheeses (2, 5). All pH 4.6 and 12% TCA soluble nitrogen values were expressed as a percentage of TN content of the cheese. An SDS-PAGE method (23) with a 10 to 20% acrylamide gradient was used to monitor and characterize the proteolysis of α_s-caseins and β-casein during cheese storage.

Microbiological Analysis

Samples of raw milk, milk after ion exchange (low calcium milk) and pasteurized standardized milk for SF cheesemaking and RFSF cheesemaking were taken for standard plate counts (SPC) and yeast and mold counts (21). Samples of SF cheese and RFSF cheese were taken after 2, 30, and 51 days of refrigerated storage.

The nonstarter colonies present in the SPC for SF and RFSF cheeses were picked and streaked a minimum of 2 times on SPC agar. Isolates were tentatively identified into genera by examining colony and microscopic morphology, and by using tests such as catalase and oxidation-fermentation tests (4). Enumeration of starter bacteria in control and RF cheeses was done by pour plate count using modified Lee's agar medium (16).

Browning Test

A Teflon® coated aluminum pan with 12 round sample wells (7 cm diameter x 3 cm high) was used for the browning test. Ground cheese samples were weighed (20 g) into each sample well in the pan. This was done in triplicate for each cheese. Ground cheese samples were allowed to warm to room temperature before heating. The pan containing the samples was put into a preheated forced air oven at 100°C for 1 h. Cheese samples were cooled to room temperature before color determination. The melted cheese fused into a solid disk that was removed from the sample well.

Color was measured using a MacBeth Color-Eye spectrophotometer (model 2020PC; Optiview, Macbeth, Newburgh, NY) that was calibrated by using a white calibration tile. Large area view (25.4

mm diameter) was used. Cheese samples were placed in a specially designed sample holder and put in the view port. Three color indices, "L" (light/dark), "a" (red/green), and "b" (yellow/blue) were taken for each sample.

RESULTS AND DISCUSSION

Cheese Making Procedures

The mean CP and casein content of the skim milk for all cheese making was 3.22% and 2.44%, respectively. In an effort to obtain more consistent fat content of final cheeses, milks were standardized at different fat levels (2.1%, 2.4%, and 2.2% for control, RF and RFSF, and SF cheeses, respectively). The four cheese types were made as described previously (8). Control cheese contains the proteases from coagulant, starter cultures, milk, and nonstarter bacteria. RF cheese contains proteases from starter cultures, milk, and nonstarter bacteria. The SF cheese contains proteases from coagulant, milk, and nonstarter bacteria. RFSF cheese contains proteases from milk and nonstarter bacteria. Therefore, the individual effects of coagulant, starter proteases and milk enzymes plus nonstarter bacteria on the proteolysis of Mozzarella cheese during refrigerated storage can be determined by comparing the proteolytic changes among the four cheese types.

Cheese Composition

Cheese moisture, fat, protein, pH, titratable acidity, and salt are shown in Table 1. It would be ideal if all four types of cheese had identical chemical composition. However, it was very difficult to control all the steps in the four different cheese-making procedures and this could not be achieved.

In our study, the moisture content for RF cheese was higher than for other cheeses. This is due to the poor syneresis in the RF cheesemaking, as reported previously (8). Visser (24) also experienced slower curd syneresis in RF Gouda cheese making than in normal cheese making, resulting in a higher moisture content in RF Gouda cheese.

Fat loss during cheesemaking was significantly higher for RF and RFSF cheeses than control and SF cheeses and caused the fat content of these cheeses to be low (Table 1). The soft gel and poor syneresis are the reasons for the larger fat loss. Most of the fat was lost into the whey, as reported previously (8). Differences in protein content are due to the differences in fat and moisture content of the cheese.

Although there were also some variations in the pH, titratable acidity, and salt content among the four cheeses, Yun et al. (27) found that for Mozzarella cheese pH ranging from 5.09 to 5.27, the rate of proteolysis was not affected by the final pH of the cheese. The moisture in the nonfat substance (MNFS) of the four types of Mozzarella cheese ranged from 55.54% in RFSF cheese to 62.54% in control cheese (Table 1). The MNFS of 34 commercial low moisture part skim Mozzarella cheese samples ranged from 55.47% to 66.38% (3). The MNFS values of all four types of Mozzarella cheese in the present study are within the range of MNFS values observed in commercial low moisture part skim Mozzarella cheese. Therefore, we felt that the changes in proteolysis during refrigerated storage of our cheese would not be affected greatly by the differences in the chemical composition among the cheeses.

Calcium concentration in cheese milk, whey, and cheese are shown in Table 2. The calcium content of milk for RF and RFSF cheesemaking was higher than for control and SF cheesemaking because calcium was added to the milks, after rennet inactivation, to induce coagulation. As expected from previous work (8), the whey and cheese from RF and RFSF cheesemakings had higher calcium content than those from control and SF cheesemakings.

Proteolysis of Cheese During Refrigerated Storage

The nitrogen components soluble in the pH 4.6 acetate buffer include high and medium molecular weight peptides derived from α_s-caseins and β-casein, as well as low molecular weight peptides and amino acids (7). Therefore, pH 4.6 soluble nitrogen is a good indication of the extent of proteolysis of cheese. On the other hand, only low molecular weight peptides and amino acids from the cheese are soluble in the 12% TCA solution (7). Thus, 12% TCA soluble protein is a good indication of the depth of proteolysis in cheese.

In addition to indicating the extent of proteolysis that has occurred in cheese, SDS-PAGE also indicates the characteristics of the proteolysis. Disappearance of the bands for individual caseins and appearance of proteolytic products with different molecular weights are distinguished easily by SDS-PAGE.

Milk Enzymes and Nonstarter Bacteria. In our experiment, the effect of milk enzymes and nonstarter bacteria could not be separated since the cheeses made were not totally free of nonstarter bacteria. However, raw milk quality was good and the conditions of cheesemaking were controlled to minimize post pasteurization bacterial contamination. The SPC of milk did not increase during the ion-exchange process, and the SPC were low after pasteurization (less than 500 cfu/ml).

Mean SPC of fresh SF and RFSF cheeses were less than 5,000 cfu/g (Table 3). After 51 d of refrigerated storage, the mean SPC decreased to <1,000 cfu/g. Thus, nonstarter bacteria in the cheeses did not increase in numbers during 51 d of storage at 4°C. At the same time, 4.5 to 6.7 x 10^8 cfu/g of starter bacteria were still viable in RF and control cheese after 51 d of storage (Table 3). Most of the nonstarter bacteria found in SF and RFSF cheeses were either a) gram positive, catalase positive, spore forming rods or b) one of two types of gram positive, catalase positive cocci. One type of cocci could ferment glucose aerobically only, while the other fermented glucose both aerobically and anaerobically. They were tentatively identified as Bacillus, Staphylococcus, and Micrococcus sp. Yeast and mold counts were <10/g in both SF and RFSF cheeses.

Proteolysis due to milk enzymes or nonstarter bacteria would be observed in the RFSF cheese. No degradation of α_s-caseins or β-casein was observed by SDS-PAGE for RFSF cheese. The rate of change of pH 4.6 soluble nitrogen, 12% TCA soluble nitrogen, and α_s-caseins in RFSF cheese are shown in Figures 1, 2, and 3, respectively. Comparing to the other three cheese types, there was almost no change in pH 4.6 and 12% TCA soluble nitrogen or α_s-caseins content of RFSF cheese during refrigerated storage. From the data presented in Figures 1, 2, and 3, it is apparent that milk enzymes and nonstarter bacteria contributed little, if any, to either the extent or depth of proteolysis during 51 d of storage of Mozzarella cheese at 4°C.

Coagulant. The nitrogen soluble in pH 4.6 acetate buffer and 12% TCA increased, while intact α_s-caseins decreased with time of refrigerated storage in control and SF cheeses (Figures 1, 2, and 3). The coagulant significantly influenced the rate of increase of pH 4.6 and 12% TCA soluble nitrogen and degradation of α_s-caseins during refrigerated storage of Mozzarella cheese. Thus, the coagulant contributed both to the extent and depth of proteolysis of Mozzarella cheese during storage at 4°C.

Creamer (9) indicated that there was rennet activity in the Mozzarella curd when the curd was held for 5 min in stretching water at temperatures below 65°C. However, no rennet activity was present in the curd that had been held for 5 min in water > 70°C. We used 60°C stretching water in our cheesemaking. Therefore, the presence of rennet activity in Mozzarella cheese is in agreement with the findings of Creamer (9).

The major caseins degraded by the coagulant used in this study (i.e., chymosin) were α_s-caseins. The β-casein was not broken down during 51 d of 4°C storage for cheese in this study. When chymosin was used as a coagulant in a previous study (28), similar results were obtained and are in agreement with

other reports (15, 19, 26). However, in industry it is common to use microbial rennets as coagulants for Mozzarella cheese manufacture instead of chymosin. The breakdown of β-casein in Mozzarella cheese made with these coagulants can be significant, particularly when Endothia parasitica protease is used (10, 28).

The SF cheese contained proteases from the coagulant, milk, and nonstarter bacteria. Since the milk proteases and nonstarter bacteria did not contribute to proteolysis as seen from the RFSF cheese, then the proteolysis observed in the SF cheese (Figures 1, 2, and 3) was due to the coagulant.

Starter Bacteria. Proteolysis that occurred in RF cheese would reflect the contribution of the starter culture enzymes. Starter culture significantly influenced the rate of changes of pH 4.6 and 12% TCA soluble nitrogen, but had no significant effect on the level of intact α_s-caseins remaining in Mozzarella cheese during refrigerated storage. Previous reports on other cheeses (19, 25) have indicated that peptidases from starter bacteria break down the proteolytic products, produced by the action of rennet, to low molecular weight peptides and amino acids. Thus, starter proteases and peptidases contribute to the depth of proteolysis. Our results support these conclusions.

Most nitrogen containing compounds that dissolve in a 12% TCA solution should also dissolve in pH 4.6 acetate buffer. Therefore, the difference between pH 4.6 and 12% TCA soluble nitrogen would indicate the net amount of medium and high molecular weight peptides present in the cheese. No significant influence of the starter proteases on the difference between pH 4.6 and 12% TCA soluble nitrogen was detected (Figure 4). The change in amount of medium and high molecular weight proteolytic products during 51 d of storage of RF cheese was about the same as RFSF cheese. Therefore, starter proteases contributed mainly the depth of the proteolysis in Mozzarella cheese during storage at 4°C.

Coagulant/starter Interaction. There was a significant interaction between coagulant and starter culture for the production of 12% TCA soluble nitrogen. This implies that it is necessary to have the proteolytic action of the coagulant on casein first, then starter proteases and peptidases break down the proteolytic products produced by the coagulant. Neither α_s-caseins nor β-casein were degraded by starter cultures present in RF cheese. In our RF cheese, the starter proteases alone could not produce changes in 12% TCA soluble nitrogen comparable to control cheese (Figure 2). This result is in agreement with Visser (25), who reported that in normal Gouda cheese the action of rennet clearly stimulated starter peptidases to produce amino acids and low molecular weight peptides. Thus, it is necessary to have both enzymes from coagulant and starter active in the cheese to achieve the depth of proteolysis observed for the control cheese.

Browning Test

Changes in "a", "b", and "L" values during 51 d of storage at 4°C are shown in Figures 5, 6, and 7, respectively. Starter proteases and peptidases in the Mozzarella cheese influenced the changes of all three color indices ("a", "b", and "L" values) of the melted cheeses. Coagulant also had some effect on the changes of "b" value in the cheese (Figure 6). The color of the melted RF cheese (at d 30) was significantly darker (lower "L" value) than other three cheeses (Figure 7). The low fat content of RF cheese (Table 1), which caused less free oil formation during the heating, might be the reason for the darker color.

There were significant differences in the changes of "a" and "b" values between cultured cheeses (control and RF) and direct acid cheeses (SF and RFSF) during the storage at 4°C (Figure 5 and 6, and Table 5). Although SF cheese had a greater extent of proteolysis than RF cheese (Figure 4), most of the proteolytic products produced by the action of coagulant in SF cheese were medium to large molecular weight peptides. Even with residual lactose present in this cheese, the Maillard reaction and browning

were minimal and resulted in low "a" value for SF cheese. On the other hand, the extent of proteolysis of RF cheese was low (Figure 4). However, most of the proteolytic products in RF cheese were low molecular weight peptides and amino acids produced by the action of starter peptidases (Figure 2). The combination of low molecular weight compounds with free amino groups and residual galactose or lactose caused extensive Maillard browning and high "a" value for RF cheese. The difference in browning (i.e. "a" value) between these two cheeses (i.e. SF and RF) indicated that the depth of proteolysis had more impact on browning of Mozzarella cheese upon baking.

Oberg et al. (18) reported that Mozzarella cheese made using proteinase positive strains of starter cultures showed more browning after cooking than cheese made using proteinase negative strains. Oberg et al. (17, 18) also compared the difference between the cook color of direct acid and cultured Mozzarella cheeses and found that the direct acid cheese appeared white, while cultured cheese developed much darker cook color. Again, this indicated that not only the residual sugar is important for development of color during cooking, specific types of proteolytic products also influence browning during cooking. Our results are in agreement with those of Oberg et al. (17, 18).

Conclusions

Endogenous milk enzymes contribute little, if any, to either the extent or the depth of proteolysis of Mozzarella cheese during 51 d of storage at 4° C. Coagulant contributes greatly to the extent of proteolysis and is the most important source of proteolytic enzyme for production of medium to large molecular weight peptides from casein in Mozzarella cheese during storage at 4° C. Starter culture proteases and peptidases contribute greatly to the depth of proteolysis and are most important for production of low molecular weight, TCA soluble, proteolytic products. However, it was necessary to have both coagulant and starter proteases present to obtain the depth of proteolysis that was observed in control cheese. The depth of proteolysis has a greater influence on the browning characteristics of Mozzarella cheese than the extent of proteolysis.

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TABLE 1. Average (n=4) chemical composition of the four types of Mozzarella cheese at 2 d of storage at 4°C.

	Cheese Type					
	Control	RF	SF	RFSF	SEM[1]	LSD[2]
Moisture, %	50.37[a]	54.75[b]	49.51[a]	49.49[a]	.65	2.08
Fat, %	19.46[a]	9.29[b]	17.53[a]	10.88[b]	.61	1.95
FDB[3], %	39.18[a]	20.55[b]	34.70[c]	21.48[b]	1.06	3.41
Protein, %	25.47[a]	29.84[b]	26.72[a]	31.42[b]	.54	1.71
pH	5.16[a]	5.27[b]	5.18[ab]	5.02[c]	.03	.10
TA[4], %	.55[a]	.43[b]	.58[ac]	.66[c]	.03	.08
Salt, %	1.33[a]	1.72[b]	1.61[b]	1.44[a]	.04	.13
S in M[5], %	2.65[a]	3.15[b]	3.25[b]	2.90[c]	.06	.20
MNFS, %	62.54[a]	60.38[b]	60.02[b]	55.54[c]	.61	1.96

[a,b,c] = Means within same row not sharing same superscripts are different (P <.05).
[1] Standard error of means; SEM = [(mean square for error)/n]$^{1/2}$.
[2] Least significant difference at P <.05.
[3] Fat content on a dry weight basis.
[4] Titratable acidity.
[5] Salt concentration in water phase of cheese.
[6] Moisture in the nonfat substance.

TABLE 2. Average (n=4) calcium concentration of milk, whey, and cheeses for the four types of Mozzarella cheese.

	Cheese Type					
	Control	RF	SF	RFSF	SEM[1]	LSD[2]
milk, mg/kg	1056.0[b]	1377.0[a]	1043.5[b]	1368.3[a]	11.17	36.43
whey, mg/kg	392.8[c]	534.5[b]	424.0[c]	602.5[a]	14.66	46.90
cheese, %	.670[b]	1.035[a]	.634[b]	1.002[a]	.031	.099

[a,b,c] = Means within same row not sharing same superscripts are different (P <.05).
[1] Standard error of means; SEM = [(mean square for error)/n]$^{1/2}$.
[2] Least significant difference at P <.05.

TABLE 4. Mean squares and (probability values) for the influence of different factors on indices of color changes during 51 d of storage at 4°C.

Factors	Color Indices[1] "a" value	"b" value	"L" Value
	---------- (x 10^{-4}) ----------		
Trial	75.88 (.15)	38.37 (.13)	53.36 (.63)
Starter	1131.48* (< .01)	179.96* (< .01)	558.14* (.03)
Coagulant	32.12 (.35)	90.72* (.04)	75.34 (.38)
Starter x coagulant	7.41 (.65)	38.63 (.15)	.59 (.94)
Error	33.46	15.83	87.81
R square	.82	.75	.50

*Factor has a significant ($P < .05$) influence.

[1] "a" value is a measure of red to green, "b" value is a measure of yellow to blue, and "L" value is a measure of light to dark.

TABLE 3. Mean (and standard deviation) total plate count (n=4) of the four types of Mozzarella cheese during storage at 4°C.

Time of storage	Cheese Type Control	RF	SF	RFSF
	---------- (cfu/g) ----------			
2 d	NA	NA	1,100 (570)	4,200 (3,300)
30 d	NA	NA	840 (360)	1,100 (930)
51 d	6.7×10^8 (1.1×10^8)	4.5×10^8 ($.8 \times 10^8$)	600 (320)	1,000 (1,000)

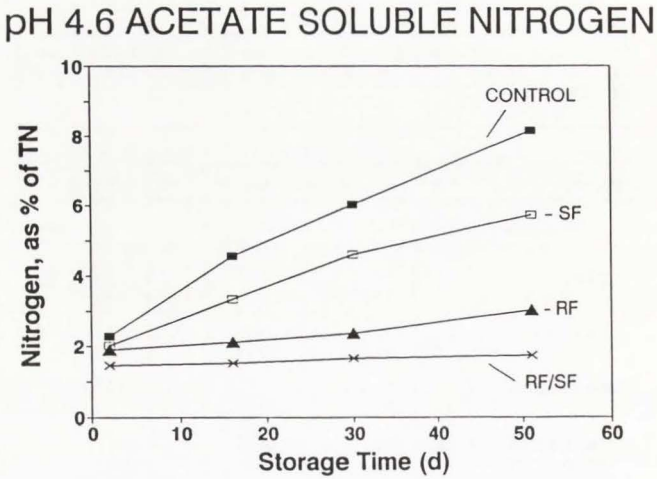

Figure 1. Changes of pH 4.6 soluble nitrogen as a percentage of total nitrogen during 4°C storage of the four types of Mozzarella cheese: control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .007.

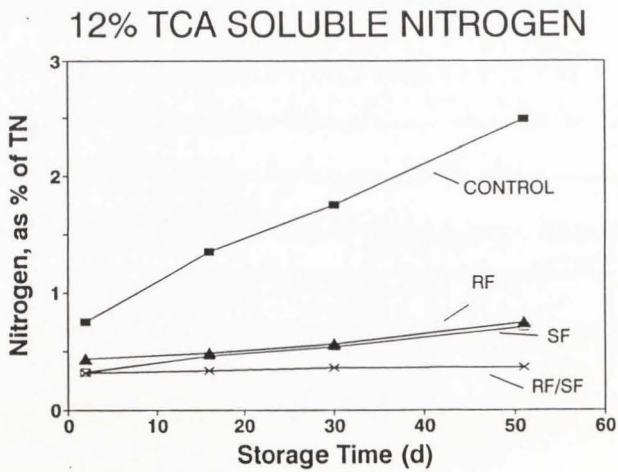

Figure 2. Changes of 12% TCA soluble nitrogen as a percentage of total nitrogen during 4°C storage of the four types of Mozzarella cheese: control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .003.

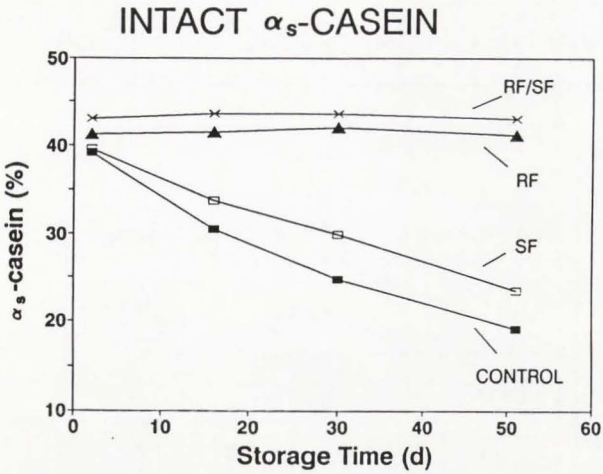

Figure 3. Changes of relative percentage of α_{s1}- plus α_{s2}-caseins during 4°C storage of the four types of Mozzarella cheese: ■ = control; control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .034.

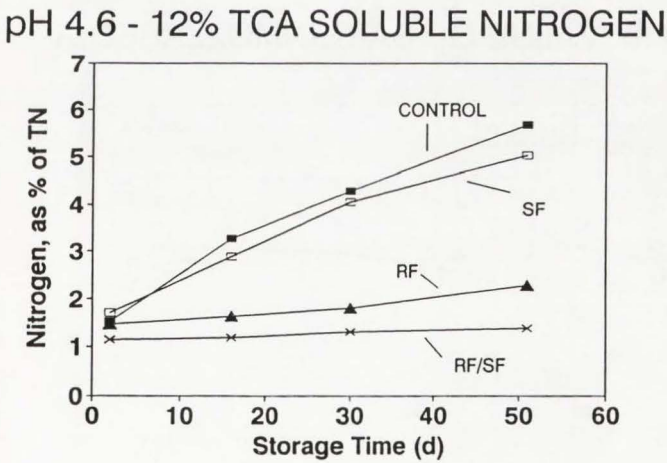

Figure 4. Changes of pH 4.6 minus 12% TCA soluble nitrogen as a percentage of total nitrogen during 4°C storage of the four types of Mozzarella cheese: control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .006.

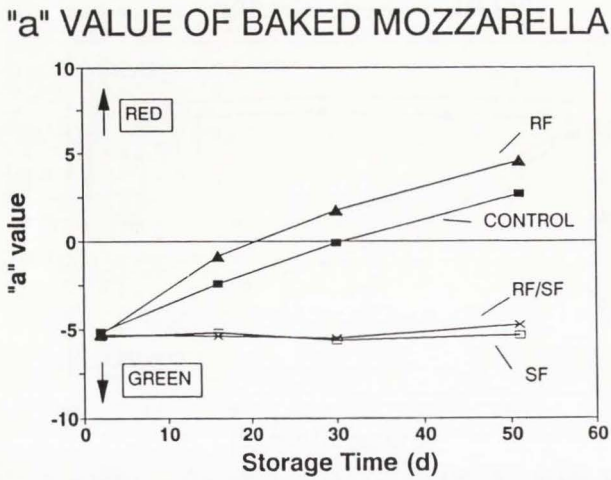

Figure 5. Changes in "a" value during 4°C storage of the four types of Mozzarella cheese (positive value is red, negative value is green): control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .029.

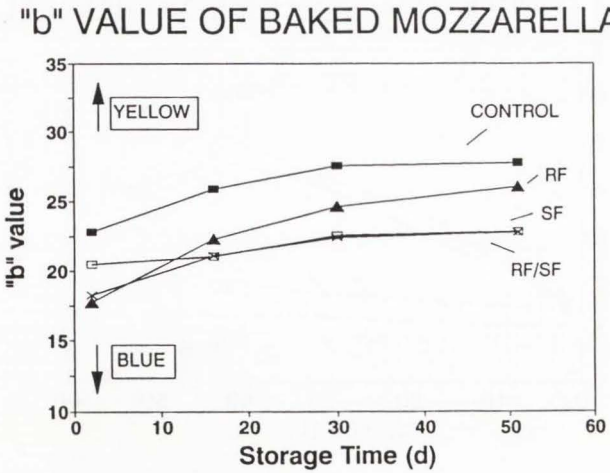

Figure 6. Changes in "b" value during 4°C storage of the four types of Mozzarella cheese (positive value is yellow, negative value is blue): control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .020.

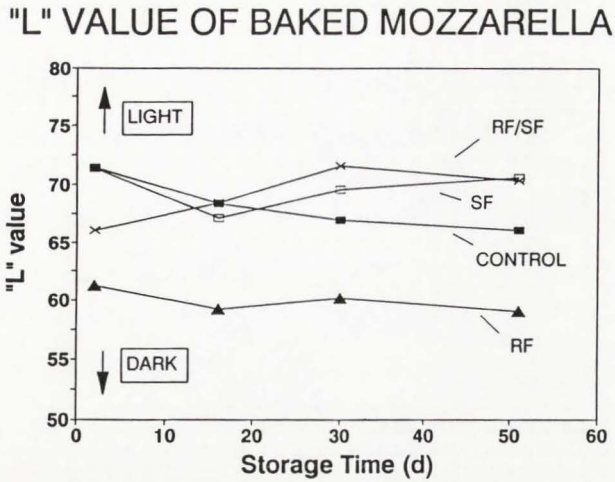

Figure 7. Changes in "L" value during 4°C storage of the four types of Mozzarella cheese (high value is lighter, low value is darker): control (■), starter-free (□), rennet-free (▲), and rennet-free, starter-free (x). SEM (of slopes) = .047.

1993-9

Specialty Cheese - Perspectives From The Marketplace

Part One
The Distributor and Importer

Jim Sebastiani
San Francisco International Cheese Imports

There are many important factors and considerations in choosing the proper distributor to handle your company's needs — not only what YOU need to consider, but also what your potential distributor will be looking for.

Consider your expenses:

- shipping costs
- time and travel expenses
- debts you will be incurring
- salary

It matters a lot when setting up your pricing structure

When choosing a distributor, get to know the potential distributor's "history" and territories (is the end-user satisfied with the distributor? Does the distributor cover your target markets?). Furthermore, consider using the distributor's private label: they're more apt to accommodate your needs if you use their label.

A very important point to remember is to spread your markets — don't cluster around one area. If your chosen distributor does not cover your entire market area, use more than one. Choose the best distributor for one particular area, another distributor for another area. Or, you may even consider a brokerage firm - but choose wisely. Sadly, there are a lot of "middle-of-the-road" brokers who won't go the distance for their clients. But there are many who will go much further.

Choose your target customers (retail, foodservice, specialty, etc.). You NEED to know who you will be targeting, so you can make the most of your advertising efforts and, more importantly, so you can make the most of the relationships you'll be developing with your clients. THEY are your "bread-and-butter" — meet their needs.

When you're calling on a potential distributor, plan your presentation thoroughly. You get only one chance to make a first impression and you want it to be a good one. Send samples before you call initially. Call while your samples are enroute to introduce yourself and your product, mentioning that samples will be arriving any day.

When you finally get a face-to-face meeting, have customer orders on-hand. They hate to turn away business! And be sure to follow-up regularly (quarterly or more often) with distributors. The more you get involved in THEIR business, the more they'll get involved in YOURS.

Marketing is vital to ANY company. Here are some general and easy suggestions:
• meet the end-user. Customers love to meet the manufacturer, and they can often offer some wonderful insights.
• offer generous introductory discounts to the end-user. An initial sampling will create interest, and introductory discounts may prompt them to try more.
• ADVERTISE!
• use colored paper for flyers/brochures. It's an eye-catcher.
• attend food shows. They're a great source for making contacts and networking. You may even share a booth with an end-user, to emphasize your involvement with your clients.
• send out LOTS of samples to LOTS of people. Nobody will know you exist if they can't see your product.
• get involved in distributors regular promotions (monthly, quarterly). Again, the more you're involved with THEM, the more they'll be involved with YOU.
• let your clients tour your facility. It increases excitement and enthusiasm for your product, while leaving a "lasting image" in their minds. Be conscious of that fact! Make sure your facility meets all safety and sanitation standards.
• hire someone to regularly call on your accounts. Even the smallest of companies can't do everything without some help. Your clients need to know they won't be forgotten as your own company grows. A field representative hired for specific areas or accounts will make your clients feel more secure using you as their source.
• offer discounts if the client orders when your field rep is visiting.

Part Two
The Retailer and Foodservice Operator

Allen Hendricks
AMH Resources, Inc.

Mr. Hendricks discussed why specialty cheeses are important in both market segments, and demonstrated how they help enhance image and profitability in both operations. He began his presentation with a historic explanation of why specialty cheeses are important to the consumer. Immigrants coming to the New World were eager to maintain their own traditions and food preferences, which meant bringing their cheese "recipes" with them.

The influx of immigrants to various regions in the United States created regional specialties which spread to other areas of the country. Today's "region-specific" menus continue to use specialty cheeses now produced domestically. These domestic reproductions easily enhance the image and profitability of dishes prepared with specialty cheeses.

First, for both the retailer and the foodservice operator, domestically-produced cheeses mean a longer shelf life yielding less shrink.

Second, an image is created. For retail this means a larger variety is available to the customer. Stores with more variety are apt to get more repeat customers. Better margins mean better profits.

For foodservice, specialty cheeses provide a special selection of products to the menu that the establishment can call their "own". Further, the number of menu items which can be prepared is directly proportional to the number of cheeses available. Again, better margins mean better profits.

Third, specialty cheeses create specialty dishes out of the ordinary. Imagine the old stand-by grilled cheese sandwich, brought to the heights of taste sensation by substituting Dill-flavored Havarti.

Lastly, allowing chefs to "play" with specialty cheeses generates some wonderful new dishes, leaving the chef feeling good about his/her "creation", and increasing the number of menu items available from the operator.

Part Three
Packaging — What Your Customers Are Looking For

Regi Hise
Wisconsin Milk Marketing Board

Mr. Hise delivered a presentation on the special considerations that should be given to the overall aspects of packaging and marketing specialty cheeses. Highlighted were examples of new specialty cheeses that have been successfully introduced.

What does "packaging" constitute? There are several factors to consider — not just "the box it comes in".

- shape and style
- cheese finish (wax, plastic coat, vacuum, etc.)
- the box
- information ON the box
- information IN the box
- re-pack labels

First of all, who is your target? Packaging needs may vary depending on your target.

- Brokers rely heavily on product sell sheets to present to potential customers.
- Distributors need sell sheets, product specifications, and information on the box.
- Retailers and Chefs/Operators make use of information ON the box and IN the box. (i.e.: how an item can best be used for increased sales)

For example, in retail it is important to consider what type of cases are used in the store for display. Not all size packages will fit in all size cases. If your focus is retail, plan your packaging with this in mind.

Furthermore, retailers need more than generic information to help sell your product to the consumer. Cooking or serving suggestions, background information about the product, sizes the product is available in, what kind of turn-around time can be expected, etc. It would be a good idea to visit your customers personally to find out this information.

Mr. Hise also introduced a new marketing tool developed by the Wisconsin Milk Marketing Board, the Wisconsin Cheesecyclopedia. This new self-directed course was developed to assist distributors, brokers, retailers, and foodservice operators in the art of buying and selling cheese. Information includes: origin, production unique to variety, standards of identity, taste, usage, serving suggestions, and menu applications. Cheese handling information includes: receiving, storage, sanitation, cutting and wrapping, staff training, signage, and merchandising. Product performance is detailed for both hot and cold applications.

The Study Course package includes 3 video cassettes containing 12 study units, a corresponding reference manual, and 6 course workbooks. Unit 1 explains "How to Use This Course" and How Cheese is Made, Graded, and Classified". Unit 2 - 12 offer specific information on individual cheese varieties categorized by degree of hardness. Upon successful completion of the Study Course and workbook, participants will receive a Certificate of Achievement.

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