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Effect of Proteolytic Activity of the Lactic Cultures on Mozzarella Cheese Quality

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EFFECT OF PROTEOLYTIC ACTIVITY OF THE LACTIC CULTURES ON MOZZARELLA CHEESE QUALITY

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Nutrition and Food Sciences

UTAH STATE UNIVERSITY
Logan, Utah

1989

ACKNOWLEDGEMENTS

I would like to express appreciation to my major professor, Dr. Gary H. Richardson, for his help and advice throughout this project. Thanks are also extended to Dr. Deloy G. Hendricks and Dr. Paul A. Savello for serving on my committee. I wish to thank Dr. Craig J. Oberg for his help and friendship. I am grateful to Mr. L. V. Moyes for technical assistance.

I would also like to thank the Western Dairy Foods Research Center for funding this project and making it possible.

I am indebted to my parents for their consistent support and encouragement throughout my education.

Most of all I express my gratitude to my wife, Hui-Ya, for her patience and continual assistance.

Wen-Hsu Wang

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ABSTRACT

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Utah State University, 1989

Major Professor: Dr. Gary H. Richardson
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The Mozzarella cheese market is growing rapidly. Major concerns with cheese meltability and color have arisen in the fast food industry. Prt^- starter culture was used in this study to improve the physical properties of Mozzarella cheese. Three tests (stretch test, melt test, and browning test) were modified to evaluate the quality of cheese.

A stretch test using the Brookfield helipath viscometer to stretch the cheese sample at 60°C was successful in distinguishing cheeses from different make procedures and from different proteolytic strains. A melt test using a glass tube to hold the cheese flow at 110°C for 60 min was used to determine meltability of cheese. A chroma meter was used to measure color change after the cheese sample was subjected to boiling water for 60 min. The b* value was used to indicate the color change.

Cheese made with Prt^- strains of Lactobacillus bulgaricus stretched less but showed longer melting flow than that from Prt^+ strains. Cheese made with Prt^- strains was lighter in color than cheese from Prt^+ strains. An inverse relationship existed between stretchability and meltability. When mixed cultures of L. bulgaricus and Streptococcus thermophilus were used, the symbiotic interaction in acid production of Prt^+ strains was more effective than mixed cultures of Prt^- strains. Stretchability of

INTRODUCTION

Mozzarella cheese is very popular throughout the world. In the United States, the per capita consumption increased 915 % from 0.40 pounds in 1960 to 4.06 pounds in 1984 (1) and is now second to Cheddar in popularity. By the year 2000, almost 900 million pounds will be produced annually in the U. S. (68). Industry quality control standards must be developed and maintained to assure retention and expansion of this market.

Proteinase negative (Prt^-) lactic starter cultures reduce proteolysis of casein, which helps improve cheese yield (37,39,67), create less bitterness (39,50,54,57,81), and increase resistance to bacteriophage (39,79,81) and inhibitory substances (33,79,81). Researchers also indicate that Prt^- cultures may reduce manufacturing times by allowing the use of higher constant cooking temperatures (88). Using Prt^- cultures to manufacture cottage cheese increases theoretical yields by 2.26% when compared with the Prt^+ parent strain (92). Yield increases up to 10% have also been reported by Ekart et al. (24). Heap and Richardson found a 5.6% yield increase in acid casein production using Prt^- cultures (37).

Khayat and Richardson (43), using the trinitrobenzene sulfonic acid (TNBS) proteolysis method to evaluate Prt^+ and Prt^- cultures, confirmed very high proteolysis in Lactobacillus bulgaricus, a primary culture of Mozzarella cheese manufacture. This observation suggests potential yield losses from using Prt^+ cultures of L. bulgaricus. They also reported that Streptococcus thermophilus has a very low proteinase activity, some strains having less than Prt^+ strains of S. cremoris. Thus, using a suitable Prt^- starter culture to replace L. bulgaricus or using only a Prt^- S. thermophilus may be encouraged and profitable. Moreover, there may exist the possibility of improving the physical properties of Mozzarella cheese that are associated with cheese maturation.

The objectives of this study are 1) to use suitable Prt^- mesophilic and/or thermophilic starter cultures that can function well under the high temperatures used in Mozzarella manufacture and 2) to evaluate the physical properties of the resultant curd.

LITERATURE REVIEW

Cultures

In 1931, Harriman and Hammer (35) showed that certain pure cultures of Streptococcus lactis that rapidly coagulate milk are composed of rapidly and slowly coagulating strains. Strains are considered fast if they coagulate reconstituted nonfat dry milk (NDM) within 16 h at 21°C from a 1% freshly coagulated inoculum and slow if they require more than 16 h to coagulate NDM (40,52,84). The fast cultures (proteinase-positive) (Prt^+) are definitely proteolytic, whereas the slow variants (proteinase-negative) (Prt^-) bring about little, if any, protein decomposition (35). Such Prt^- variants are generated at a relatively high frequency of divisions (1 to 2%) and do not revert to the parent Prt^+ strain (14,30,35,73,96).

Studies have shown that the membrane proteinase of the slow mutant and the parent have similar properties, while the intracellular proteinase of the spontaneously generated slow mutant differs from the parent intracellular proteinase in every property examined (101,103,104). This intracellular proteinase is considered responsible for the mutant's 'slowness.' However, the high occurrence of spontaneous loss and its irreversible nature suggest that it could be due to loss of plasmid-carried genes for some or all of the cell-wall-bound proteinase activity (56,61,73).

The proteinase system is needed by lactic cultures to obtain certain nitrogenous constituents from milk proteins thereby allowing the culture to grow properly in milk. The limited growth and acid production of Prt^- can be improved to the Prt^+ level by the addition of various protein fractions (more readily available form of nitrogen) such as liver fractions, yeast extract, pancreas extract, and peptone (2,8,30,45,55,74,102). Also, nucleic acid derivatives are found to be stimulatory for Prt^- variants (46).

Prt^- strains have several potential advantages in cheesemaking: 1) less casein is proteolyzed (37,67), 2) the bitter-flavor defect is reduced (39,50,54,57,81), 3)

bacteriophage attack and antibiotic inhibition are minimized (33,39,79,81), and 4) acid-production is enhanced at elevated temperatures (88).

pH-Control Systems

Bulk starters progressively lose activity when stored at low pH, which results in irregular acid production during cheesemaking (36,72). A pH-controlled whey-base bulk starter system was developed by Richardson et al. (82). The medium pH is maintained near 6.0 during starter ripening by injections of anhydrous or aqueous ammonia. An ammonia injection recorder tracing provides cheesemakers with a continuous history of culture activity (82). The numbers of bacteria produced in the system are about ten times more than are produced in conventional culture systems (80). This system results in elevated and consistent acid-producing activity of starter cultures without overripening, less frequent bulk starter preparation, and reduction of phosphates in the medium without compromising phage-inhibitory benefits (5,39,82). Reduced inoculum levels and media-cost savings are also reported (31,39,82,106).

Another system used is an internal pH-controlled medium that uses built-in buffers to neutralize acid production by the growing cultures and ensures maintenance of the pH above 5.2 to prevent or minimize acid injury to the starter cells (39,58,105). The medium utilizes a combination of ammonium phosphates and trimagnesium phosphate that solubilizes slowly to neutralize acid produced by the starter (85,86). This system offers some benefits including built-in pH control, phage neutralization, improved holding characteristics, and comparable starter activity (58,105). Sufficient Prt^- cell masses can be successfully propagated under continuous pH control if adequate stimulants are provided (62,78,81).

Mozzarella Cheese Manufacture

Mozzarella and pizza cheese belong to the pasta filata family. They differ in that pizza cheese contains less moisture than Mozzarella cheese does. Traditionally,

Mozzarella cheese is made from the high-fat milk of the water buffalo. However, the cheese has been successfully made from cow's milk, and greater mechanization has been applied in this process in recent years. The renneted acid curd, which ripens to pH 5.2, is heated in hot water (80°C), stretched, and molded. The conversion of dicalcium paracasein to monocalcium paracasein by lactic acid during ripening gives a smooth stretching quality to the cheese (48). The fat/protein ratio and the pH of the unheated curd must be carefully controlled to minimize loss of fat and protein during stretching and to prevent a tough, grainy texture (48). Generally, curd above pH 5.4 can't stretch and below pH 5.1 has difficulty retaining fat (47,48).

Acid in the cheese can result from heat-resistant lactic starters including Streptococcus thermophilus and Lactobacillus bulgaricus, or from direct introduction of lactic, acetic, and hydrochloric acids (9). It is reported that Mozzarella can be successfully manufactured from direct acidification without major defects (9).

Physical Properties Measurements

The textural characteristics of milk curd and cheese were studied extensively in Switzerland and England during the 1940s and summarized by Baron et al. (18,51). Since then, automated testing systems, a texture profile analysis (TPA) technique, and a variety of instruments and methods for determining textural parameters have been developed and applied to a large number of cheese samples and cheese varieties (51). However, few studies have been reported on the relationships among manufacturing parameters, compositional data, and rheological properties of cheese (18).

Instrumental or objective measurements can be classified as fundamental, empirical, and imitative (93,94). Fundamental tests measure fundamental rheological properties and provide a scientific basis for the development of more meaningful empirical tests (93). Empirical tests measure parameters that practical experience indicate to be

related to textural quality (93). Imitative tests imitate the conditions to which the material is subjected in practice (94).

Finished cheese has the most complex structure of any major dairy product. The heterogeneous nature of cheese structure and the ill-defined force distributions make it arduous to accomplish fundamental measurements (70,76). However, empirical methods are simple and useful to the cheese manufacturer if results are interpreted carefully (94).

Since Mozzarella cheese is usually consumed in the melted state, its melting characteristics are major factors in evaluating quality and acceptability. The melted state behaves in a viscoelastic nature, which is extremely temperature dependent and related to cheese composition and microstructure (44,70). Methods commonly used to measure cheese meltability, as described by Schreiber [Kosikowski(48), Park et al.(70)] and Arnott et al. (4,70), are based on heating a disk of cheese of specified dimensions in a petri dish at a specified temperature and time followed by measuring the decrease in disk height or increase in disk area. Instead of using a petri dish, Olson and Price (69) employed a glass tube to hold the cheese flow during the melt test. They claimed that the method eliminates film formation and permits more accurate measurement. Lee et al. (51) determined meltability of various cheeses by recording the temperature at which flowability became measurable by a Brookfield viscometer. Smith et al. (90) used a capillary rheometer to determine the flow curves of melted cheese.

Recently, the application of a Brookfield viscometer for measuring melted Mozzarella cheese consistency was successfully reintroduced by Kindstedt and Rippe (44,83). They used a helipath viscometer attached to a strip chart recorder to obtain a continuous permanent record of the data. A T-bar spindle connected to the viscometer is lifted through the tempered cheese column and cuts a helical path as it rotates. When the spindle rises above the cheese surface, a strand of melted cheese may be formed between the spindle and the cheese surface depending on individual melting properties. The strand is stretched by the rising spindle. Resistance of the melted cheese and resistance of the

strand to spindle rotation can be related to viscosity (liquid-like properties) and stretchability or elasticity, respectively, which show in the viscometer profile that provides qualitative information regarding melted cheese viscoelastic properties.

Browning Reactions

There are four types of browning reactions in foods: Maillard, caramelization, ascorbic acid oxidation, and phenolase browning (28). Maillard reaction is a kind of nonenzymic browning that involves the interaction of proteins or amines with carbohydrates (28). It takes place during heating or prolonged storage.

Lactose and casein are two principal reactants in the browning of milk systems (34,71,95). Serum proteins appear to have little effect on the formation of brown color (34). It has been reported that high processing temperatures and high sugar in Cheddar cheese cause browning in process cheese (95). Accumulation of lactose or galactose accompanied by proteolysis creates the potential for the Maillard reaction (6). A positive correlation between galactose content and brown color intensity has been found in heated process and Mozzarella cheese (6,41). Also, galactose is more reactive in the browning reaction than glucose and much more than lactose (75). Browning of cheese due to high galactose content can be lessened by using starter cultures that ferment galactose (41).

A quick and accurate method (7) to predict the tendency of cheese to undergo browning after processing has been developed. The method employs a Hunterlab colorimeter to analyze cheese samples for three color indices, L*, a*, and b*, as compared to a control sample. L* is a brightness variable; a* and b* are chromaticity coordinates in which a* denotes green to red and b* signifies blue to yellow in the color space (13). Color deviation (dE), which represents total color difference is calculated as the square root of the sum of the squares of the three components representing the difference between L*, a*, and b* readings of the sample and the standard, as shown by the equation $dE = [(dL^*)^2 + (da^*)^2 + (db^*)^2]^{1/2}$, and serves to evaluate the tendency of browning (13).

MATERIALS AND METHODS

Culture Maintenance

Strains of Streptococcus cremoris, S. thermophilus, and Lactobacillus bulgaricus were selected for this study based on their proteolytic activity (63). These strains were checked for their ability to grow at 40°C to determine their suitability for manufacture of Mozzarella cheese. The Prt^+ strains were propagated in sterile10% NDM. The Prt^- variant strains were propagated in sterile10% NDM with 0.1% yeast extract added. Sufficient seed cultures were frozen and stored at -40°C to prevent proteinase activity changes during the study. Working cultures were subcultured weekly by inoculating 1% of a freshly coagulated culture into the appropriate sterile substrate. Cultures were stored at 4°C and incubated at 40°C until freshly coagulated prior to use or transfer.

Media

Low heat NDM (Danish Creamery Assn., Fresno, CA 93775) was reconstituted at 10% (10 g NDM in 90 ml de-ionized water), sterilized (121°C for 15 min), and stored at 4°C. Stimulated NDM was reconstituted as above except that it was fortified with 0.1% yeast extract (Becton Dickinson and Co., Cockeysville, MD 21030).

Culture Activity

Culture activity was determined by measuring the pH drop over 2, 4, and 6 h in inoculated NDM enriched with 0.1% yeast extract at 40°C. Inoculum levels of 2, 4, 6, 8, and 10% were evaluated. Change in pH was monitored with a Ross combination electrode (Orion Research, Inc., Cambridge, MA 02139) and a Beckman model 60 pH Meter (Beckman Instruments, Inc., Fullerton, CA 92634).

Bulk Starter Preparation

Bulk starter cultures of S. cremoris were prepared using the Utah State University Lactic Culture System (80). Whey-based medium consisting of 5% (w/v) whey powder (Gossner's Foods, Inc., Logan, UT 84321), 0.4% AYE-Light, and 0.1% N-Z-Amine Type E casein hydrolyzate (Humko-Sheffield, Oneonja, NY 13820) (106) was steamed at 90°C for 20 min, cooled to 27°C and inoculated with 1% of freshly coagulated culture. The medium pH was controlled at about 6.0 by addition of 30% ammonium hydroxide (Fisher Scientific Co., Fair Lawn, NJ 07410) (91) to the medium.

Bulk starter cultures of S. thermophilus and L. bulgaricus were prepared in 10% CR starter media (Miles Lab., Inc., Madison, WI 53701) plus 1% yeast extract, which was heated to 90°C and held at this temperature for 40 min. The media were then cooled rapidly to 40°C (incubation temperature) and inoculated with 1% of the culture. The final pH of a properly ripened starter was between 4.1-4.3 (15). This final pH was usually reached within 6-7 h at 40°C.

Simulated Mozzarella Cheese Manufacture

Small vats (6 l) of cheese were made following procedures previously developed (25,48,77). Raw milk from Utah State University Dairy Products Lab was standardized to a casein/fat ratio of 1:2 and pasteurized at 63°C for 30 min. The milk was cooled to 32°C, and a 2% inoculum of starter culture was added. Milk in the 20.5 cm x 20.5 cm x 25 cm vat was ripened at 32°C for about 1 h. 1.5 ml single-strength rennet (Chr. Hansen's Laboratory, Inc. Milwaukee, WI 53214) was diluted 1 : 20 with cold tap water and added. A smooth, thick curd formed in 20-30 min. The curd was cut and heated to 41°C in 30 min with periodic agitation. When pH decreased to 5.9-6.0, the whey was drained. The curd patties were handled like Cheddar until a pH 5.2-5.3 was reached, at which time they were milled, mixed, and molded in 80-82°C hot water until they became smooth and elastic. The molded curd was placed in a 800-ml beaker and cooled with ice

water to firm the curd. It was then placed in 25% salt brine for 8 h and kept at 4°C for testing. Cheese quality was determined by a stretch test, melt test, and browning test at 1, 7, 14, and 28 days following manufacture. Cheese made from direct acidification of milk served as the control (9). Six liters of the milk with the same treatment as above were kept at 4.4-7.2°C, acidified to pH 5.6 with lactic acid (J. T. Baker chemical Co., Phillipsburg, NJ), warmed to 37°C, and set with 1.5 ml rennet. The curd was cut, held at 37°C for 80 min, and heated to 49°C in 5 min. It was then drained, molded, and brine-salted as described above.

Stretch Test

A Brookfield LVT-Helipath viscometer system (Brookfield Engineering Lab., Inc. Stoughton, Mass. 02072) equipped with a T-bar spindle (TE with 1.075 cm length) was used to evaluate the stretchability of cheese (Figure 1). Fifteen grams of shredded cheese were weighed, tamped down into a test tube (25 mm x150 mm), and placed in a 60°C water bath for 10 min. The helipath stand was turned on to lower the viscometer. The spindle gradually penetrated into the tempered cheese sample and reached the bottom of the test tube. At that time, the helipath stand was turned off, and the viscometer was activated at a speed of 1.5 rpm until a full-scale reading was reached or a dial reading was stabilized. Then, the helipath stand was turned on to raise the viscometer. The wire spindle cut a helical path up through the cheese sample with a fresh cut surface at each rotation. When the spindle rose above the cheese surface, a strand of melted cheese would be formed between the rising spindle and the cheese surface, depending on the melting properties of the individual cheese sample. Figure 2 shows a strand of melted Mozzarella cheese stretched by the rising spindle. Dial readings were recorded for each cutting cycle. Sixteen visual readings were obtained in the 10 min required for the helipath system to traverse from the lower to upper limits.

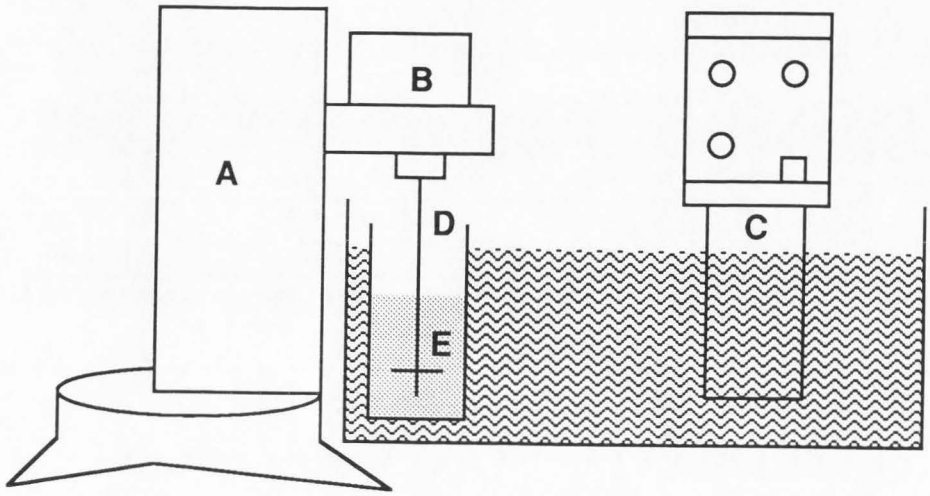

Figure 1. Schematic diagram of the helical viscometer for stretch test.
A. Helipath stand.
B. LVT viscometer.
C. Temperature controller.
D. TE spindle.
E. Cheese sample.

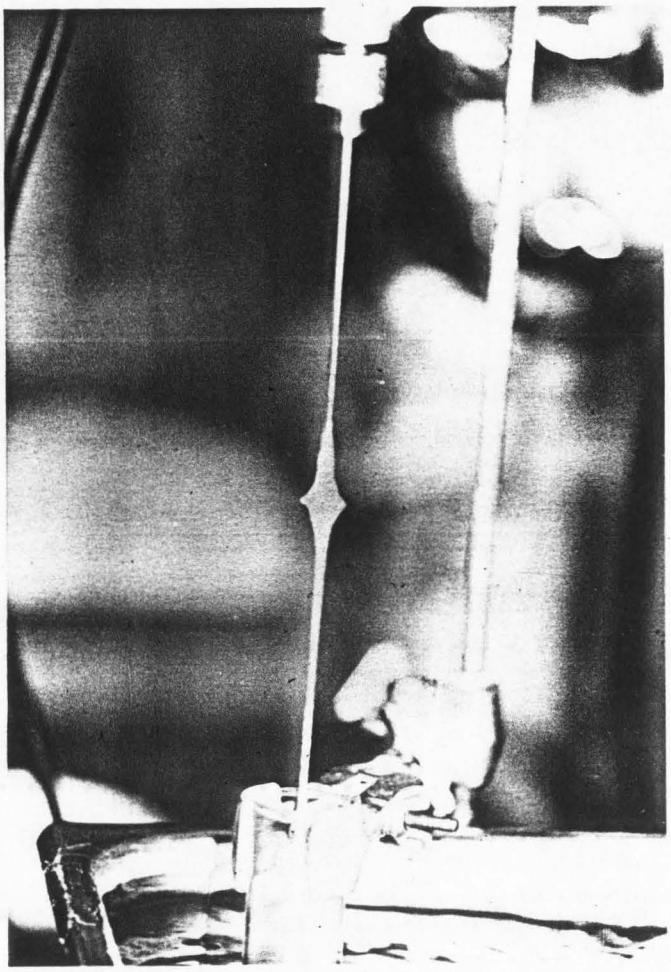

Figure 2. A strand of melted Mozzarella cheese stretched by the rising spindle.

Browning Test

Test tubes containing grated cheese were put into boiling water for 60 min and analyzed for color changes using a chroma meter CR-100 (Minolta Corporation, Meter Division, Ramsey, NJ 07446). The b* index (blue to yellow)(13) was used to indicate the color change of each cheese sample. The chroma meter was calibrated using the standard white reflector plate included with the meter. The selector switch was set to Illuminant C, and L*a*b* was selected as chromaticity-measuring mode. The bottom of the test tube was held in contact with the measuring head of the meter. Eight readings were made at the bottom of the tube, rotating the sample 45 degrees between readings. The larger the b* number, the darker the cheese sample.

Melt Test

The method described by Olson and Price (69) was used to measure meltability. Fifteen grams of diced cheese (5 mm x 5 mm x 5 mm) was placed in one end of a Pyrex glass tube (30 mm x 250 mm). The cheese end of the tube was closed with a number 7 solid rubber stopper, and the opposite end was closed with a rubber stopper of the same size but with a hole. The tube was tempered at 4°C for 30 min in a vertical position with the cheese end at the bottom. The tube was then placed in a horizontal position on the tilt-control rack in an oven at 110°C for 60 min. After the tube was cooled to room temperature, the distance of cheese flow was measured in centimeters.

Statistical Analysis

A factorial design was used to analyze differences in the physical properties of cheese made from different proteolytic strains and to control the extraneous variability due to different batches of milk being used. The equation for an additive model for this design could is:

$Y_{ijk} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk}$

where μ: overall mean of all experiments

a_i: effect due to different proteolytic strains

b_j: effect due to different batches of milk

c_k: effect due to age of cheese

ab_{ij}: interaction due to strains and batches of milk

ac_{ik}: interaction due to strains and age of cheese

bc_{jk}: interaction due to batches of milk and age of cheese

abc_{ijk}: interaction among three factors

Fisher's Least Significant Difference was used to determine which pairs of strains were different when the F test of analysis of variance was significant.

RESULTS AND DISCUSSION

Culture Activity

Two Prt$^-$ variants of Streptococcus cremoris, UC310 and UC85, demonstrated inability to decrease the pH adequately for Mozzarella cheese manufacture. The pH only dropped to 6.0-6.1, whereas the normal requirement is 5.2-5.3 in 5 h.

Some Prt$^-$ strains of Streptococcus thermophilus, WT16A, WT16-6, WT13B, and WT13L, were able to make Mozzarella cheese satisfactorily but did not show significant differences in cheese qualities from their Prt$^+$ parents, WT16 and WT13 (Appendix Table 28,29).

When Prt$^-$ strains of Lactobacillus bulgaricus were grown in CR starter medium plus 1% yeast extract, only 2% inoculum was needed for normal Mozzarella cheese-making. Prt$^-$ strains WB100, WB102, WB131, WB132, and WB133 and Prt$^+$ strains WB111, WB117, WB118 and WB104 were tested. During Mozzarella cheese manufacture, most of the Prt$^-$ strains reduced the pH much faster during 4 h incubation than Prt$^+$ strains, although they were not as active as Prt$^+$ strains at the beginning (Figure 3).

Figure 3 shows that Prt$^-$ strains WB100 and WB102 reached the required pH of Mozzarella cheese after 6 h incubation, while Prt$^+$ strains WB111 and WB104 required 7 h to attain the desired pH. Prt$^-$ strains of L. bulgaricus functioned better at the higher temperature than Prt$^+$ strains, because the temperature of the cheesemaking procedure was 32°C during the first 3 h and raised to 41°C thereafter. When L. bulgaricus and S. thermophilus were combined, the cheesemaking time was shortened to 4-5 h because of their symbiotic interaction (Figure 4). The mixed cultures of Prt$^+$ strains (WT16-WB111, WT16-WB104) showed better activity during the whole manufacturing procedure than those of Prt$^-$ strains (WT16A-WB100, WT16A-WB102). The symbiotic interaction of mixed Prt$^+$ cultures seemed to be superior to that of mixed Prt$^-$ cultures.

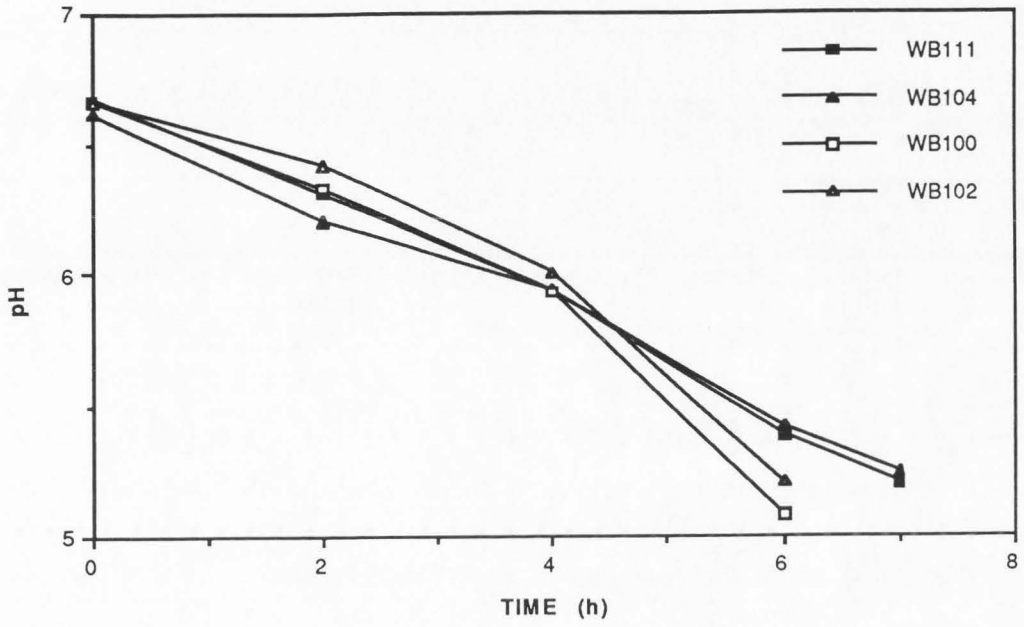

Figure 3. The pH changes of Mozzarella cheese manufactured employing different proteolytic strains of Lactobacillus bulgaricus.

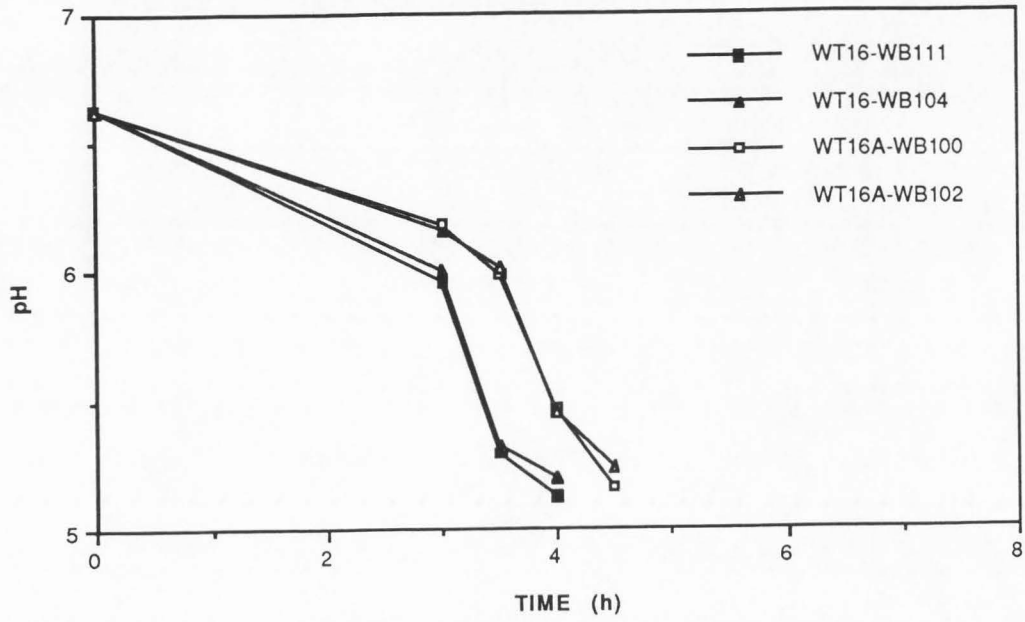

Figure 4. The pH changes of Mozzarella cheese manufactured employing different mixed cultures of Streptococcus thermophilus and L. bulgaricus.

Stretch Test

A preliminary study examined different types of cheeses and determined whether different stretching properties could be detected. Mild Cheddar, old Cheddar (aged 12 months), Swiss cheese, process cheese, cheese food, cheese spread, and Mozzarella cheese were tested. Viscometer profiles were obtained by plotting LVT dial readings against time.

Figure 5 shows a standard profile of a stretchable cheese. The off-scale region of the profile (A) indicates high viscosity of the cheese sample through which the spindle rotated. On exiting the cheese surface (B), the rising spindle stretched the cheese strand and the resistance force on the spindle allowed a distinctive tail region (C) to be formed. Fresh Mozzarella cheese caused the entire profile to remain off-scale because of the rubbery texture (Figure 6).

Figures 7-12 exhibit profiles of a variety of cheese products. If no tail region were found these cheese products were unstretchable or had weaker stretchable strands than Mozzarella cheese. It is believed that interactions of curd protein molecules are the basis for the properties of stretchability and elasticity that characterize Mozzarella cheese (44). The profiles of mild Cheddar, old Cheddar, and Swiss cheese reveal that these curd interactions are not as strong as those of Mozzarella, and intense proteolysis might be responsible for the weak viscosity of old Cheddar. For process cheese products, curd protein interactions are largely lost because of the addition of calcium-binding emulsifying salts during manufacture (44,89). The low viscosity of cheese food and cheese spread might be due to their high moisture, high acidity, and ground-up properties.

The superiority of the LVT viscometer over the MVT instrument reported by Kindstedt et al. (44,83) was evident in its ability to measure weak cheese strands as shown in Figures 7, 8, and 9. Those would probably not be measurable on a less

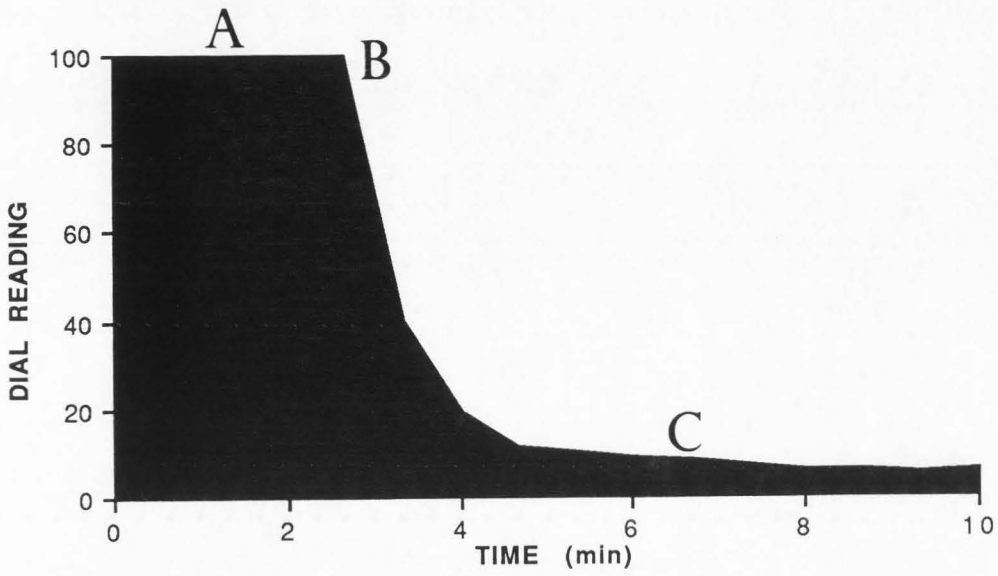

Figure 5. Typical viscometer profile of a stretchable Mozzarella cheese.
A. Spindle rotated through cheese sample.
B. Spindle exited cheese sample.
C. Cheese strand was stretched by the spindle.

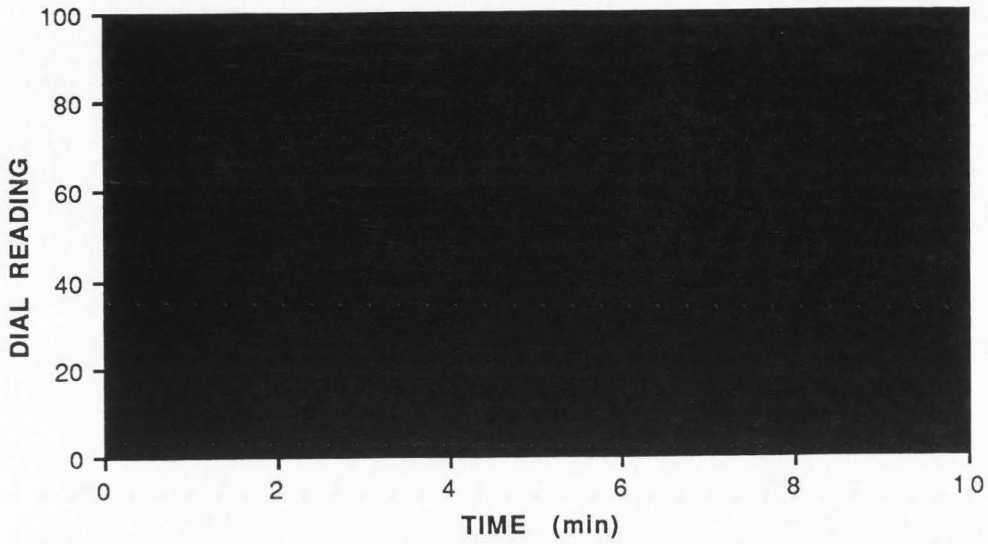

Figure 6. Viscometer profile of a fresh Mozzarella cheese (1 day old).

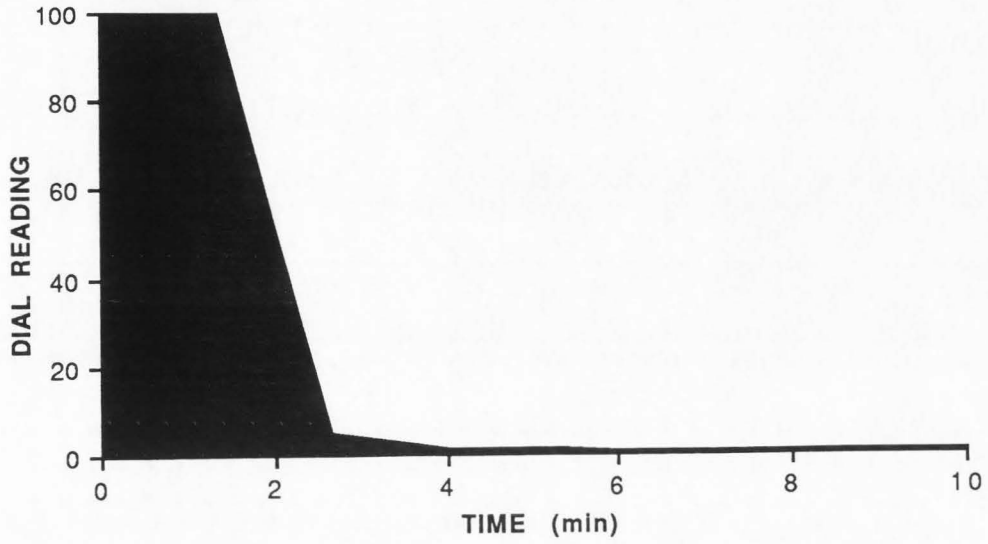

Figure 7. Viscometer profile of a mild Cheddar cheese.

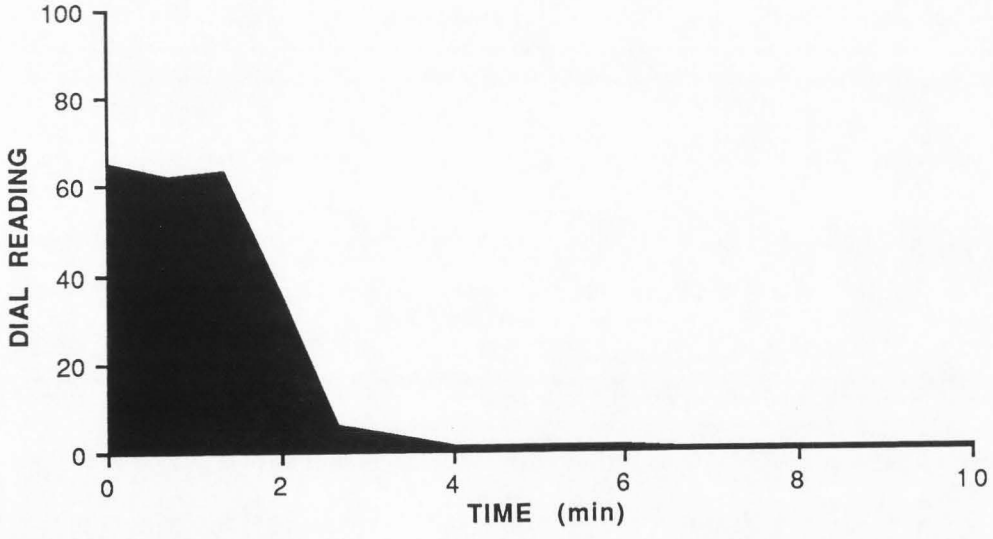

Figure 8. Viscometer profile of a 12-month-aged Cheddar cheese.

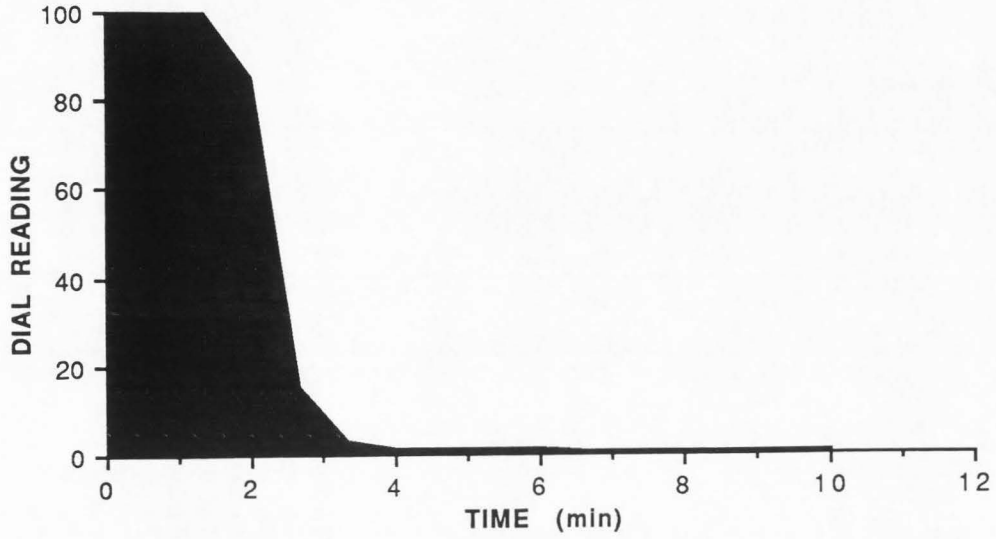

Figure 9. Viscometer profile of a Swiss cheese.

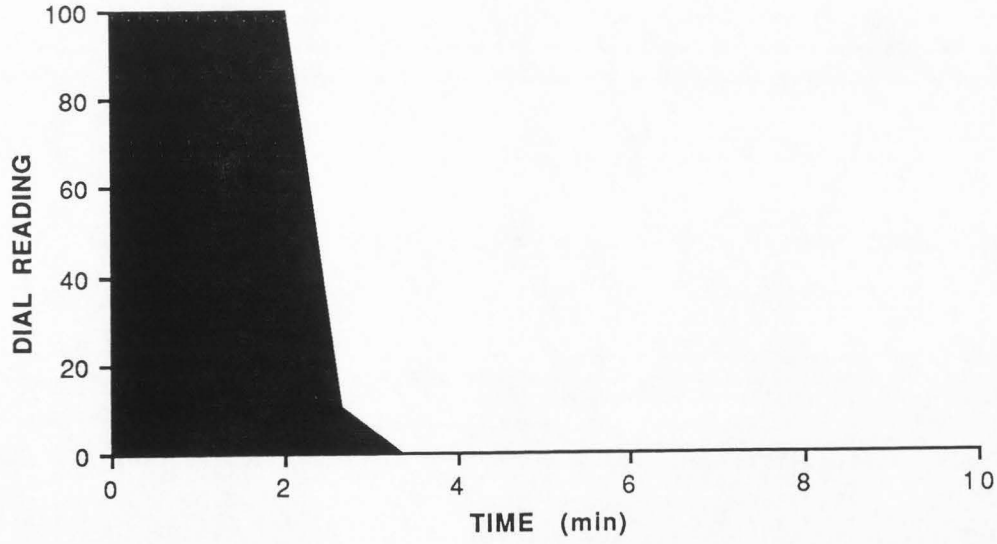

Figure 10. Viscometer profile of a process cheese.

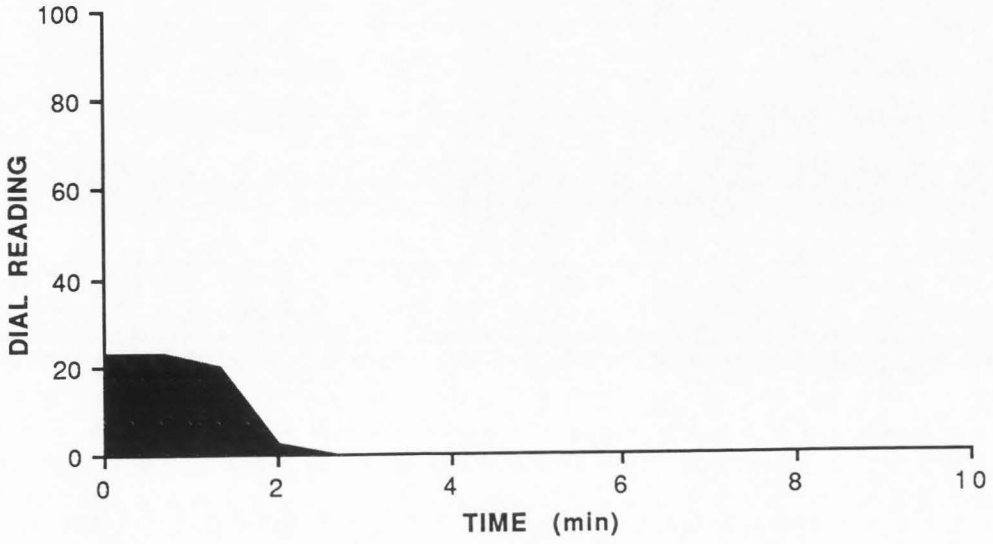

Figure 11. Viscometer profile of a cheese food.

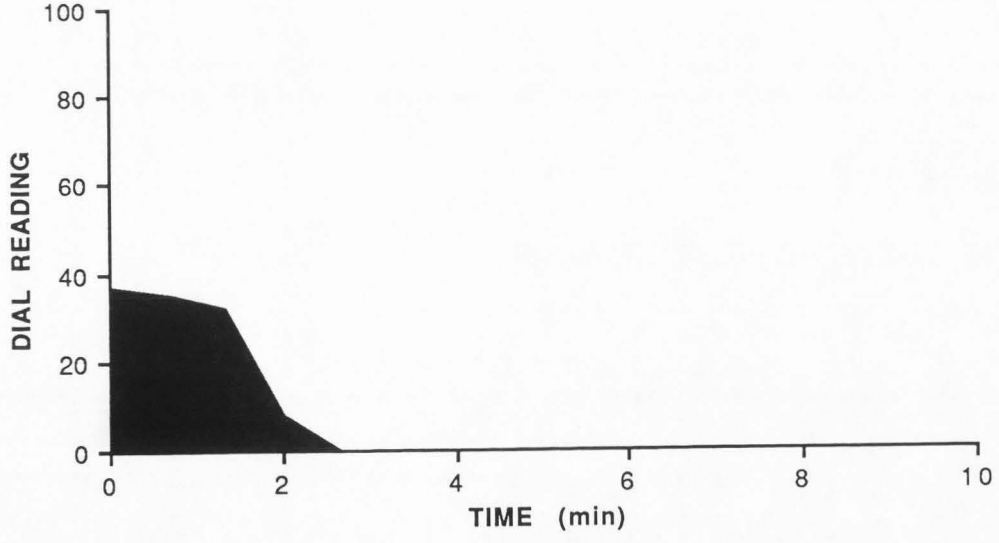

Figure 12. Viscometer profile of a cheese spread.

sensitive instrument model.

It was easy to identify stretchable from unstretchable cheese by the visible tail region of the viscometer profile. There was a problem with converting the qualitative description to a quantity value that could be statistically analyzed to discern small differences between stretchable cheeses manufactured with different proteolytic strains and enzymes. Since the tail region is of most interest in this stretch test, instrument readings were selected from the time the spindle left the surface of the cheese mass and totaled to obtain the stretchability data.

The time (t_{100}) when the dial reading first shifted on-scale was used to begin the instrument readings. On a typical viscometer profile (Figure 13), t_{100} was the point between off-scale and on-scale regions and served to separate these two regions. As an indicator of stretchability, it was essential for t_{100} not to incorporate any other factors besides stretching. There were two points at which the dial reading could move into on-scale region: first, when the spindle still rotated in the cheese sample and viscosity of the melted cheese was not high enough to keep the dial reading in the off-scale region; and second, once the spindle left the cheese surface, the dial reading would drop into the on-scale region unless that cheese sample was very young. Therefore, while t_{100} was used as a criterion to compare stretchability, it could combine factors attributed by viscosity and therefore would not be a pure indicator for stretchability comparison. Furthermore, research shows that t_{100} is not always in proportion to stretchability. Figures 14-15 reveal that the t_{100}'s of these two Mozzarella cheese samples were almost the same, implying that the samples had the same stretchability, but actually the tail regions were significantly different. Accordingly, use of t_{100} to compare stretchability is not suitable.

The area of the tail region of the viscometer profile proved to be a better indicator of stretchability. The sum of the dial readings (S_{dial}) from 6 to 10 min was arbitrarily chosen to determine the approximate area of the tail region and quantitatively characterize

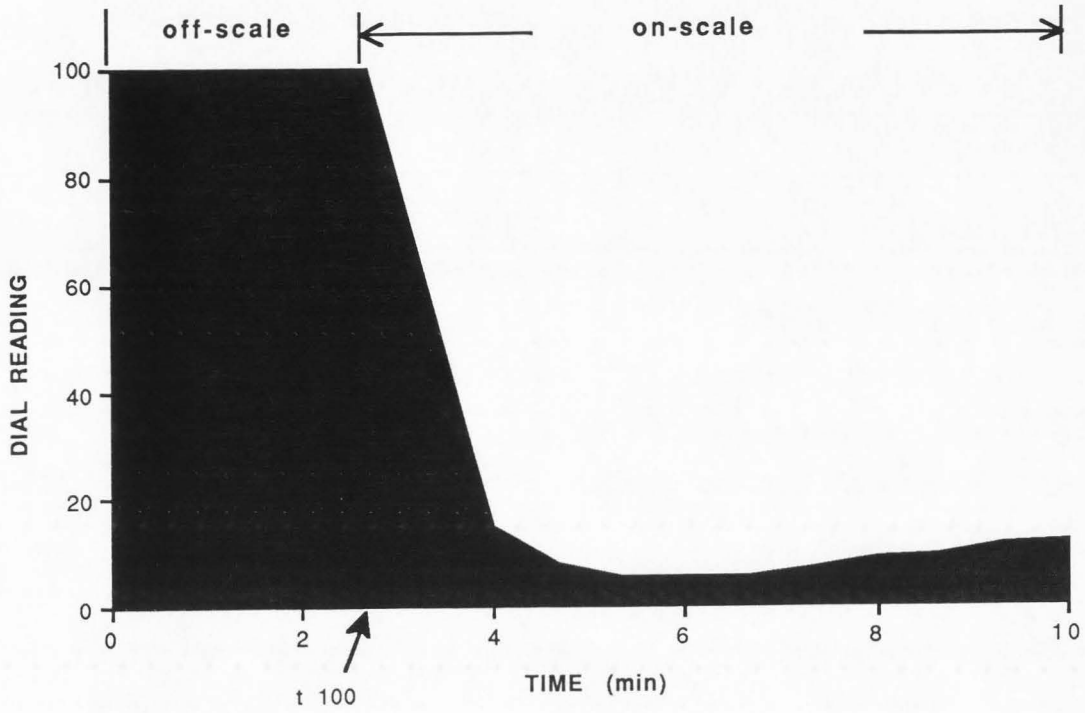

Figure 13. t_{100} shown as the point between off-scale and on-scale regions in a viscometer profile of a stretchable cheese.

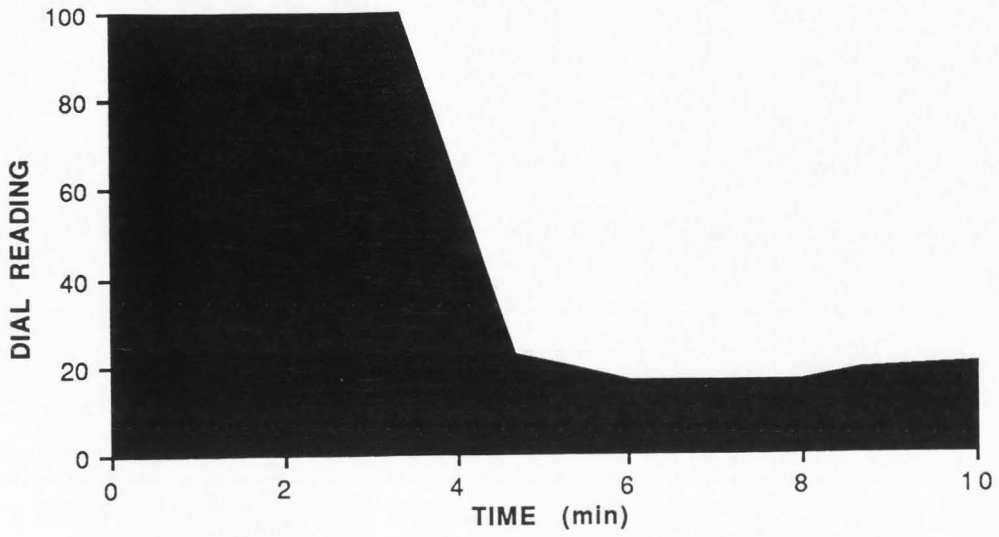

Figure 14. Viscometer profile of a 14-day-aged Mozzarella cheese.

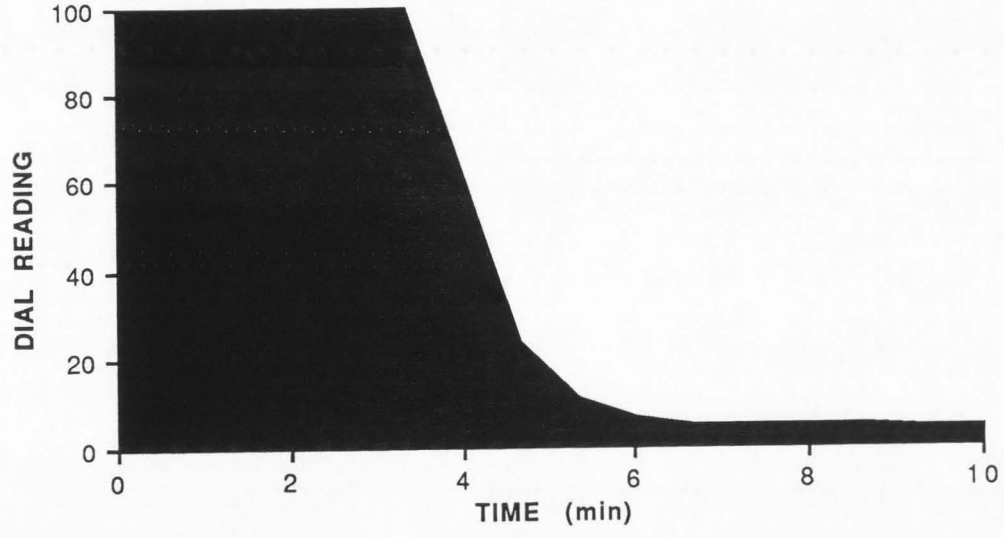

Figure 15. Viscometer profile of a 28-day-aged Mozzarella cheese.

stretchability of the cheese. Since S_{dial} covered most of the tail region and was exclusively freed from the rheology associated with below the sample surface readings, it proved a better index for stretchability than t_{100}.

Table 1 includes stretchability data comparing the S_{dial} of a Mozzarella cheese tested at 1, 7, 14, and 28 days following manufacture. Decline of stretchability was quantifiably identified by using S_{dial}. At day 1, although the spindle exited the cheese surface, the very green Mozzarella cheese still gave off-scale readings. That's why the value of S_{dial} was 700, since there were seven readings taken in 6-10 min. As aging increased, the tail region of the profile became narrow and S_{dial} correspondingly decreased. Since Mozzarella cheese has a filamentous structure (11) and does not melt uniformly at 60°C, the accumulation of cheese curd masses around the rotating spindle is affected by the size of cheese sample as well as by the cheese curd itself. Therefore, the cheese sample must be prepared in a very uniform manner to minimize the error factors of its structural inhomogeneity and the method variability.

Mozzarella cheeses made with Lactobacillus bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and the direct acidification procedure were tested for their stretchability at 1, 7, 14, and 28 days following manufacture. Figure 16 shows that stretchability decreased with aging. Enzymatic proteolysis did not seem to be serious during the first week, but it influenced stretchability significantly thereafter. Stretchability of the cheese with direct acidification diminished rapidly during the first week and gradually in the following weeks. This loss must be attributable to enzyme coagulant activity. Cheese made with Prt^- strains lost stretchability rapidly during the second week, while the cheese from Prt^+ strains still resulted in a high S_{dial}. The cheese made with Prt^+ strains stretched better than Prt^- strains and direct acidification. The stretchability between Prt^+ and Prt^- strains was statistically different at $\partial = 0.05$ when cheese was aged over 14 days (Appendix Table 19).

Table 1. Stretchability of Mozzarella cheese tested at 1, 7, 14, and 28 days after manufacture shown as sum of dial reading (Sdial).

age (days)	reps	Sdial (mean)	S.D.	C.V.(%)
1	4	700		
7	4	600	123	20.5
14	4	431	119	27.6
28	4	82	23	28.0

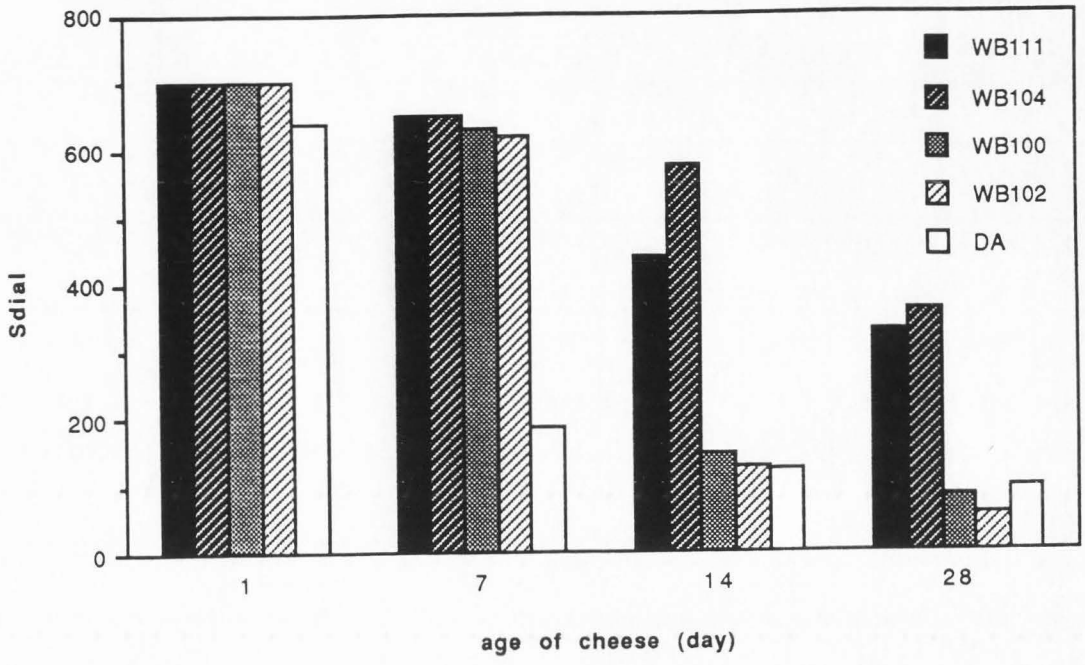

Figure 16. Comparison of stretchability of Mozzarella cheeses made with L. bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and direct acidification.

Stretchability has been defined as the ability of curd protein molecules to interact in a unidirectional fibrous manner (44). Creamer (17) suggested that the stretching characteristics of Mozzarella cheese might be correlated to the relatively higher concentration of intact casein and large peptides present in the cheese. The degradation of cheese protein by enzymatic proteolysis during aging is believed to be the main factor responsible for the loss of stretchability. Since Prt^+ strains are more proteolytic than Prt^- strains, they would result in protein degredation more extensively and theoretically make the cheese lose stretchability more rapidly than the Prt^- strains. However, the opposite results occurred, suggesting that proteolysis from enzyme coagulant would play a greater role in this phenomenon. To minimize the proteolytic action from enzyme coagulant, porcine pepsin is a better choice than rennet, because it is largely inactivated during early stages of cheese manufacture (26).

Melt Test

The Schreiber test (48) for melting quality of process cheese was first evaluated. The method requires that a disk of cheese with specific dimensions be heated at 232°C for 5 min. The increase of disk area is then measured. There were some difficulties encountered with this method in the present study. First, cheese tended to bubble under this high heating temperature. Second, the temperature varied greatly during opening and closing the oven door. The temperature range varied as much as 190-220°C. Third, the temperature never did reach 232°C within 5 min. If the heating time was increased, the bubble problem was more serious, and a surface film formed.

Therefore, a method using lower temperature for longer time was developed based upon the method by Olson and Price (69). Fifteen grams of diced cheese (5 mm x 5 mm x 5 mm) was used instead of a cheese cylinder, because heat transfer appeared to be faster and more even, and the test condition of diced cheese more closely resembled the cheese product on pizza. The required temperature (110°C) was reached within 5 min, and the

temperature varied 105°C± 5°C as samples were placed in the oven. The temperature remained constant after 110°C was reached. Heating for 60 min was required to completely melt the cheese and to minimize variability due to transient temperature changes. The comparison made between the original method of Olson and Price and the modified one is shown in Figure 17. The correlation coefficient for the regression line is 0.88.

Figure 18 shows the meltability of Mozzarella cheese made with L. bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and direct acidification and tested at 1, 7, 14, and 28 days following manufacture. Figure 19 exhibits cheese flow of a batch of 14-day aged Mozzarella cheese. Different lengths of cheese flow due to the use of different proteolytic strains was observed.

Meltability of the cheese increased as aging increased. Cheese made with direct acidification melted best on the first day and stabilized during aging. There was no difference in meltability between cheeses made from Prt^+ strains and from Prt^- strains on the first day. After 7 days' aging, the cheese from Prt^- strains melted better than that from Prt^+ strains or from direct acidification. The difference is significant at $\partial = 0.05$ (Appendix Table 20).

During cheese ripening, there are three types of enzymes mainly involved in the proteolysis, milk proteinases, rennet or other enzyme coagulants, and enzymes from starter culture. Alkaline proteinase (plasmin), the major native proteinase in milk, is heat-resistant to pasteurization conditions and some UHT treatments (17,29,99) and might exist in the cheese after severe processing temperature. Creamer (16) showed that B-casein is broken down by alkaline proteinase during ripening of Cheddar and Gouda cheese, while Castberg and Morris (10) believe the influence on cheese ripening is minimal. The activity of alkaline proteinase in Mozzarella cheese might contribute little to proteolysis possibly because of the very small amounts of the enzyme and the low pH of the cheese.

Although most of the rennet activity is lost in the whey at draining, a small part remains in the curd and represents an important part in the proteolysis of cheese during

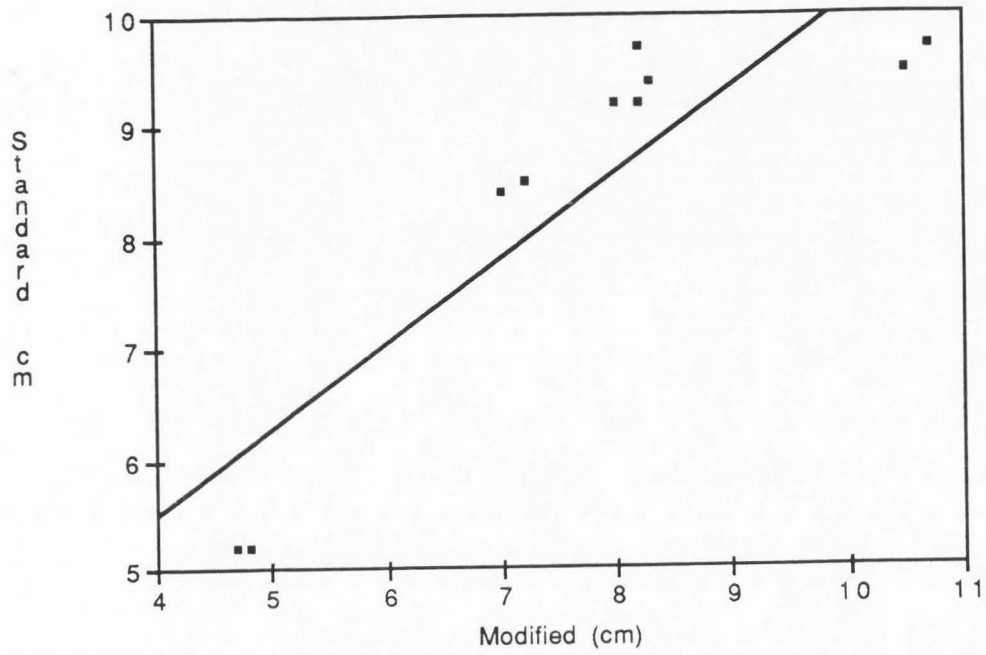

Figure 17. Comparison of modified melt test with method of Olson and Price (standard). The correlation coefficient for the regression line was 0.88.

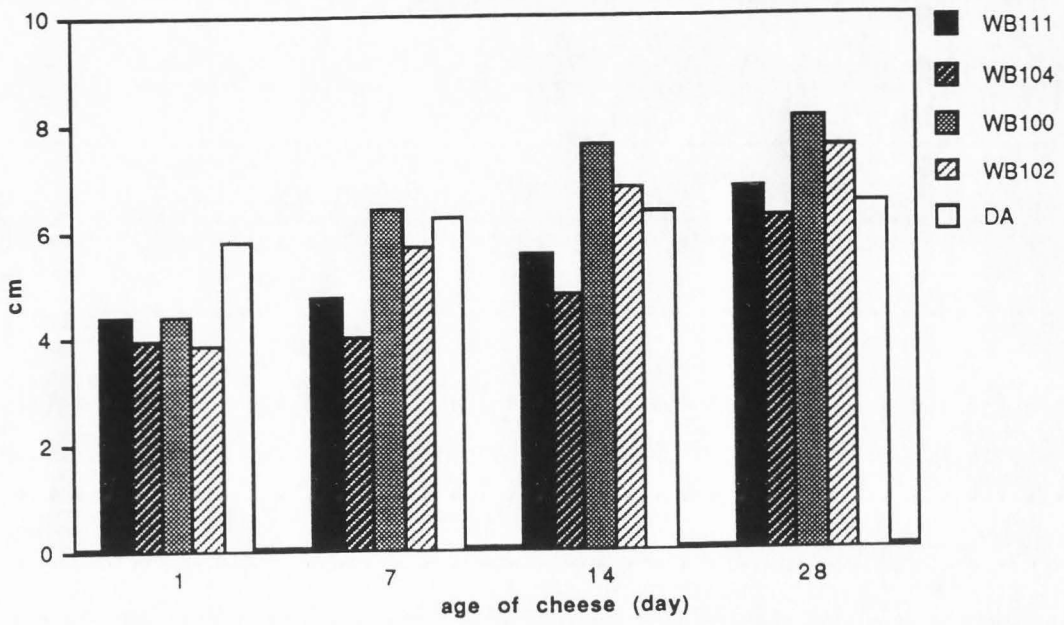

Figure 18. Comparison of meltability of Mozzarella cheeses made with L. bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and direct acidification.

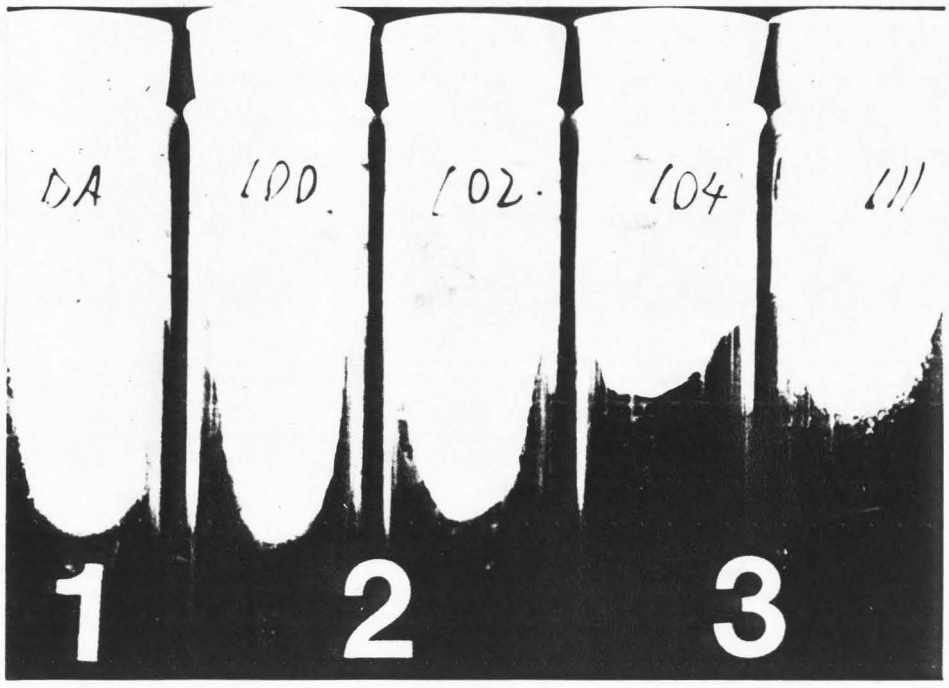

Figure19. Cheese flow of 14-day-aged Mozzarella cheese made with 1. direct acidification method, 2. L. bulgaricus Prt^- strains (WB100,WB102), and 3. Prt^+ strains (WB111,WB104) in the melt test.

ripening (23,26,29,38,99,107). Rennet has a strong and early action on a_{s1}-casein and causes a primary, limited degradation of the casein to form large peptides (19,23,32,60,65,66,100). It has been reported that 40% of the a_{s1}-casein of aseptic curds is broken down by rennet after 24 h (23). For Mozzarella cheese, the degradation rate of a_{s1}-casein would be decreased, since rennet activity is influenced by the high cooking temperature (32). This might be part of the reason direct acidified cheese loses stretchability sharply in a week. Since B-casein undergoes very little proteolysis by rennet (60,66,100), the loss of stretchability of direct acidified cheese seems to be the consequence of degradation of a_{s1}-casein by rennet. It is suggested that a_{s1}-casein might be a main factor in maintaining the stretching characteristics of the cheese. This result is supported by the popular model of cheese microstructure, that is, a_{s1}-casein interacts strongly with other casein molecules and is a link in the protein network (18,53). When the a_{s1}-casein is degraded, the protein network loses its strength. Creamer (17) also attributed the improved stretch of Mozzarella cheese to more intact a_{s1}-casein. de Jong (19,20) and Noomen (60) pointed out that the change in the consistency of 'Noordhollandse Meshanger' cheese is caused by the breakdown of a_{s1}-casein.

Since B-casein undergoes very little proteolysis by rennet (60,66,100), it is likely responsible for the more constant meltability of the direct acidified cheese during 28 days' ripening.

In the study of the acidified cheese, it is suggested that a_{s1}-casein is an influential factor to stretchability, while B-casein is related to meltability. This observation partly agrees with the inference of de Jong (22) that a_{s1}-casein is a structure former of the casein submicelle, while B-casein does not play an important structural role in the casein micelle. Additional evidence is needed to explore the exact mechanism.

There is still very little information about the proteolytic enzyme system of Lactobacillus bulgaricus, but it is believed that the majority of lactic acid bacteria possess both intra- and extracellular proteinase/peptidase activity (10,32). Clearly, the

proteinase(s) is located primarily in the cell wall, since the size and charge of casein molecules prevent their diffusion into the cell wall (97). Argyle et al. (3) showed that L. bulgaricus appears to have proteolytic activity associated with the cell wall. It was also reported that B-casein is hydrolyzed more easily than a_{s1}-casein by proteinase preparations (12) and intracellular proteinases (64) from L. bulgaricus. The cell-wall-bound (extracellular) proteinase/peptidase hydrolyzes large casein aggregates to smaller peptides, which in turn penetrate the cell wall and serve as substrates for the intracellular proteinase/peptidase system.

Slow acid-producing characteristics of Prt^- strains is due to the loss of all or most of their surface-bound proteinase activity (56,61,73). Thus, Prt^- strains are expected to degrade casein modestly and to improve the physical properties of Mozzarella cheese. However, the results from this study of the stretch and melt tests suggest that Prt^- strains are more proteolytic than Prt^+ strains. This indicates that some treatments during cheese manufacture might influence the structure of the bacterial cell and the enzyme system. Since the temperature of the molding process was as high as 80°C, it was possible that the bacterial cells were killed, disrupted with coincidental release of intracellular proteinase/peptidase. It had been reported (49) that maximal intracellular dipeptidase activity is attained when 90% of the starter population is inactivated. Therefore, intracellular proteinase/peptidase would dominate during cheese ripening, and Prt^- was not necessarily the strain that broke down the casein poorly, since the intracellular proteinase/peptidase released might be more proteolytic than that of Prt^+. Different intracellular proteinase activity has been observed between a slow acid-producing mutant of Streptococcus lactis and its parent (101,104). Lawrence et al. (50) found relatively high amounts of free amino acids in Cheddar cheese made with the 'slower' strains from Streptococcus cremoris and suggested that the peptidase activity of non-bitter (slower) strains is greater than that of bitter (faster) strains. The peptidase responsible for

degradation of bitter peptides is an intracellular enzyme released from cell lysis (57,97). In that case, Prt^- might degrade more protein to weaken the cheese structure.

While Prt^- variants of lactic streptococci are deficient in cell-wall-bound proteinase, these cells contain peptidase activities and peptide transport systems that appear to be similar to those in the parent cells (97). Other workers have reported that the mutant strain has less intracellular enzyme activity than the parent strain (42,87) or even shows no intracellular proteolytic activity (27). Therefore, Prt^+ is more proteolytic than Prt^- no matter what intracellular enzymes might be released from cell lysis. From this point, the results of this study show that the higher the proteolytic activity of the strain, the more stretchable of the cheese. But the meltability did not parallel this. Further, there seems to be an inverse relationship between meltability and stretchability (Figure 16,18), as both are affected by protein degradation but exhibit different results, possibly due to different mechanisms of protein molecule interaction.

The moisture content and pH values of cheese have been found to influence both the relative rates of breakdown of the different caseins and the nature of the degradation products formed (4,16,19,21,59). The lower moisture content of the starter cheeses compared to that of acidified cheese decrease the rennet activity. Therefore, protein degradation of the starter cheeses by rennet is gradual during the first week. Then, the synergistic proteolytic action of rennet and bacterial enzymes on caseins (23) increase the degradation rate to the extent that some of the starter cheeses exhibit poorer stretchability than acidified cheese. The moisture content also regulates the rheological behavior of the mass of partly broken down protein (22).

Browning Test

It was convenient to use the cheese sample spent in the stretch test for the browning study. During heating, the test tube was covered with aluminum foil to prevent excess loss of moisture. Air bubbles within the cheese expanded, which made a loose

cheese body. After heating, it was necessary to pack the cheese to tighten the loose body and to remove the moisture left in the bottom of the tube. Otherwise, color reflection through the bottom of the test tube from the measuring head would interfere. The b* index was found to represent the real situation of color change when the cheese sample readings were compared with visual differences in the preliminary study (Appendix Figure 28).

Browning tests of Mozzarella cheeses made with L. bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and direct acidification are shown in Figure 20. Figure 21 exhibits the color change of a batch of 14-day-aged Mozzarella cheese in the browning test. Cheeses made with Prt^+ strains demonstrated darker color than those with Prt^- strains, while the cheese from direct acidification showed the least color change of all samples (Appendix Table 21).

Increased titratible acidity during cheese manufacture indicated lactose could be converted to lactic acid by all strains, but metabolism of galactose, a reducing hydrolyzate form, exists in only some strains (41,98). If galactose could not be utilized, it would accumulate in the cheese and react with amino compounds to form a darker color than the cheese without galactose accumulation. Darker color of the cheeses with Prt^+ strains (WB111,WB104) suggests that the galactose metabolism of Prt^+ strains might be inferior to that of Prt^- strains (WB100,WB102). Galactose has been found to be much more active in browning reactions than lactose (75). Since lactose was not utilized in the direct acidified cheese, it remained unchanged. This cheese had the lightest color of all samples. However, the amount of amino groups must be hydrolyzed as being the major factor controlling this reaction.

Effect of Mixed Cultures

Traditionally, Streptococcus thermophilus is used to combine with L. bulgaricus as a mixed culture to make Mozzarella cheese. A S. thermophilus Prt^+ strain (WT16)

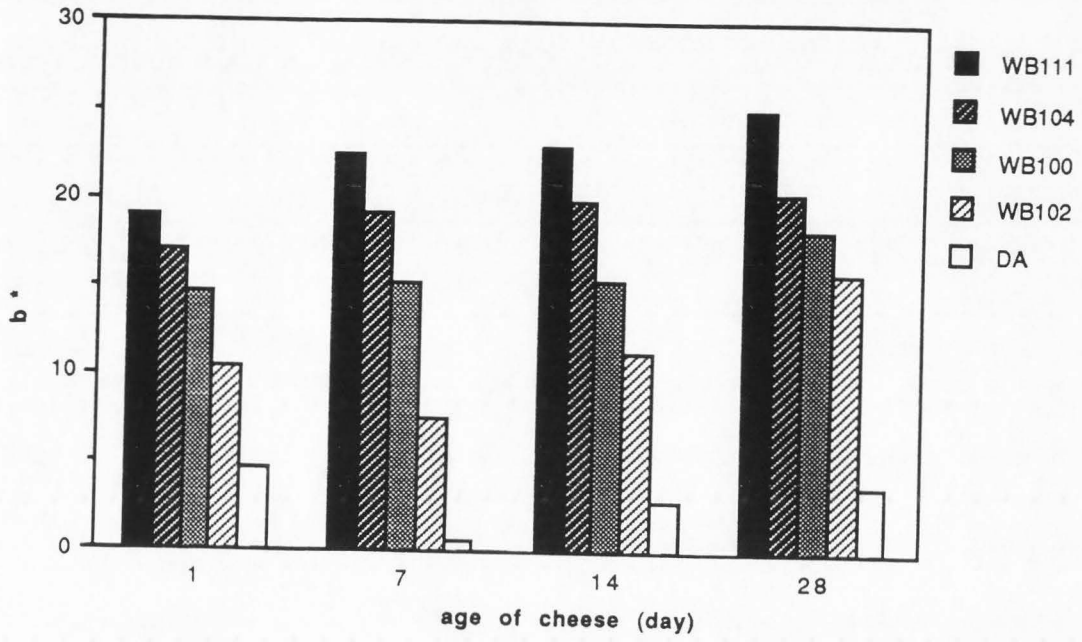

Figure 20. Comparison of browning test of Mozzarella cheeses made with L. bulgaricus Prt^+ strains (WB111,WB104), Prt^- strains (WB100,WB102), and direct acidification.

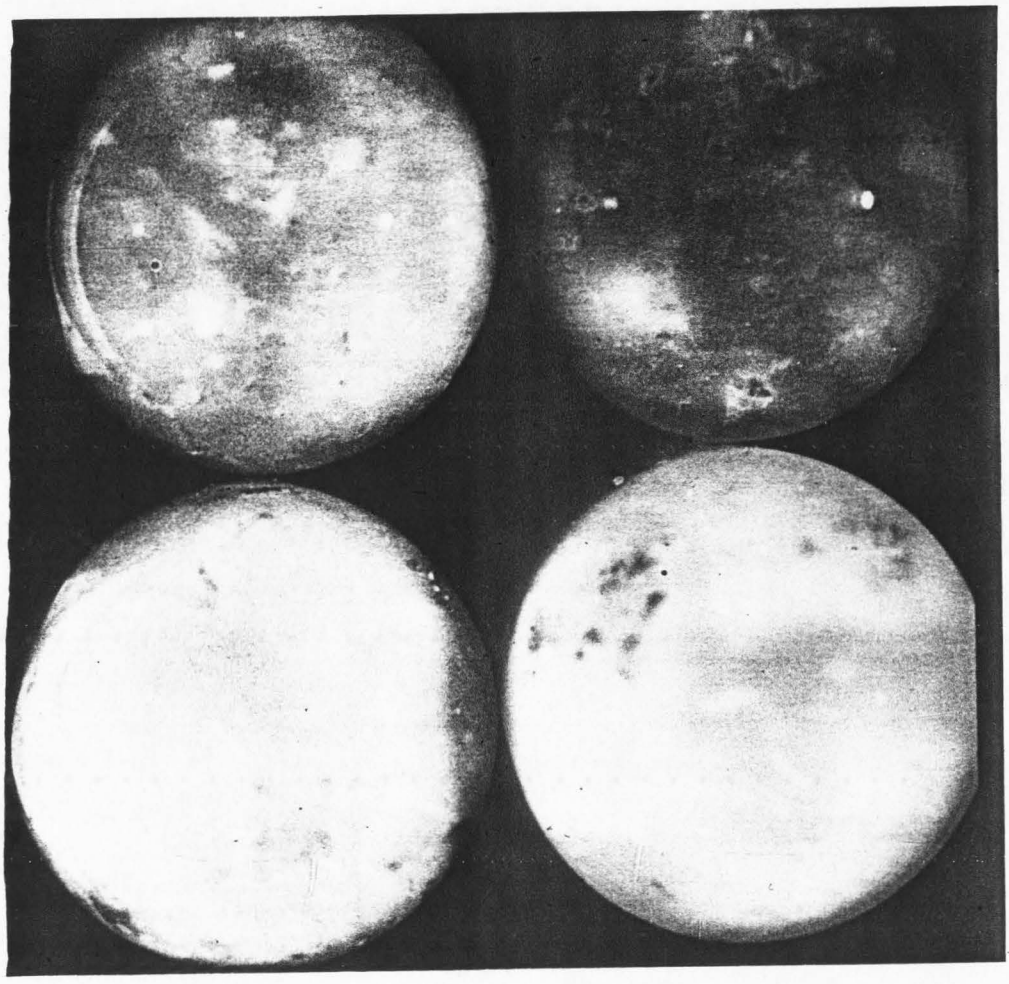

Figure 21. Color change of 14-day-aged Mozzarella cheese made with L.bulgaricus Prt^+ strains, WB104 (top left), WB111 (top right), Prt^- strain, WB102 (bottom left), and direct acidification method (bottom right) in the browning test.

combined with each of L. bulgaricus Prt^+ strains (WB111,WB104) and a S. thermophilus Prt^- strain (WT16A) combined with each of L. bulgaricus Prt^- strains (WB100,WB102) were used to make Mozzarella cheese. The inoculum ratio of S. thermophilus (coccus) to L. bulgaricus (rod) was 40/60 and 30/70. A pure culture of each strain was grown separately and inoculated into the cheese milk according to their ratios.

Stretchability of cheeses made with mixed cultures from either Prt^+strains (WT16-WB111,WT16-WB104) or Prt^- strains (WT16A-WB100, WT16A-WB102) was lost sharply (Figure 22,23). Both mixed cultures of Prt^+ strains and mixed cultures of Prt^- strains showed stretchability much poorer than single strains of either Prt^+ or Prt^- L. bulgaricus (Figure16). The symbiotic relationship between S. thermophilus and L. bulgaricus is believed to increase the proteolytic activity of mixed cultures. The degree of stretchability loss is critical for mixed cultures of Prt^+ strains more than for mixed cultures of Prt^- strains, when compared with their respective single strain of L. bulgaricus. This indicated that the symbiotic interaction of Prt^+ strains are more effective than that of Prt^- strains. This result agrees with their acid production during Mozzarella cheese manufacture (Figure 4).

As observed in the stretch test, stretchability of the cheese was lost severely when the mixed culture of coccus and rod was used. The melt test showed the same response that the cheese from mixed cultures exhibited longer melting flow than that from single strain (Figure 24,25). Moreover, the mixed cultures of Prt^+ strains showed longer melting flow than either mixed cultures of Prt^- strains or than single strain, either Prt^+ or Prt^-. Again, this phenomenon suggests that the symbiotic interaction of Prt^+ strains is much more active than that of Prt^- strains.

The results of the browning tests of Mozzarella cheese made with mixed culture are shown in Figures 26,27. The mixed cultures of Prt^+ strains exhibit darker color than those of Prt^- strains. The b* value of the direct acidified cheese is quite constant. Since the loss of stretchability with aging shows that rennet was still active within the acidified

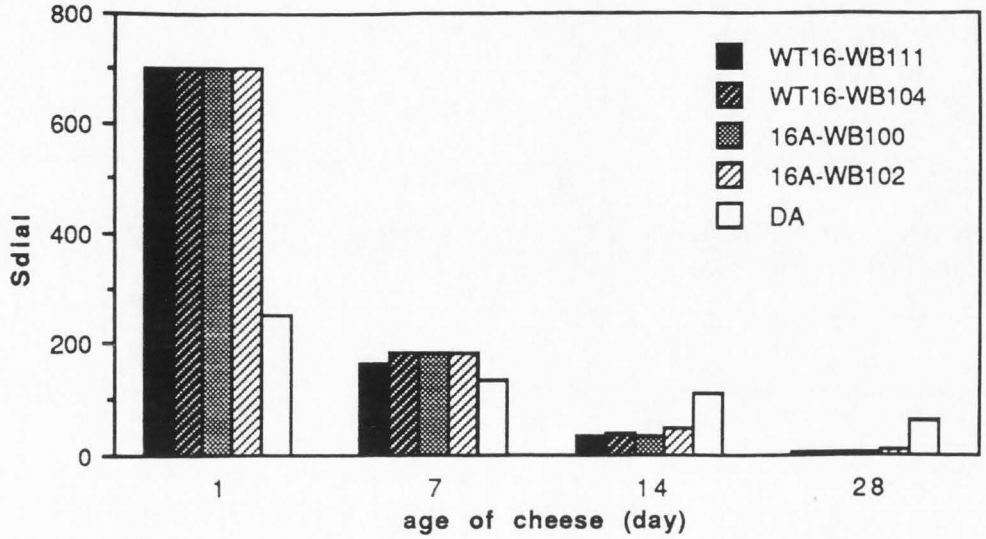

Figure 22. Comparison of stretchability of Mozzarella cheeses made with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 40/60).

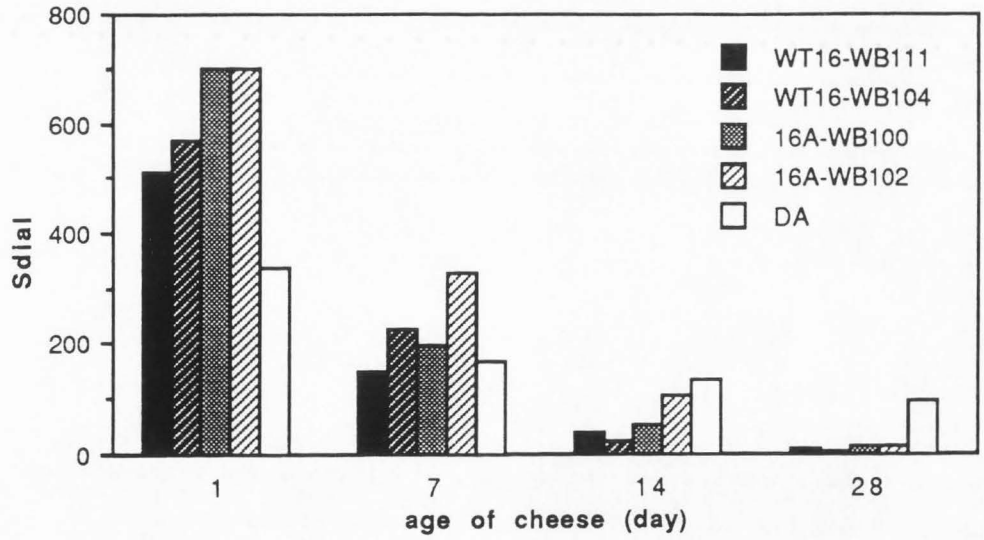

Figure 23. Comparison of stretchability of Mozzarella cheeses made with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 30/70).

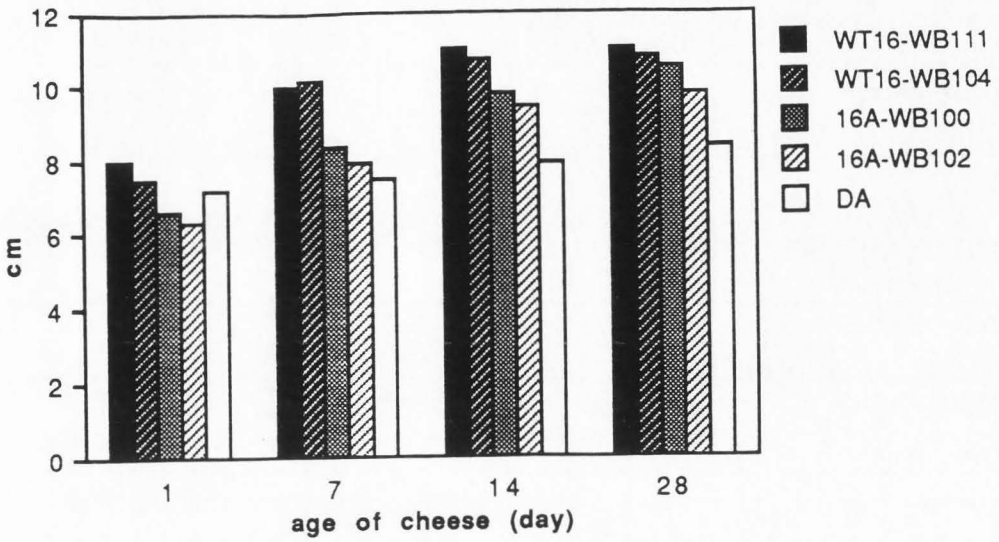

Figure 24. Comparison of meltability of Mozzarella cheeses made with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 40/60).

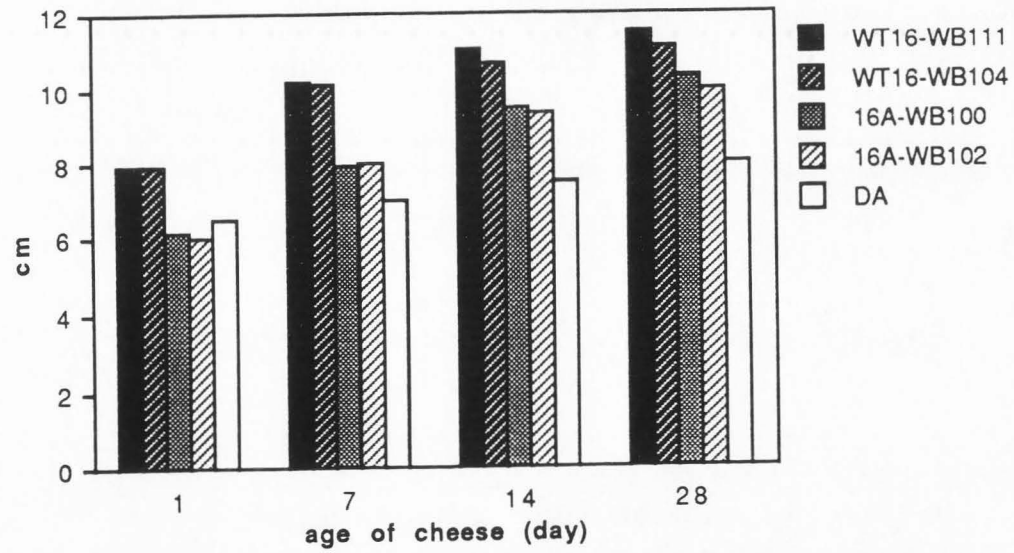

Figure 25. Comparison of meltability of Mozzarella cheeses made with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 30/70).

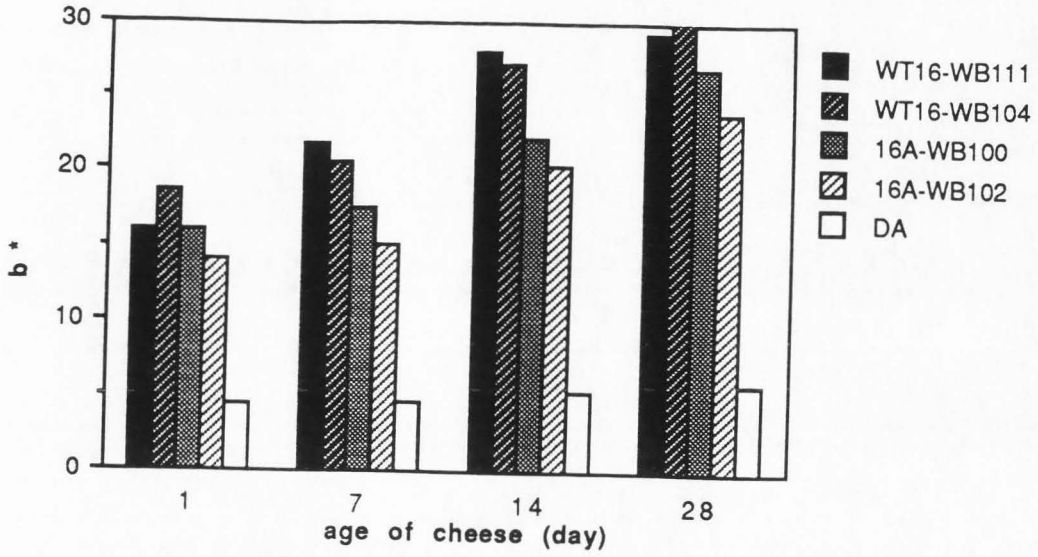

Figure 26. Comparison of browning test of Mozzarella cheeses made with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 40/60).

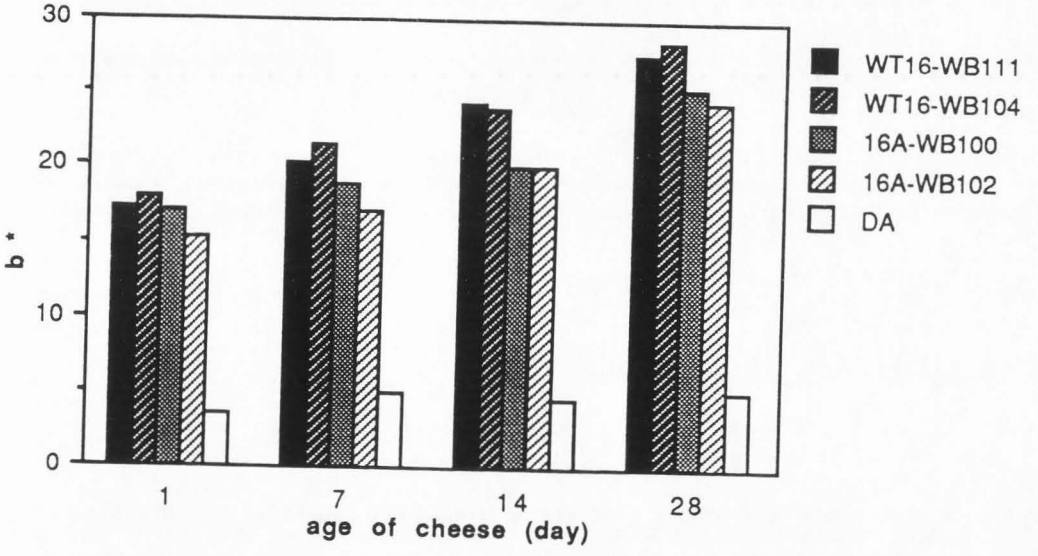

Figure 27. Comparison of browning test of Mozzarella cheesesmade with mixed cultures of S. thermophilus and L.bulgaricus (ratio of coccus/rod = 30/70).

cheese, the protein degradation was expected to contribute to browning reaction. However, with no lactose utilization the acidified cheese did not get darker with aging. The stable b* value of the acidified cheese suggests on the one hand that amino compounds do not play as important a role as milk sugar in the browning reaction and that the activity of lactose in the browning reaction is quite inert. However, galactose is quite active in producing browning products even independent of the presence of amino acids (75). That might be the reason why the rate of browning of starter cheese is enhanced, shown as the higher b* value, compared to acidified cheese.

CONCLUSIONS

1. A stretch test was developed based upon an LVT Brookfield viscometer. It appeared more sensitive than an MVT device.
2. Two melt tests were evaluated. The modification of the Olson and Price method appeared to provide more readable data in this study.
3. A browning test was developed, and the b* value of a Minolta CR-100 meter was used to indicate the degree of browning.
4. Mozzarella cheese manufactured with Prt^- strains of L. bulgaricus demonstrated less stretchability but longer melting flow than the cheese from Prt^+ strains. Direct acidified cheese lost stretchability rapidly but kept constant meltability during aging.
5. In the browning test, the cheese made with Prt^- strains was lighter in color than cheese from Prt^+ strains. Direct acidified cheese had the lightest color of all cheese samples.
6. Mixed Prt^+ cultures demonstrated more active symbiotic interaction than mixed Prt^- cultures, so the effects of proteolysis appeared enhanced.

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APPENDIX

Table 2. The pH[1] change of Mozzarella cheese manufactured from different strains of Lactobacillus bulgaricus.

	time (h)				
strain	0	2	4	6	7
WB111	6.67	6.31	5.93	5.39	5.20
WB117	6.69	6.36	5.98	5.51	5.32
WB118	6.70	6.46	6.07	5.59	5.36
WB104	6.61	6.20	5.94	5.42	5.24
WB100	6.66	6.33	5.93	5.08	
WB102	6.66	6.42	6.00	5.21	
WB131	6.69	6.31	5.89	5.19	4.81
WB132	6.69	6.47	6.11	5.41	4.97
WB133	6.70	6.35	5.71	4.88	4.46

[1] Means of three trials

Table 3. The pH[1] change of Mozzarella cheese manufactured from mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60).

	time (h)				
strain	0	3	3.5	4	4.5
WT16-WB111	6.62	5.96	5.30	5.13	
WT16-WB104	6.62	6.00	5.32	5.20	
WT16A-WB100	6.62	6.18	5.99	5.47	5.16
WT16A-WB102	6.62	6.16	6.01	5.46	5.24

[1] Means of three trials

Table 4. The pH[1] change of Mozzarella cheese manufactured from mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70).

	time (h)				
strain	0	3	3.5	4	4.5
WT16-WB111	6.62	5.77	5.37	5.16	
WT16-WB104	6.62	5.82	5.42	5.14	
WT16A-WB100	6.62	6.13	6.07	5.37	5.27
WT16A-WB102	6.62	6.11	6.00	5.40	5.28

[1] Means of three trials

Table 5. pH of resultant curds of Mozzarella cheese made with different strains of Lactobacillus bulgaricus.

	strain			
trial	WB111	WB104	WB100	WB102
1	5.29	5.30	5.19	5.20
2	5.29	5.27	5.31	5.28
3	5.31	5.28	5.25	5.29

Table 6. pH of resultant curds of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus.

		WT16/WB104		WT16A/WB102	
trial	coccus/rod	WT16/WB111	WT16A/WB100		
1	40/60	5.13	5.20	5.14	5.22
	30/70	5.07	5.12	5.27	5.18
2	40/60	5.10	5.12	5.17	5.25
	30/70	5.16	5.14	5.24	5.15
3	40/60	5.18	5.16	5.23	5.20
	30/70	5.20	5.19	5.17	5.15

Table 7. ANOVA for moisture content of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

Source	df	Mean Squares	F-ratio	Sig. level
Between				
Strain	4	4.9656067	2.217	.1401
Error	10	2.2401533		
Total	14			

Table 8. ANOVA for moisture content of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

Source	df	Mean Squares	F-ratio	Sig. level
Between				
Strain	4	4.5113100	4.401	.0261
Error	10	1.0251800		
Total	14			

Table 9. ANOVA for moisture content of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) andwith direct acidification.

Source	df	Mean Squares	F-ratio	Sig. level
Between				
Strain	4	2.2157733	.366	.8276
Error	10	6.0572600		
Total	14			

Table 10. ANOVA for stretchability of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	187661.78	52.871	3.84
Batch	2	88167.11	24.840	4.46
Strain x Batch	8	3549.453		
Time	3	806557.89	112.847	4.76
Time x Batch	6	7147.274		
Strain x Time	12	44017.274	8.782	2.18
Error	24	5012.4652		
Total	59			

Table 11. ANOVA for meltability of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	6.716250	50.977	3.84
Batch	2	0.405500	3.078	4.46
Strain x Batch	8	0.1317500		
Time	3	17.589944	231.616	4.76
Time x Batch	6	0.0759444		
Strain x Time	12	1.2236944	32.179	2.18
Error	24	0.0380278		
Total	59			

Table 12. ANOVA for color change of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	686.34250	47.474	3.84
Batch	2	116.05017	8.027	4.46
Strain x Batch	8	14.457250		
Time	3	42.16594	6.610	4.76
Time x Batch	6	6.379278		
Strain x Time	12	9.058167	4.693	2.18
Error	24	1.9302500		
Total	59			

Table 13. ANOVA for stretchability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	203353.0	124.142	3.84
Batch	2	2878.6	1.757	4.46
Strain x Batch	8	1638.064		
Time	3	1117244.7	514.473	4.76
Time x Batch	6	2171.630		
Strain x Time	12	35928.059	24.8825	2.18
Error	24	1447.2640		
Total	59			

Table 14. ANOVA for meltability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	10.608125	4.700	3.84
Batch	2	11.932542	5.286	4.46
Strain x Batch	8	2.2572812		
Time	3	26.790778	74.872	4.76
Time x Batch	6	0.3578194		
Strain x Time	12	1.0143194	5.740	2.18
Error	24	0.1767257		
Total	59			

Table 15. ANOVA for color change of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	733.49114	283.620	3.84
Batch	2	10.95852	4.237	4.46
Strain x Batch	8	2.586178		
Time	3	279.19587	37.524	4.76
Time x Batch	6	7.440406		
Strain x Time	12	15.890381	10.564	2.18
Error	24	1.5041679		
Total	59			

Table 16. ANOVA for stretchability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) andwith direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	24743.67	2.313	3.84
Batch	2	102839.57	96.307	4.46
Strain x Batch	8	10697.833		
Time	3	884868.31	26.905	4.76
Time x Batch	6	32888.339		
Strain x Time	12	23383.558	2.568	2.18
Error	24	9106.5617		
Total	59			

Table 17. ANOVA for meltability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) and with direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	17.892979	3.304	3.84
Batch	2	1.309292	0.242	4.46
Strain x Batch	8	5.4155417		
Time	3	30.506931	69.516	4.76
Time x Batch	6	0.4388472		
Strain x Time	12	0.8230069	2.671	2.18
Error	24	0.3081528		
Total	59			

Table 18. ANOVA for color change of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) and with direct acidification.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	4	695.68885	370.138	3.84
Batch	2	1.98713	1.057	4.46
Strain x Batch	8	1.8795392		
Time	3	174.44337	17.851	4.76
Time x Batch	6	9.7723667		
Strain x Time	12	9.0431764	5.136	2.18
Error	24	1.7606014		
Total	59			

Table 19. Multiple range analysis for stretchability of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

age (day)	strain	rps	Means[1]
	WB111	3	700[a]
	WB104	3	700[a]
1	WB100	3	700[a]
	WB102	3	700[a]
	DA	3	636[a]
	WB111	3	650[b]
	WB104	3	650[b]
7	WB100	3	627[b]
	WB102	3	617[b]
	DA	3	187[a]
	WB111	3	439[c]
	WB104	3	574[b]
14	WB100	3	146[a]
	WB102	3	128[a]
	DA	3	123[a]
	WB111	3	328[b]
	WB104	3	357[b]
28	WB100	3	87[a]
	WB102	3	57[a]
	DA	3	97[a]

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 112.

Table 20. Multiple range analysis for meltability of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

age (day)	strain	rps	Means[1]
	WB111	3	4.4[a]
	WB104	3	3.9[a]
1	WB100	3	4.4[a]
	WB102	3	3.8[a]
	DA	3	5.8[b]
	WB111	3	4.7[b]
	WB104	3	4.0[a]
7	WB100	3	6.4[d]
	WB102	3	5.7[c]
	DA	3	6.2[cd]
	WB111	3	5.5[b]
	WB104	3	4.8[a]
14	WB100	3	7.6[d]
	WB102	3	6.8[c]
	DA	3	6.3[c]
	WB111	3	6.8[a]
	WB104	3	6.2[a]
28	WB100	3	8.1[b]
	WB102	3	7.5[b]
	DA	3	6.5[a]

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 0.68.

Table 21. Multiple range analysis for color change of Mozzarella cheese made with different strains of Lactobacillus bulgaricus and direct acidification.

age (day)	strain	rps	Means[1]
	WB111	3	19.0^{c}
	WB104	3	17.1^{bc}
1	WB100	3	14.7^{bc}
	WB102	3	10.4^{ab}
	DA	3	4.8^{a}
	WB111	3	22.6^{c}
	WB104	3	19.2^{bc}
7	WB100	3	15.2^{b}
	WB102	3	7.6^{a}
	DA	3	0.7^{a}
	WB111	3	22.9^{d}
	WB104	3	20.0^{cd}
14	WB100	3	15.5^{bc}
	WB102	3	11.2^{b}
	DA	3	2.8^{a}
	WB111	3	25.1^{c}
	WB104	3	20.3^{bc}
28	WB100	3	18.3^{bc}
	WB102	3	15.9^{b}
	DA	3	3.8^{a}

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 7.2.

Table 22. Multiple range analysis for stretchability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

age (day)	strain	rps	Means[1]
	WT16-WB111	3	700[b]
	WT16-WB104	3	700[b]
1	WT16A-WB100	3	700[b]
	WT16A-WB102	3	700[b]
	DA	3	250[a]
	WT16-WB111	3	162[a]
	WT16-WB104	3	183[a]
7	WT16A-WB100	3	184[a]
	WT16A-WB102	3	185[a]
	DA	3	133[a]
	WT16-WB111	3	34[a]
	WT16-WB104	3	38[a]
14	WT16A-WB100	3	34[a]
	WT16A-WB102	3	48[a]
	DA	3	109[a]
	WT16-WB111	3	3[a]
	WT16-WB104	3	6[a]
28	WT16A-WB100	3	6[a]
	WT16A-WB102	3	8[a]
	DA	3	64[a]

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 76.

Table 23. Multiple range analysis for meltability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

age (day)	strain	rps	Means[1]
	WT16-WB111	3	8.0[a]
	WT16-WB104	3	7.5[a]
1	WT16A-WB100	3	6.6[a]
	WT16A-WB102	3	6.3[a]
	DA	3	7.2[a]
	WT16-WB111	3	10.0[a]
	WT16-WB104	3	10.1[a]
7	WT16A-WB100	3	8.3[a]
	WT16A-WB102	3	7.9[a]
	DA	3	7.5[a]
	WT16-WB111	3	11.0[b]
	WT16-WB104	3	10.7[b]
14	WT16A-WB100	3	9.8[ab]
	WT16A-WB102	3	9.4[ab]
	DA	3	7.9[a]
	WT16-WB111	3	11.0[a]
	WT16-WB104	3	10.8[a]
28	WT16A-WB100	3	10.5[a]
	WT16A-WB102	3	9.8[a]
	DA	3	8.3[a]

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 2.8.

Table 24. Multiple range analysis for color change of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 40/60) and with direct acidification.

age (day)	strain	rps	Means[1]
1	WT16-WB111	3	15.9^{bc}
	WT16-WB104	3	18.7^{c}
	WT16A-WB100	3	15.9^{bc}
	WT16A-WB102	3	14.1^{b}
	DA	3	4.4^{a}
7	WT16-WB111	3	21.9^{c}
	WT16-WB104	3	20.6^{c}
	WT16A-WB100	3	17.5^{b}
	WT16A-WB102	3	15.1^{b}
	DA	3	4.7^{a}
14	WT16-WB111	3	28.1^{c}
	WT16-WB104	3	27.1^{c}
	WT16A-WB100	3	22.2^{b}
	WT16A-WB102	3	20.4^{b}
	DA	3	5.4^{a}
28	WT16-WB111	3	29.3^{cd}
	WT16-WB104	3	30.3^{d}
	WT16A-WB100	3	26.9^{bc}
	WT16A-WB102	3	24.0^{b}
	DA	3	5.9^{a}

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 3.0.

Table 25. Multiple range analysis for stretchability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) and with direct acidification.

age (day)	strain	rps	Means[1]
	WT16-WB111	3	511^{ab}
	WT16-WB104	3	570^{b}
1	WT16A-WB100	3	700^{c}
	WT16A-WB102	3	700^{c}
	DA	3	337^{a}
	WT16-WB111	3	150^{a}
	WT16-WB104	3	229^{a}
7	WT16A-WB100	3	198^{a}
	WT16A-WB102	3	330^{a}
	DA	3	169^{a}
	WT16-WB111	3	40^{a}
	WT16-WB104	3	23^{a}
14	WT16A-WB100	3	55^{a}
	WT16A-WB102	3	104^{a}
	DA	3	137^{a}
	WT16-WB111	3	8^{a}
	WT16-WB104	3	5^{a}
28	WT16A-WB100	3	14^{a}
	WT16A-WB102	3	15^{a}
	DA	3	97^{a}

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 195.

Table 26. Multiple range analysis for meltability of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) and with direct acidification.

age (day)	strain	rps	Means[1]
	WT16-WB111	3	7.9[a]
	WT16-WB104	3	7.9[a]
1	WT16A-WB100	3	6.2[a]
	WT16A-WB102	3	6.0[a]
	DA	3	6.5[a]
	WT16-WB111	3	10.2[a]
	WT16-WB104	3	10.1[a]
7	WT16A-WB100	3	7.9[a]
	WT16A-WB102	3	8.0[a]
	DA	3	7.0[a]
	WT16-WB111	3	11.0[a]
	WT16-WB104	3	10.7[a]
14	WT16A-WB100	3	9.5[a]
	WT16A-WB102	3	9.3[a]
	DA	3	7.5[a]
	WT16-WB111	3	11.5[a]
	WT16-WB104	3	11.1[a]
28	WT16A-WB100	3	10.3[a]
	WT16A-WB102	3	10.0[a]
	DA	3	7.9[a]

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 4.4.

Table 27. Multiple range analysis for color change of Mozzarella cheese made with mixed cultures of Streptococcus thermophilus and Lactobacillus bulgaricus (ratio of coccus/rod = 30/70) and with direct acidification.

age (day)	strain	rps	Means[1]
1	WT16-WB111	3	17.3^{bc}
	WT16-WB104	3	17.9^{c}
	WT16A-WB100	3	17.0^{bc}
	WT16A-WB102	3	15.3^{b}
	DA	3	3.6^{a}
7	WT16-WB111	3	20.7^{cd}
	WT16-WB104	3	21.5^{d}
	WT16A-WB100	3	18.8^{bc}
	WT16A-WB102	3	17.1^{b}
	DA	3	4.9^{a}
14	WT16-WB111	3	24.3^{c}
	WT16-WB104	3	24.0^{c}
	WT16A-WB100	3	20.0^{b}
	WT16A-WB102	3	20.0^{b}
	DA	3	4.7^{a}
28	WT16-WB111	3	27.7^{cd}
	WT16-WB104	3	28.6^{d}
	WT16A-WB100	3	25.4^{bc}
	WT16A-WB102	3	24.5^{b}
	DA	3	5.2^{a}

[1] Means with the same letter are not significantly different at ∂=.05.
[2] LSD = 2.6.

Table 28. ANOVA for meltability of Mozzarella cheese made with different strains of Streptococcus thermophilus.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	5	2.43379	2.677	3.33
Batch	2	909.46379	1000.516	4.10
Strain x Batch	10	0.9089947		
Time	3	49.73530	13.256	4.76
Time x Batch	6	3.7517894		
Strain x Time	15	2.0161485	2.678	2.01
Error	30	0.7528666		
Total	71			

Table 29. ANOVA for color change of Mozzarella cheese made with different strains of Streptococcus thermophilus.

Source	df	Mean Square	F-ratio	$F_{0.05}$
Strain	5	0.52100	1.151	3.33
Batch	2	440.67402	973.902	4.10
Strain x Batch	10	0.452483		
Time	3	21.65682	0.999	4.76
Time x Batch	6	21.669361		
Strain x Time	15	0.824408	1.612	2.01
Error	30	0.5114812		
Total	71			

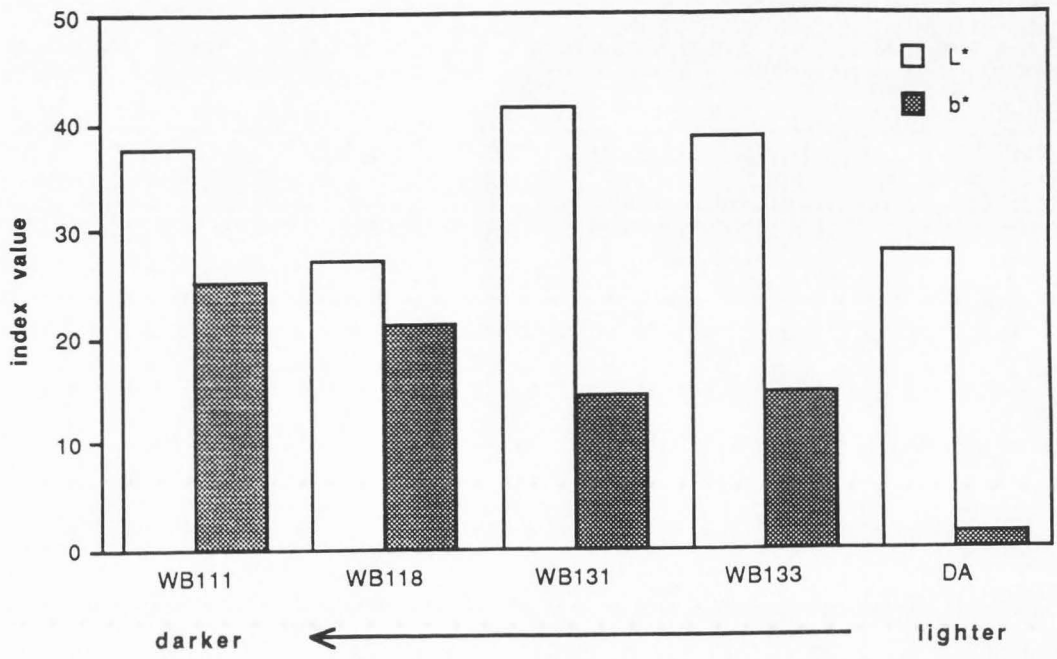

Figure 28. Comparison of b* index and L* index in representing the color change as Mozzarella cheese samples with visual difference in color were measured.