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Proceedings from the 32nd Marschall Italian and Specialty Cheese Seminar

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PROUD OF THE PAST
FOCUSED ON THE FUTURE

PROCEEDINGS FROM THE 32ND MARSCHALL ITALIAN & SPECIALTY CHEESE SEMINAR

PRESENTED BY
RHÔNE-POULENC DAIRY INGREDIENTS
MADISON, WISCONSIN

IN CONJUNCTION WITH
SEMINAR EXHIBITORS AND NON-EXHIBITING HOSTS

SEPTEMBER 20 & 21, 1995
DANE COUNTY EXPOSITION CENTER · MADISON, WISCONSIN
MARSCHALL
ITALIAN &
SPECIALTY
CHEESE
SEMINAR

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The world of Italian Cheese. There never has been a selection of cheese that has changed the world’s appetite more than Italian Cheese.

The broad range of delightful flavors ranging from Romano, Parmesan, and Asiago, to Provolone, Mozzarella and Ricotta have tantalized the taste buds world over. As the world has learned, there are no geographical limits to flavor delight. Whether it's South America, Japan, China, Soviet Union or in the “Good Ole USA”, people respond to the highly flavorful Italian Cheese varieties.

For a minute this morning, let’s turn back the clock of history and see just how the building blocks of the Italian Cheese Industry got started. You will find in the review some of the reasons the Italian Cheese appeal is so strong. You will find that the growth of these important cheeses didn't come about by accident, but by decades of “hit or miss” trials conducted mostly by individuals, not by large corporations. Sacrifices were made by men and women in their pursuit of flavor and tradition.

To the ethnic Italian, cheese is a great deal more than something to eat. Cheesemaking is an art and a tradition handed down through centuries from parent to child. Each generation took its responsibility by following precisely the direction given by their predecessor in making their fine quality cheese.

One interesting quality learned by me, in my years of exposure to the industry, was the reluctance of the ethnic Italian cheesemakers to accept innovations and scientific advancement in their cheese endeavor. They apparently felt bound by laws, traditions and customs not to alter their methods of manufacture for fear change might somehow adversely affect the flavor, body or texture of their cheese. In view of the popularity and success of the Italian Cheese varieties on the world market, they apparently may have been right. But when you consider the different flavors, cheese bodies and textures of each Italian variety, one may start to understand their attitude to change.

In Italy, some of the common practices for instance is to use only raw milk. The milk is usually not refrigerated and used as fresh as possible. In most instances, the use of pumps and centrifuges are forbidden. Creaming pans are used instead.

Whey starters have been used successfully in Italy for years - but with good reason. Whey starters work much better in raw, fresh nonrefrigerated milk that has not been exposed to pasteurization, separation, clarification or pumping. The whey starter can be traced back to around 1890 in Italy. (The Italian Cheese Industry didn't get started in the U.S. until 1899.)

In Italy, prior to 1890, no cultures were used in the production of hard/soft type cheeses. The natural bacterial flora of fresh daily raw milk was sufficient for making cheese.

From an economic aspect, cheese production in Italy, especially made from natural bacterial flora of the milk, must have brought on some moments of uncertainty due to the contamination of anaerobic spore-forming bacteria (late gas producers). Much cheese damage and inconsistencies were experienced. Around the end of the 19th century an expert by the name of Notari became convinced that the action counteracting that of clostridia could be obtained after inoculating the milk with lactic starter and raising its acidity at the moment prior to the setting of the vat. Thus was born the whey starter.

The whey starter of today is quite different then that of yesteryear. The whey starter of around 1900 was predominantly lactic streptococcus when the whey acidity was low. But when the whey acidity was high, it was certain that some form of bacillus (rod) was in the majority compared to the coccus.
Mesophilic lactobacilli were also commonly used in whey starters around the turn of the century because cheesemakers used lower inoculation temperatures which also resulted in lower levels of acid development.

By 1930, studies made by Bondiole were aimed at controlling the coccus and rod ratio in whey starter. The temperature of the bulk was used to manipulate the ratios. (Recognizing ratio control was important in cheese making.)

Later in 1943, it was observed that the *L. Bulgaricus* type rod was found in whey starter. In 1962, Ballazzi showed that the species always present in whey starter and usually predominant was *L. Helveticus*.

The evolution of the whey starter from the mesophilic to the thermophilic lactobacillus is a story in itself.

It is believed that each whey starter was a system or culture program by itself. Each program adjusted to various factors acting in different conditions to produce a common desirable price of cheese.

In Italy, the "whey starter is king." It does everything the cheesemaker desires and who will argue with success? Italy has proven it can provide cheese to the world that is unique and flavorful.

But in the U.S., the traditional values of the "Motherland" could not be realized due to requirements of pasteurization of milk. The high expectations of Italian immigrant cheesemakers were soon to be changed.

As early as 1899, Giuseppe Pollio built a driftwood fire on a beach in New York's Coney Island, hung a kettle from a make-shift tripod and began processing Ricotta cheese by the traditional open-fire method. One year later Guiseppi moved indoors to a small store and added Mozzarella to his product line.

Pasquale Frigo came to Lemont, IL in 1913, sent there by his father from Italy to continue the family tradition of Italian cheesemaking in the new world.

From its humble beginning in Coney Island, the domestic Italian Cheese Industry staggered and stumbled for approximately 25 years. The development of the U.S. Italian Cheese Industry in the early days had to survive the strong competition from imports, overcoming obstacles of cheese color, flavor and cheese made with milk from sheep, goat and buffalo.

Today, the U.S. domestic manufacturers have great confidence in their products and firmly believe they can match or exceed the quality of any import.

Where have the domestic cheesemakers gotten their confidence?

1) Learning from the past.
2) Using "Good Ole American Technology."
3) A "winning attitude."
4) Open mindedness.

Like the "Old World" Italian cheesemakers, the immigrants from Italy possessed the same quantity of sheer determination for cheese quality, too. Although, prior to 1963, the U.S. Italian Cheese Industry was made up of privately held family operations and the industry research was principally done by each group (family). The uniqueness of this approach was that each company would develop its product line based on previous changes/results, thus rewards were based on acceptance by the general public as to what cheese quality feature was most desirable.
In 1964, an event happened that would change the U.S. Italian Cheese Industry forever... the inception of the “first Marschall Invitational Italian Cheese Seminar.”

Prior to 1964, the U.S. Italian cheesemakers were a loosely knit group held together mostly by family ties rather than common interests. With the end of WWII and the influx of GI's who had tasted the flavors of Italy, the demand for Italian cheese varieties mushroomed. Increased cheese demand due to the popularity of pizza created sufficient challenges to the infant industry.

A young man by the name of Stan Ferris at Marschall Dairy Products, Inc. recognized the need for a professional gathering to aid in the technical and communication support of a growing industry. The ICS, as it is commonly referred to, was the first meeting of its kind to cater to the support of a specialized group. The Italian Cheese Industry from that point on used the meeting as a vehicle of communication in directing its needs economically as well as politically. The marriage of Marschall Products and the U.S. Italian Cheese Industry has lasted nearly 31 years. (North America, Australia, South America)

Thirty-one years - that's a long time. A coincidence or not, the amazing fact is that the Italian Cheese Industry has had 31 years of continued growth too. Marschall Products (Rhône-Poulenc Dairy Ingredients, Inc.) congratulates the U.S. Italian Cheese Industry on its continuing growth. We hope our investment in products and services and research and development have contributed to that success.

Some of the contributions made by Rhône-Poulenc started as early as 1964.

1964 - Introduction of the first frozen non-concentrated culture in 1 ml. vials.

1967 - Frozen concentrated cultures used for direct inoculation of bulk starter.
   - Rennet paste application - combination of lipase and coagulant enzyme.
   - Old World flavor with New World technology, off setting the stop action by the USDA on imported pastes from Italy.

1973 - Introduction of Thermostar Starter Medium/CR Cultures, a program especially designed to provide phage protection and limited ratio control of the coccus and rod culture.

1978 - Custom packaging of blended lipase enzymes to meet consumer needs or vat requirements.
   - Direct-to-the-vat thermophilic cultures, thermorod and thermococcus.
   - pH control of coccus and rod
   - pH control equipment - CT2000, CT2000SS

By 1986, nearly 65% of all the Mozzarella cheese made in the U.S. was using the Thermostar Starter Program. The industry, unlike the Italian cheesemakers of Italy, made the change from whey/milk starters to the commercial program.

Why the change? It was felt that Marschall brought a meeting, research, quality ingredient products, technical service and, most important, trust to the industry. It was felt that Marschall recognized what the industry needed and responded accordingly.
The growth of the Italian Cheese Industry and Marschall Products simultaneously were like a partnership, each benefiting from the relationship. Rhône-Poulenc Dairy Ingredients is proud to continue the relationship.

**What's in the future?**

- Changes in bulk starter production that will provide the user complete control over the coccus and rod ratios.
- Systems that will provide and insure full development of the maturity of each coccus and rod culture strain grown.
- The ability to selectively pick cultures ripening systems to meet your plant’s requirements.
- Access to complete sterile air environment by all starter programs.

In today’s cheesemaking world, it’s more important than ever to completely understand your starter make-up and its potential. Rhône-Poulenc has been the forerunner in developing new products and applications and we **intend to stay in front!**

Bill Knoespel and Dr. Doug Willrett will discuss in more detail some of the advancements we have for future consideration. We ask that you attend these talks; we feel they have something to offer everyone.

You may be asking yourself why the presentation on whey starters was delivered. It’s because, to the Italian Cheesemaker, the cheese he produced was his livelihood and he adjusted his starter until he got what he wanted. He was not fortunate enough to have the research capabilities of today, but he got the job done. Rhône-Poulenc Dairy Ingredients offers you the same opportunity plus the most state of the art starter ripening system available today. There is only one thing missing - RISK!

THANK YOU!
EXPLORING THE CURRENT AND FUTURE CULTURE APPLICATIONS FOR ITALIAN AND SPECIALITY CHEESE

Craig J. Oberg, Ph. D., Department of Microbiology,
Weber State University, Ogden, UT
Coauthors: Dr. Jeff Broadbent, Dr. Don McMahon,
Shelby Caldwell, Roxanne Fecera and David Perry.

Abstract

Now that Mozzarella cheese production rivals Cheddar cheese, intensified production demands are being placed on thermophilic starter cultures. Bacteriophage problems have increased requiring novel solutions. The use of new starter organisms, genetic engineering, and development of phage resistant mutants are recent approaches. Thermophilic pediococci with introduced lactose utilization genes show promise to combat phage problems. Consumer demands for low-fat, reduced-fat and non-fat Mozzarella cheese also require new cultures to provide proper physical properties in cheeses with a very different micro-environment. Culture adjuncts can be used to accelerate development of cheese functionality when fat is removed. Exocellular polysaccharide producing cultures have shown promise in the production of low fat Mozzarella, particularly in modifying cook properties.

Review of Thermophilic Cultures

Thermophilic starter cultures are differentiated from mesophilic starters by their ability to grow at higher temperatures (optimal growth from 40 to 52°C vs. 22 to 30°C) (Thunell, 1986). Thermophilic lactobacilli commonly used for Mozzarella cheese production include Lactobacillus delbrueckii ssp. bulgaricus and Lactobacillus helveticus. Among bacteria presently classified as streptococci, Streptococcus thermophilus is the only dairy starter to be used for Mozzarella cheese.

All thermophilic lactobacilli are lactose-positive, but only L. helveticus and L. acidophilus also ferment galactose (Kandler, 1986). Considerable variation among rates of acid production from carbohydrate fermentation were detected within subspecies. These differences have important implications for cheese make times and the quality of finished product. Investigators suggested that slower acid drive in Mozzarella is accompanied by slower conversion of dicalcium para-casein to monocalcium para-casein, which yields less stretch in the finished product (Kosikowski, 1977). Conversely, if curd pH continues to drop below the acceptable range of 5.1 to 5.3, more calcium is lost from the curd, monocalcium para-casein is converted to para-casein, and the curd retains less fat and exhibits poor stretch.

Commercial cultures can be purchased as either frozen concentrates or freeze-dried concentrates. Bulk sets of either type are used to inoculate bulk tanks while DVS (direct vat sets) are inoculated directly into the cheese milk. Cultures can contain individual strains, single rod or coccus strains, or can contain a mix of rods and cocci. The majority of cultures contain known strains and are called defined strains.

Commercial culture media is composed of either a whey-based or milk-based medium with supplemental salts, buffers, and growth stimulants added. The vast majority of media is buffered either internally by salts or externally by liquid neutralizers. Most media currently used is whey-based and externally buffered, either by continual injection or a one-step neutralization.
One of the most immediate needs within the dairy fermentation industry is the methodical characterization of commercial thermophilic cultures, particularly for utilization and metabolism of sugars and for proteolytic capabilities. Thunell (1986a) listed 14 traits to be characterized in a well-defined thermophilic starter: acid production rates, phage sensitivity, strain compatibility, salt tolerance, off flavor, proteolysis, freeze-thaw stability, phosphate tolerance, bacteriocin production and sensitivity, type of lactic acid produced, growth medium preferences, plasmid stability, optimal growth conditions, and sugar fermentation profiles. Barach (1987) also listed a set of features to be characterized for each strain, dividing these into two categories: starter-related and cheese-related features. They also included specific screening for galactose utilization and for an oxidation-reduction profile. Redox potential was related to the "pink ring" defect in aged Italian cheese. Although expensive to do, such characterizations are required for the development of predictable and consistent starter systems that provide uniform, high quality products. Because of their common application, a series of well-defined dairy thermophilic bacteria would benefit Italian cheese makers.

Confusion may exist within the industry as to which *Lactobacillus* species is utilized for the rod portion of commercial thermophilic blends. Often, *L. delbrueckii* ssp. *bulgaricus* is thought to be present, but surveys revealed the presence of *L. helveticus* in some commercial blends (Oberg, unpublished data). A rapid method to determine which species is present involves the capability to ferment maltose, because *L. delbrueckii* ssp. *bulgaricus* cannot ferment maltose, whereas *L. helveticus* can utilize maltose. Galactose is not used as an indicator sugar because it yields variable results.

Thermophilic lactobacilli and streptococci are much more sensitive to antibiotics in milk than other lactococci (Reinbold, 1989). Antibiotic contamination of milk can have serious effects on antibiotic-sensitive consumers, so antibiotic-sensitive organisms provide a valuable safeguard. Decreased culture activity in the vat may occur as a result of this increased antibiotic sensitivity, which is occasionally blamed on other factors including phage attack. Better tests are required to pinpoint causes of slow acid production.

NaCl tolerance is variable among thermophilic lactobacilli and cocci, particularly compared with mesophilic lactococci. Barach (1987) reported that most strains of *S. thermophilus* have no significant growth at 1.5% NaCl in 6 h. However, at 24 h most strains could grow in the presence of 2.5% NaCl. Thunell (1989) also noted that *S. thermophilus* strains were more NaCl-sensitive than other lactic acid bacteria and found that acid production stopped at 2% NaCl. This may have serious implications on final cheese pH if manufacturers switch from brining to dry salting in the production of Italian cheeses.

Freezing tolerance varies significantly between thermophilic rods and cocci. Rods are more sensitive to freezing and to frozen storage than are cocci. In a survey of frozen, commercial, mixed thermophilic cultures, some were very deficient in rods. This may be due to the freezing sensitivity of lactobacilli and implies significant variation between the coccus:rod ratio packed by the culture manufacturer and that obtained by the cheese producer.

The majority of lactobacilli, including thermophiles, are more sensitive to phosphates than are streptococci or lactococci. Consequently, inhibitory concentrations of phosphate should be determined for the lactobacilli strains utilized prior to the selection of a bulk starter media. Traditional phage inhibitory media formulations may not provide optimal growth.

Thermophilic starter cultures are generally composed of two different genera grown together in the same bulk culture tank. Symbiosis and competition occur simultaneously during growth and yield a particular ratio of cocci to rods in finished thermophilic starter inoculum. Rate of acid production in mixed coccus and rod cultures is greater than the
sum of the two single culture’s acid production. Rajagopal and Sandine (1990) note that the majority of mixed thermophilic cultures were also more proteolytic than the sum of the individual cultures. Contributions made by each culture to this interaction have been examined and several components identified. Lactobacilli degrade casein, supplying peptides and amino acids to the weakly proteolytic streptococci, which in turn produce formic acid and carbon dioxide to stimulate growth of lactobacilli (Radke-Mitchell, 1986). Investigators suspect other stimulatory compounds and interactions remain to be delineated. Others suggest that catabolism of urea by \textit{S. thermophilus} releases carbon dioxide which stimulates the growth of lactobacilli. Successful symbiotic growth obviously requires compatible strains in the pair or the final rod to cocci ratio and activity of each culture may be deleteriously affected.

Use of Proteolytic Adjunct Cultures in Low-Fat Mozzarella Cheese

The popularity of pizza and interest by consumers to lower fat in their diet has prompted research to develop an acceptable low-fat Mozzarella cheese with appropriate functionality for pizza. Textural characteristics, as well as melt properties, are significantly affected by decreasing the fat content of Mozzarella cheese (Tuckey, 1974; Tunick, 1991). An increase in toughness can be observed as the fat is removed from Mozzarella cheese which is the result of an increase in protein concentration as well as differences in the distribution of moisture within the protein matrix (Merrill, 1994). Medium firmness, sufficient melt, adequate stretchability and ease of shredding, all desirable properties of Mozzarella cheese, vary with the age of the cheese, pH, moisture content, salt content, and type of starter culture (McMahon, 1993). Tunick (1991) showed that low fat (9% fat), high moisture Mozzarella did not have physical characteristics comparable to part-skim Mozzarella cheese until after 6 weeks of refrigerated storage.

Dejong (1976) attributes the softening of cheese over time to the hydrolysis of the proteins in the cheese. Changes affecting the properties of stretch and melt in Mozzarella have also been associated with proteolysis in the cheese (Oberg 1991a, 1991b). Tunick (1991) established that the degradation of \textit{a}_51-casein in low fat, high moisture Mozzarella resulted in the cheese having textural properties comparable to that of high fat Mozzarella. The objective of this study was to examine the effects of a proteolytic positive (Prt+) lactose deficient (Lac\textsuperscript{d}) mesophilic adjunct culture on the physical properties of low fat Mozzarella cheese. We used three different levels of adjunct culture; .25\%, .50\%, and 1.0\%, along with a conventional starter culture.

In order to keep the make time of the experimental cheeses consistent it was necessary to adjust the starter culture because the adjunct culture had a significant effect on the cheese make time probably due to increased stimulation of the starter cocci by peptides produced by the adjunct culture. Average make time for the cheese was consistent with industry practices, ranging from 2.3 to 2.5 h. Moisture content in the cheeses manufactured with the adjunct culture ranged from 61\% to 63\%, while the control had a moisture of about 60\% (Table 1). Total protein in the cheeses made with adjunct culture was 23\% to 24\%, slightly lower than the control but corresponding to the increased moisture. Percent fat remained fairly consistent in all of the cheeses.

A significant difference between treatments was found for melt. Improved melt among the adjunct treated cheeses can be seen at 1, 7 and 14 d after manufacture with the 0.50\% level of adjunct culture demonstrating the best overall melt (Figure 1). By 28 d after manufacture, all the cheeses demonstrated similar melt characteristics. There were no significant differences in the viscosity of the cheese among treatments, but time did play a significant role in affecting changes in viscosity.
Gels from SDS-PAGE analysis of the protein breakdown in the cheeses between 1 and 28 d of refrigerated storage show the disappearance of the \( \alpha_{s1} \)-casein band in the control cheese as well as the cheese made with 0.50% adjunct culture. The appearance of a band between the \( \alpha_{s1} \) and \( \beta \)-casein bands becomes more visible over the 28 d storage period. This band is assumed to be \( \alpha_{s1} \)-I casein. Differences in proteolysis among treatments could not be visually determined from the gels. This gel method did not prove sensitive enough to measure differences in proteolysis between the cheeses. We are now using capillary gel electrophoresis for analysis and initial work indicates we can monitor the breakdown of individual caseins in the cheese. This should allow for the study of cultures that preferentially breakdown alpha or beta casein and how these cultures affect the physical properties of the cheese.

Several modifications were made to the Utah State University Reduced Fat Mozzarella cheese manufacturing procedure (Merrill, 1994) to enhance moisture retention in the low-fat cheese. These modifications included keeping the curd in the whey during acid production, decreasing the cook temperature, and eliminating the cheddaring step. These modifications were made in an effort to maintain the moisture levels in the cheese with fat levels below 9%. The decreased cook temperature also facilitated the survival of the starter and adjunct cultures, their proteases and rennet, which may all have an effect on the breakdown of \( \alpha_{s1} \)-casein.

Differences in the softness and textural characteristics of the cheese body manufactured with the adjunct culture were observed. By 14 d after manufacture cheese made with the adjunct culture became sticky and more difficult to shred than the control cheese (low-fat with out adjunct culture). Significant differences in the melting properties of the cheese also suggests differences in the proteolytic breakdown between the control and adjunct-treated cheeses. The increased melt displayed by the adjunct treated cheeses directly after manufacture is a desirable characteristic since Mozzarella cheese typically used on pizza is less than 1 wk old. The cheeses made with the Prt+ Lacd adjunct culture were softer than the control cheese from 1 d after manufacture and continued to increase in softness throughout the storage period, presumably due the greater degree of proteolysis. Softness was particularly apparent when the cheeses were shredded for use on pizza.

The cheeses made with the Prt+ Lacd adjunct culture displayed overall desirable functionality for low-fat Mozzarella cheese. The Prt+ Lacd adjunct culture that was used affected meltability and may possibly be used to decrease the length of storage time necessary to achieve desirable melt and softness in body and texture. Optimizing the adjunct culture inoculum level or selecting other cultures with similar properties may result in additional improvements.

**Moisture Retention Cultures**

In low-fat Mozzarella cheese there is a decreased amount of fat globules causing the casein strands to become more compact as the curd is forming (Oberg, 1993). Increased compaction of the protein network decreases the amount of space available for water, making it difficult to retain moisture in the final cheese product. Lower moisture levels in low-fat Mozzarella cheese results in cheese with a tough rubbery texture, along with poor melting and stretching properties (Mistry, 1993).

Modifications in the make procedures of reduced-fat and low-fat Mozzarella cheese are most commonly used to retain higher moisture levels in the cheese. A wide variety of exocellular polysaccharides (EPS) are produced by lactic acid bacteria, including many of the thermophilic organisms. Cultures that produce these exopolysaccharides have been used to
improve rheological behavior and texture in other fermented dairy products because they can bind free water and slow whey separation (Cerning, 1990; Schellhaass, 1985). The objective of this study was to determine if EPS-producing starter and EPS-producing adjunct cultures could be used to retain more moisture in a low-fat (6%) Mozzarella cheese. Cheeses were made with non-EPS cultures, EPS cultures, and the addition of a mesophilic EPS-producing adjunct culture. In addition, we wanted to see if differences in moisture could affect the melt property of the cheese.

All cheeses were at similar fat levels (6.0-6.4%) that were appropriate for a low fat cheese (Table 2). Protein content of the cheeses were similar, with the higher moisture cheeses having slightly less protein on a wet basis. Cheese moisture varied depending on the type of cultures used to make the cheese. Both the starter culture, and the adjunct culture significantly affected moisture retention in the cheese (Table 2). Using the EPS starter culture increased moisture by 3%. No whey leakage was observed in any of the cheeses over 28 d of storage.

Use of the EPS starter culture significantly increased cheese melt, while the EPS adjunct culture did not. The cheese that melted the most was made using the EPS starter culture, plus the EPS adjunct culture (Figure 2). Cheese made with the EPS starter culture (without the adjunct) had the next highest melt values at all days of storage. Adding the EPS adjunct culture to the control (non-EPS) starter culture did not increase the meltability of the cheese. Storage time significantly affected cheese melting. All cheeses showed an increase in melt between d 1 and d 7, while between d 14 and d 28 there was a decrease in melt.

It is interesting that using an EPS starter culture increased both cheese moisture and cheese melt, while adding an EPS adjunct culture with a non-EPS starter culture had no effect on cheese melt even though cheese moisture was increased. This is probably related to the nature of the exopolysaccharide produced by the different cultures. Some cultures release the exopolysaccharide into the surrounding environment while others may keep it attached to the cell as a capsule. Microscopy shows that the capsule on the EPS starter cultures remains attached to the cell. In general, the higher the cheese moisture the greater the cheese melt (Figure 3). Because the EPS adjunct culture did not influence cheese melting the same as the EPS starter culture, it suggests they have different effects on the cheese microstructure.

Studies of Mozzarella cheese microstructure by Oberg (1993) showed that much of the water in the cheese is contained in columns surrounded by the protein network. The removal of the fat causes these columns to become more narrow, creating less space for water in the cheese curd. In addition to binding free water, the production of EPS material may help to hold the protein network apart producing larger columns that are able to hold more water. Starter cultures producing an exopolysaccharide can be useful in increasing moisture retention in low-fat Mozzarella cheese. A similar effect may be observed in low moisture part skim Mozzarella cheese. Increasing the moisture levels of low-fat Mozzarella cheese can improve cheese melting properties. Exopolysaccharide-producing cultures could be used as a strategy for producing low fat cheeses without the use of expensive fat replacers.

**Development of Alternative Starter Cultures**

Starter cultures for the manufacture of Italian cheeses like Mozzarella or Provolone typically contain *Streptococcus thermophilus* and *Lactobacillus helveticus* or *L. delbruekii* subsp. *bulgaricus*. Explosive growth in the Mozzarella cheese industry over the past 20 years has led to an increased incidence of bacteriophage attack on *S. thermophilus* (Thunell, 1989). Bacteriophages of starter lactobacilli have also been isolated in cheese plants, but these viruses appear far less frequently. For these reasons, one method to control bacteriophage problems in
Mozzarella plants may be to expand the number of phage-unrelated starter cocci available for strain rotation practices. One approach to this objective may be to replace *S. thermophilus* in Italian starter blends with suitable lactic cocci from a different genus or species.

Pediococci are homofermentative lactic acid bacteria which, from an industrial perspective, include species primarily important for meat and vegetable fermentations (Garvie, 1986). These bacteria sometimes dominate populations of nonstarter lactic acid bacteria in ripened cheese (Bhowmilk, 1990), and some strains have even been used as adjunct cultures to improve attributes of Cheddar and Mozzarella cheese. Unfortunately, pediococci typically are unable to ferment lactose, a feature which clearly restricts more widespread application of these bacteria in milk fermentations. As an example, lactose-positive *P. acidilactici* and *P. pentosaceus* may be suitable replacement cocci for *S. thermophilus* in Italian starter blends because these bacteria grow at 45°C and each has a long history of safe consumption in human food (Altay, 1994; Kim, 1992). The development of gene transfer systems for pediococci in recent years (Altay, 1994) has provided new opportunities to investigate this and other applications for pediococci in milk fermentation.

This study constructed Lac⁺ *P. acidilactici* and *P. pentosaceus* strains by transformation with a naturally-occurring 35 kilobase (kb) *Lactococcus lactis* lactose plasmid, pPN-1. Lactose-positive transformants were investigated for stability of the Lac⁺ phenotype, the ability to acidify milk, and other important dairy starter properties. Results indicated Lac⁺ *Pediococcus* spp. have good potential as replacement cocci for *S. thermophilus* in Italian starter blends.

Plasmid pPN-1 was obtained from *Lactococcus lactis* C2 by transduction to the plasmid-free bacterium *Lactococcus lactis* LM2302 (McKay, 1973). Lysates of the transductant *Lactococcus lactis* PN-1 were analyzed by agarose gel electrophoresis and found to contain a single 35 kb plasmid, designated pPN-1, which indicated approximately 20 kb of the original C2 lactose (lac) plasmid had been lost (McKay, 1982).

Electroporation was used to transform the parental strains with intact pPN-1, then Lac⁺ transformants isolated from each strain were examined for pPN-1 uptake. Lysates of Lac⁺ *P. acidilactici* ATCC 12697 and *P. pentosaceus* ATCC 25745 transformants, designated *P. acidilactici* SAL and *P. pentosaceus* SPL-2, contained a new plasmid molecule which co-migrated through 0.6% agarose gels with CsCl₂-purified pPN-1.

Lac⁺ expression in *P. acidilactici* SAL and *P. pentosaceus* SPL-2 was evaluated in MRS-L broth (containing only lactose as a sugar source). Both transformants grew much better than respective wild-type strains in MRS-L broth (Figure 4). As expected, improved growth by Lac⁺ transformants in MRS-L was accompanied by increased acid production (Figure 5). After nine sequential transfers (> 175 generations) in MRS-L broth, approximately 95% of *P. acidilactici* SAL CFU remained Lac⁺ (Figure 6). In contrast, growth for a similar period in MRS-G reduced the Lac⁺ population to less than 20%. Because Mozzarella starter blends frequently include *L. helveticus*, Lac⁺ transformants were tested for production of compounds which inhibited growth of *L. helveticus* LH100. Agar overlay tests indicated *L. helveticus* LH100 growth was not inhibited by any of the *Pediococcus* spp.

Lac⁺ transformants alone showed weak ability to acidify non fat dry milk, but 1:1 combinations (1% total inoculum) with LH100 produced final milk pH values notably lower than those obtained with SAL, SPL-2, or LH100 pure cultures (Figure 7). Acid production by 1% *Pediococcus*/LH100 strain combinations was also slower than that noted with the positive control, a 1% *S. thermophilus* TA061/LH100 blend (1:1). These differences were substantially reduced, however, when higher numbers of pediococci and lactobacilli (1% versus 0.5% each) were added to the milk.

*P. acidilactici* SAL or *P. pentosaceus* SPL-2 were tested for susceptibility to bacteriophages
in 835 separate whey samples collected from North American cheese producers. At least 440 of these samples contained more than $10^5$ PFU per ml, but bacteriophages able to attack SAL or SPL-2 were not detected.

Those results demonstrated Lac$^+$ Pediococcus spp. transformants SAL and SPL-2 acquired pPN-1 and were able to express lac genes encoded by that molecule. Experiments to evaluate pPN-1 stability in SAL and SPL-2 indicated a high proportion of transformants retained the plasmid when cells were grown on lactose. Although pediococci rapidly became Lac$^-$ in glucose broth, Lac$^+$ stability should not be a problem in industry because dairy starter cultures are grown in milk or whey based media.

Restriction endonuclease analysis of pPN-1 showed the lac operon was intact, but deletion events had removed some of the DNA needed for expression of the lactococcal cell wall proteinase. Those results demonstrated weak or absent caseinolytic activity was the primary basis for slow milk coagulation and acidification by Lac$^+$ Pediococcus spp. transformants. While it may be feasible to identify or construct proteinase-positive pediococci, low caseinolytic activity in these bacteria should not preclude their use as substitute cultures for S. thermophilus, because the latter species also is typified by relatively weak proteolytic activity.

In Italian starter blends, growth of S. thermophilus also generates metabolites which promote increased numbers of lactobacilli. Symbiotic growth between starter cocci and rods is a characteristic and desirable property of these blends because it provides a synergistic increase in lactate production. Synergistic lactate production also occurred when Lac$^+$ pediococci were paired with L. helveticus LH100.

As expected, bacteriophage sensitivity tests showed P. acidilactici SAL and P. pentosaceus SPL-2 were resistant to bacteriophages in whey samples obtained from North American cheese plants which used Lactococcus lactis, S. thermophilus, and Lactobacillus spp. starters. In summary, Lac$^+$ pediococci have good potential as replacement cocci for S. thermophilus in Italian starter blends, and this application may facilitate development of new strain rotation schemes to combat S. thermophilus bacteriophage problems in Mozzarella cheese plants.

**Projections For The Future**

Phage problems will continue to increase and demand new and innovative strategies. Phages will develop for the Lactobacilli and eventually become as serious as the coccal phage. The use of new bacterial genera and species, along with the development of phage resistant strains will be needed. Browning during cooking and blister formation will need to be addressed. The use of less proteolytic cultures to inhibit the formation of amines which react with the galactose will need to be developed. As the market increases for reduced-fat, low-fat, and non-fat Mozzarella cheese new cultures will have to be developed to address the functionality problem associated with these cheeses. Cultures that help retain more moisture, selectively breakdown certain protein fractions, and produce compounds that mimic the role of milk fat will be developed.

**Acknowledgments**

We thank the National Dairy Promotion and Research Board, the Utah State University Agricultural Experiment Station, and the Western Center for Dairy Protein Research and Technology for funding this research. We also thank Donald V. Sisson for his assistance with statistical analysis and Gary Pedersen for gel photography.
Table 1. Mean percentages of moisture, fat, protein and make time in (h) for low fat Mozzarella made with different levels of adjunct culture.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture</th>
<th>Fat</th>
<th>Protein</th>
<th>Make Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M SEM</td>
<td>M SEM</td>
<td>M SEM</td>
<td>M SEM</td>
</tr>
<tr>
<td>control</td>
<td>59.6 0.47</td>
<td>7.0 0.29</td>
<td>25.2 0.28</td>
<td>2.5 0.17</td>
</tr>
<tr>
<td>0.25% adjunct</td>
<td>60.8 1.4</td>
<td>6.4 0.08</td>
<td>24.1 0.78</td>
<td>2.3 0.15</td>
</tr>
<tr>
<td>0.50% adjunct</td>
<td>62.8 0.61</td>
<td>6.2 0.17</td>
<td>22.8 0.67</td>
<td>2.3 0.15</td>
</tr>
<tr>
<td>1.0% adjunct</td>
<td>61.7 1.3</td>
<td>6.2 0.14</td>
<td>23.4 1.4</td>
<td>2.4 0.16</td>
</tr>
</tbody>
</table>

Table 2. Composition of low-fat Mozzarella cheese made using exopolysaccharide (EPS) cultures compared to control (non-EPS) cultures.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Starter</th>
<th>Adjunct</th>
<th>% Moisture</th>
<th>% Fat</th>
<th>% Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X SEM</td>
<td>X SEM</td>
<td>X SEM</td>
</tr>
<tr>
<td>1</td>
<td>non-EPS</td>
<td>-</td>
<td>58.2 0.50</td>
<td>6.3 0.33</td>
<td>26.3 1.70</td>
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<tr>
<td>2</td>
<td>EPS</td>
<td>-</td>
<td>61.0 0.47</td>
<td>6.2 0.20</td>
<td>25.9 0.63</td>
</tr>
<tr>
<td>3</td>
<td>non-EPS</td>
<td>EPS</td>
<td>60.9 0.12</td>
<td>6.2 0.20</td>
<td>26.5 0.60</td>
</tr>
<tr>
<td>4</td>
<td>EPS</td>
<td>EPS</td>
<td>62.2 0.38</td>
<td>6.4 0.20</td>
<td>24.7 0.63</td>
</tr>
</tbody>
</table>

Figure 1. Mean (±SEM) melt measurements (centimeters) of low fat Mozzarella cheese made without the addition of the Prt+ Lac^d adjunct culture, solid bar, with .25% inoculum of the Prt+ Lac^d adjunct culture, diagonal striped bar, .50% inoculum of adjunct, open bar and 1.0% inoculum of adjunct, diagonal dotted bar.
Figure 2. Effect of culture type on meltability of low-fat Mozzarella cheese during 28 d storage at 4°C. Cultures used were non-EPS starter, open bar; EPS starter, light bar; non-EPS starter plus EPS adjunct, dark bar; EPS starter plus EPS adjunct, striped bar. Error bars represent individual standard errors for each mean.

Figure 3. Correlation between cheese moisture and cheese melt on d 1 for three replicates of low-fat Mozzarella cheese.
Figure 4. Growth in MRS-L by lactose-positive (Lac\(^+\)) Pediococcus spp. transformants and wild-type strains. Open symbols represent wild-type *P. acidilactici* ATCC 12697 (○) and *P. pentosaceus* ATCC 25745 (●), closed symbols denote the respective Lac\(^+\) transformants *P. acidilactici* SAL and *P. pentosaceus* SPL-2.

![Growth curve](image)

Figure 5. Acid production in MRS-L by lactose-positive (Lac\(^+\)) *Pediococcus* spp. transformants and wild-type strains. Open symbols represent wild-type *P. acidilactici* ATCC 12697 (○) and *P. pentosaceus* ATCC 25745 (●), closed symbols denote the respective Lac\(^+\) transformants *P. acidilactici* SAL and *P. pentosaceus* SPL-2.

![Acid production curve](image)
Figure 6. Percentage of lactose-positive *P. Acidilactici* SAL (∅, u) and *P. pentosaceus* SPL-2 (∅, n) after serial transfer at 37°C in MRS broth that contained lactose (∅, ∅) or glucose (u, n) as the carbohydrate source.

Figure 7. Acid production by various bacteria in 9% reconstituted skim milk (RSM). Graph A shows pH changes in RSM inoculated with 1% *Pediococcus acidilactici* SAL (u), 1% *Lactobacillus helveticus* LH100 (ii), 0.5% SAL plus 0.5% LH100 (o), 1% SAL plus 1% LH100 (∆), and 0.5% *Streptococcus thermophilus* TA061 plus 0.5% LH100 (∅). Graph B shows similar data for RSM inoculated with 1% *Pediococcus pentosaceus* SPL-2 (u), 1% *Lactobacillus helveticus* LH100 (ii), 0.5% SPL-2 plus 0.5% LH100 (o), 1% SPL-2 plus 1% LH100 (∆), and 0.5% *Streptococcus thermophilus* TA061 plus 0.5% LH100 (∅).
REFERENCES


HOW THE MICROSTRUCTURE OF MOZZARELLA CHEESE IS RELATED TO FUNCTIONAL PROPERTIES

Donald J. McMahon, Ph.D., Department of Nutrition & Food Sciences, Utah State University, Logan, UT

It is well known that the stretch and melt characteristics of Mozzarella cheese are important aspects of its appeal to consumers. Although there have been a number of recent investigations of factors that affect these properties, our knowledge of why Mozzarella cheese stretches and melts is still very limited. This limitation in our knowledge of how to control functional properties has meant that it has been difficult to apply a scientific method to the development of new cheeses, especially as new markets have opened for reduced fat and fat-free cheeses.

Many researchers have turned to tools such as electron microscopy as a way of obtaining information about the internal structure of cheese that may be useful in understanding chemical and functional properties. While electron microscopes allow objects to be examined under magnifications from 150 to 150,000 times, such observations must be interpreted with caution. Not only the researcher, but the people who read published articles that contain micrographs, should be aware that before a cheese sample can be examined in the microscope it must undergo considerable sample preparation to allow such high magnifications be reached. Sometimes, objects may appear different in micrographs than they do in real life. Such “artifacts” should always be reported when micrographs are being interpreted.

There are two types of electron microscopes: transmission and scanning. As their names suggest, each looks at objects in different ways. In transmission electron microscopy (TEM) the electrons are passed through a solid object and areas that are “electron dense” appear in the micrograph as dark areas (Figure 1, 2). When the electrons pass freely through the sample (without being intercepted by dense atoms in the sample) such areas are observed as being white. To prepare cheese for TEM a time-consuming procedure is usually followed (1). The proteins must first be fixed, with an agent such as glutaraldehyde or formaldehyde, so that they remain in their original positions. They are then treated with osmium which fixes the fat (however, saturated fats are not fixed) and stains the proteins. Then the samples are dehydrated by transferring them into ethanol, followed by an organic solvent such as propylene oxide. Then, because only a very thin section of material can be analyzed by TEM, the sample is embedded in a resin and cut into sections 50 to 100 nm thick. When viewed by TEM, the sample appears as a cross section (Figure 3) although it must be realized that it is really a composite picture comprising all parts of the sample in the section that scatter electrons and preventing them being transmitted through the sample.

When milk is renneted, the casein micelles in milk become unstable and aggregate. Curd is formed when this aggregation process reaches a time at which a gel network is formed. As will be discussed later, it is the continuation of this protein aggregation process that drives the observed changes in cheese microstructure. After the curd has been cut to stimulate syneresis and whey expulsion, the individual micelles continue to fuse together. Whereas, they started out as individual proteins strands (4), by the time the curd is ready to have the whey drained, the micelles are observed by TEM (Figure 1) to have merged into thicker strands as previously shown (8) with fat globules and whey filling up the spaces between them. After the whey is drained, these strands become thicker and whey and bacteria become trapped between them. The cocci in the cheese curd are most often observed as diplococci and Figure 3 shows a “cross section” through a bacterium at high magnification.
It can also be seen that no individual micelles can be observed but rather the proteins appear relatively homogeneous. High magnifications have commonly been obtained with TEM but only recently (6) has high resolution high magnification images been obtained by scanning electron microscopy (SEM).

When SEM is used to observe microstructure of cheese, rather than “looking” through the sample, a stream of electrons is fired at the surface of the sample and an image obtained is based on electrons that are scattered back from the surface. After the cheese curd has been stretched in hot water, it can be seen that the proteins have been drawn into large (5-20 mm) fibres that are oriented in the direction that the cheese was stretched. (Figure 4). In such micrographs it appears that there are voids between the protein fibres but this is an artifact caused by sample preparation.

While preparation of cheese samples for SEM is not as tedious (nor does it require the meticulous skills needed to make thin sections) as for TEM, there are still a number of steps that must be followed. The proteins are fixed (and so they shouldn’t change in appearance) but usually the fat is washed out of the sample during dehydration and other procedures that are used to make the cheese more conductive by impregnating it with heavy metals. This allows higher resolution to be obtained (6). If the surface of a sample cut from a block of cheese is examined by SEM, it will typically have a “smeared” appearance as seen in Figure 4. This limits the amount of information gained and so SEM samples are usually frozen and fractured so as to present a “clean” surface and to show the internal structure of the cheese (Figure 5). After cooking and stretching, the channels between the protein fibers appear smooth and empty. This is because during sample preparation the fat and most of the bacteria are washed away from the fractured surface. In the actual cheese, these channels are filled with close-packed fat globules.

When the cheese has been cooled, and especially after overnight refrigeration, the surfaces of the protein fibers are seen to be dimpled (Figure 6). Previously (Figure 5) the cheese had been hot when sampled and so the fat was liquid, but when it cooled and solidified, there was pressure being exerted onto the fat globules by the protein matrix. Some bacteria can be seen remaining in the serum channels as well as being embedded in the protein matrix. Those that are embedded in the protein matrix (Figure 6) are unable to grow as there is no available moisture. It is only within the serum channels that “free” water exists and where bacteria can grow. By this stage of cheese manufacture the cocci dominate the microflora independent of the initial rod:cocci ratio. As already mentioned, most of the cocci are usually observed in the diplococci form and very few rods are observed. The membranous material observed in the channels is another artifact and is the remnant of proteins in the fat globule membranes.

It is the “free” water in the serum channels that is measured as “expressed moisture” by centrifuging. Initially, about 30% of the total cheese moisture is expressible (2). However, this decreases to 0% after two weeks of refrigerated storage of the cheese. At the same time, the functional properties of the cheese are changing and Mozzarella cheese is normally stored for one to two weeks to develop good melting properties. This has been attributed to the effect of proteolysis on the cheese matrix. What we observe with SEM after two weeks storage of low moisture part skim mozzarella cheese (Figures 7 and 8) is that the dimpled appearance of the serum channels has become more pronounced. Instead of shallow indentations (as in Figure 6) the indentations are almost complete hemispheres made up on material from the protein matrix. In Figure 8 (upper right) can be seen how close packed the fat globules and bacteria were and how far the protein matrix has penetrated.
Some researchers (3, 9) have also observed this change in appearance of fat globules (or more precisely the voids left when fat was extracted) but attributed it to some process of aggregation of fat globules. The micrographs (Figures 6, 7, 8, 9, and 10) clearly show that there has been no change in the distribution of the fat globules but rather it is all a function of the continuing change in protein structure. These changes were initiated when renneted was added to the milk, further enhanced when the curd was fermented and the pH lowered, and the cheese proteins were still changing when the cheese was brined and packaged.

As shown in Figure 2 and Figure 6, fat in cheese plays a role of preventing the protein from completely coalescing. In the cheese curd, wherever there is a fat globule it prevents the chains of para-casein micelles from joining together into thicker strands. At this stage the fat is evenly distributed throughout the curd (8) but when the proteins are heated and mechanically forced to flow, the fat globules are pushed together. At the typical pH of Mozzarella cheese the para-casein proteins are "sticky" in nature and have a tendency, when in contact, to fuse together. Much of this fusion comes about through hydrophobic interactions and is a continuation of the aggregation process that started when the milk was renneted. This process of protein fusion continues until the proteins are physically interrupted by other components of the cheese that do not have the same hydrophobic properties. In Mozzarella cheese, this role is played by the fat globules.

The aqueous surface of the fat globule membrane is very hydrophilic and if it is intact there is no interaction between the fat globules and the para-casein. If the fat has been homogenized, then it will interact because of the presence of casein micelles that become adsorbed to the fat surface during homogenization. When the fat globules are as packed as closely as they can, they exert a pressure back onto the protein strands preventing them from coming together. The protein is thus formed into fibers that are separated by the fat/serum channels. After twenty-one days of storage, the protein matrix has extensively flowed into the serum channels so that the fat globules are completely surrounded (Figures 9 and 10).

A problem with removing fat from cheese is that there is no longer anything to prevent the protein strands from completely coalescing. Thus, insufficient water is retained in the cheese and the cheese does not have the desired melt properties. Furthermore, the protein still undergoes the same changes during storage and would fill what ever channels are present, compounding the melt problems. It would then need to be aged for a longer time to allow proteolysis to alleviate the problem of protein flowability so that the cheese melts to an acceptable level. Some strategies used for maintaining moisture in reduced fat cheeses include using a higher processing temperature, pre-acidifying the milk to pH 6, cutting with larger knifes, using less agitation and cooking to a lower temperature (7).

When the cheese is heated and melted a different microstructure is observed (Figure 11). Instead of there being fibers, the protein matrix has flowed together into a relatively homogeneous mass with many small and large pockets which hold the fat globules. Melting and elasticity of mozzarella cheese are critical functional properties (5) for its use in cooking (especially on pizzas). A key component of achieving good melting is to have adequate moisture content in the cheese. Understanding the way in which the cheese microstructure is formed allows strategies to be developed for controlling its functional properties.
Figure 1. Mozzarella cheese curd prior to draining whey.

Figure 2. Mozzarella cheese curd after dry stirring.
Figure 3. Mozzarella cheese curd after dry stirring at high magnification showing loss of micelle identity and cross section of bacterium.

Figure 4. Surface of mozzarella cheese after hand stretching.
Figure 5. Mozzarella cheese after mechanical stretching.

Figure 6. Mozzarella cheese after brining and overnight cooling.
Figure 7. Mozzarella cheese after 14 days refrigerated storage (low magnification).

Figure 8. Mozzarella cheese after 14 days refrigerated storage (high magnification).
Figure 9. Mozzarella cheese after 21 days refrigerated storage (low magnification).

Figure 10. Mozzarella cheese after 21 days refrigerated storage (high magnification).
References


Introduction

Recently, research aimed at enumerating and cataloging traditional Italian dairy products reported that more than 400 different cheeses are produced in Italy. Although these cheeses are produced from different milks (cow, water buffalo, goat and sheep) and through the use of very different technological conditions, they are all generally produced from raw milk and without the use of industrial starter cultures of lactic acid bacteria (LAB). Moreover, fermentation and ripening of traditional Italian cheeses are not the result of a single strain; rather, they come from the interaction of different strains of lactic acid bacteria and enterococci present in natural whey and milk cultures or in raw milk. Therefore, the quality of these products, is strictly dependent on the microbial associations responsible for the fermentation. For these reasons, the "biodiversity" of LAB involved in cheese production is considered a fundamental factor for the maintenance of the characteristic features and quality of traditional dairy products.

The purpose of the work presented here was the isolation of new starter cultures composed of wild LAB and enterococci with unique features such as proteolytic activity, acid production, aroma and bacteriocin, and the study of strain-species composition in the microbial associations of LAB and enterococci found in natural cultures for cheese production. To obtain "wild" LAB possessing the desired technological properties, strains were isolated from high quality Fontina, Toma, Grana and Mozzarella produced using traditional techniques. All the samples were collected from cheese produced without the addition of commercial starter cultures. The choice of this environment was dictated by the absence in these cheese factories of selected industrial starters.

The main objectives of our study on LAB biodiversity from traditional Italian cheese were:

- Isolation and characterization of new strains of LAB possessing interesting technological properties for the dairy industry.
- Examination of LAB biodiversity within natural cultures.
- Study of the complex association of natural dairy cultures.
- Isolation of strains from natural environments (traditional dairy productions) and their maintenance in culture collection.
- Design of starter cultures composed of strains isolated from this project.

Strains of LAB and enterococci were isolated from the following traditional dairy products:

- Fontina: a cheese from Valle d'Aosta in the alpine region of Italy, produced without the use of any starter cultures.
- Grana cheese
- Mozzarella
- Toma: very similar to Fontina, produced on the Alps from partially skimmed milk without starter cultures.
Taxonomic Identification

The taxonomic identification of bacteria isolated from the natural microflora involved in cheese fermentation has been limited by the complexity of the bacterial associations. Since these organisms often have similar nutritional and environmental growth requirements, it is often difficult to distinguish them using physiological tests and selective media. Moreover, the application of genetic analyses, such as the DNA-DNA hybridization, for taxonomic characterization of a wide number of strains has been hampered by the time required to process each sample.

Recently the use of new molecular techniques, such as rRNA-targeted oligonucleotide probes, have been proposed for the taxonomic identification of LAB strains. The oligonucleotide probes are based on conserved domains within the genes encoding 16S and 23S rRNA, that are widespread in many Eubacteria, as well as variable regions unique to individual species. Several DNA sequences were analyzed to identify the targeted domains for the protocols of colony hybridization that are currently available for LAB. In addition to DNA hybridization techniques, PCR (Polymerase Chain Reaction) offered new opportunities for the characterization of bacterial species. Specific segments of the rRNA can be used as primers for amplification in PCR experiments.

The following protocols for taxonomic identification were developed and applied in this research:

- colony hybridization with the RNA targeted oligonucleotides.
- amplification of the 16s and 23s genes, by using sets of primers derived from the conserved regions, southern blotting and hybridization with the species specific probes.
- "species specific amplification," using one primer derived from the conserved area and the other from the species specific region.
- amplification of the specific domains of rRNA and sequence analysis.

The strains isolated from Fontina, Grana, Mozzarella and Toma were first characterized by means of traditional physiological tests, then further analyzed using molecular techniques for taxonomical identification. This approach allowed the taxonomical identification of the cheese isolates and the characterization of species composition within the natural cultures involved in cheese fermentation.

When the data from the physiologic and the genetic characterizations of lactic cocci isolated from Fontina, Toma and Mozzarella were compared, a discrepancy was observed. In general, the results showed that the number of enterococci was much lower using molecular taxonomic methods as compared to the standard physiological tests. Strains were isolated from these high quality cheeses that were genetically (23S sequence) characterized as Streptococcus thermophilus, but exhibited physiological properties similar to enterococci (for example: salt resistance, growth at 10°C, or growth at pH 9.5). These results demonstrate that within the complex natural microflora found in traditional cheese production, there exist atypical strains with properties ideally suited for technological purposes that are genetically distinguished as one species, but exhibit physiological features common to two different bacterial groups.
Strain Typing by PCR

The fundamental role of starter bacteria in the natural culture for cheese production has long been studied and clearly demonstrated. However, very little information on the microflora strain composition is available. Furthermore, the growth dynamics of these strains during the different phases of cheese fermentation are still unknown. Although several authors have speculated on the complexity of natural dairy fermentations, these studies have been hampered by the lack of suitable methodology for LAB strain identification and typing. Several techniques for bacterial strain typing have been described that are based on digestion of genomic DNA with restriction enzymes with analysis using conventional and pulsed field gel electrophoresis. A major limitation of these systems towards the study of complex microbial ecosystems is the low number of samples that can be analyzed. Recently, PCR protocols have been developed for strain typing. The PCR method is based on the generation of randomly amplified polymorphic DNA (RAPD) fragments from the bacterial chromosome. The RAPD fragments are then analyzed by agarose gel electrophoresis resulting in a unique pattern or strain fingerprint. Our laboratory has recently developed a RAPD technique specific for LAB and enterococci isolated from dairy products, vegetable fermentations and intestinal microflora. This protocol enables us to generate RAPD fingerprints from a low amount of bacterial cells and to process up to 50 different samples in one day. We have used this technique to characterize strains from Fontina and Toma during the initial days of cheese ripening. By comparing the RAPD fingerprint against a database containing the physiological and genetic characteristics of these isolates, we were able to determine the re-isolation frequency of a given strain during the different steps of cheese production. This type of analysis creates a map of the strain/species composition of natural cultures in cheese production.

The RAPD strain typing method also enabled us to study the microflora strain composition of whey cultures used for Grana cheese production; specifically, the evaluation of individual Lactobacillus strain growth dynamics in this natural fermentation. In order to determine the strain composition of the whey starter culture, bacteria were isolated at different stages of the fermentation. The RAPD profiles demonstrated that the microflora is composed primarily by a few number of thermophilic and acidophilic lactobacilli strains. Varying the environmental conditions (pH and temperature) caused changes in the pool of dominant strains that were observed. These results have highlighted some aspects regarding the lactic and enterococcal microflora of traditional cheese:

A The number of strains derived from natural dairy fermentation is less than the number of isolates. By comparing the RAPD fragments on gel electrophoresis, it is possible to observe that different colonies isolated from the same cheese production are replicas of the same strains. This means that in natural fermentations there are a few "dominant" strains that drive the cheese fermentation.

B RAPD-PCR is a good monitoring technique. It is possible to define the "dominant" strains and observe the dynamics of growth and colonization during the different steps of cheese production.

C Environmental changes cause variations in the ratio among the bacteria composing the pool of dominant strains.
Conclusions

Natural starter cultures play a fundamental role in the production of Italian traditional cheese. According to cheese makers and experimental observations, the substitution of natural cultures with industrial starters result in a decrease in the organoleptic characteristics of these products. Our research on the natural microflora responsible for the fermentation of traditional Italian cheese using molecular techniques for bacterial characterization has highlighted several topics:

- The strains isolated from traditional Italian cheese show unique physiological and technological properties.
- Within the natural microflora there is elevates biodiversity. The strains isolated from natural cultures are often “atypical”. For example, atypical strains of *S. thermophilus* harbouring some physiological characteristics of enterococci (such as the salt resistance or the growth at low temperatures) were found in several Italian traditional cheeses.
- Enterococci have a relevant role in the acidification and ripening process of cheese such as Fontina and Toma produced without the addition of starter cultures. The role of enterococci was also observed in several cheeses produced from raw milk using whey or milk natural cultures (e.g. traditional Mozzarella cheese).
- The conditions imposed by cheese making technology has led to the selection of LAB strains particularly adapted to a whey-cheese environment. Closely related strains belonging to the same species but isolated from different cheese fermentation exhibit very different physiological features.
- The fermentation of natural dairy cultures is driven by a limited number of strains. The use of the RAPD for strain typing allows one to monitor the growth dynamics of these dominant strains. Varying the environmental conditions (pH, temperature, a_w, salt) changes the pool of dominant strains that can be observed.
- The results obtained from the application of molecular techniques to strain typing enables the creation of strain progression maps involved in natural cultures. It has also allowed the design of starters for use in industrial processes that mimic the activity of natural cultures in traditional cheese making.

Notes

In addition to this study, we have also applied genetic engineering techniques to obtain LAB with new and improved features for dairy fermentation. One example is the heterologous gene cloning and expression of the lipase gene from *Staphylococcus hyicus* into strains of *Lactobacillus*. The activity of these modified lactobacilli were evaluated in a cheese model. “Gnotoxicen” cheese (produced under sterile conditions to avoid microbial contamination) was produced using the lipase expressing lactobacilli. The fatty acid content was followed during a three month ripening period. The amount of released long chain fatty acid was found to be two times greater than the cheese produced using the parental (non-genetically modified) *Lactobacillus* strain controls.
EXPLORING FIELD PROBLEMS AND SOLUTIONS FOR ITALIAN AND SPECIALTY CHEESE

Mark E. Johnson, Ph.D., Wisconsin Center for Dairy Research, Madison, WI

In the cheese monograph "American Cheese Varieties" by Reinbold and Wilson published in 1965, the authors state that "most defects in American variety cheeses may be traced to improper moisture content, acidity and the growth of undesirable microorganisms." They go on to say that those general factors may be related to poor workmanship, milk quality, starter, and sanitation. Of course these conclusions apply to all cheeses and things haven't changed. The same problems keep rearing their ugly heads and the same solutions are reiterated. However, the cheesemaker must face these problems within the confines demanded by new equipment, new starters, new technologies, greater competition and increased demands for higher yields, reproducible quality and longer shelf-life under conditions not conducive for maintaining cheese quality.

Each year we ask participants in the Wisconsin Cheese Technology Short Course what cheese defects they would like us to specifically address. Each year the majority of the defects are the same as in previous years. The solutions to the problems may, however, change with the technology with which the cheesemakers have to work. This presentation will address the most common cause of cheese problems and the solutions to them.

Cheese Structure

Since the most mentioned defects concern the body of the cheese, it is important that the structure of the cheese is discussed. During coagulation, the caseins in milk form a continuous network that is punctuated with fat and interlaced with serum. The firmness or hardness of the cheese is due to the protein concentration and structural integrity. Moisture and fat, in essence, dilute the protein and "lubricate" the protein allowing the protein molecules to flow or move more easily in relation to each other. Indeed, higher moisture/higher fat cheeses tend to exhibit the defects of body, and functional properties of cheese that are attributable to characteristics of the protein i.e. soft body, free oil release, and excessive melt. Therefore the following discussion must be taken in context with both cheese moisture and fat level as they relate to the level of protein.

The nature of the protein network is the key to unlocking the mysteries of body defects (or desired attributes) in cheese. The protein network is comprised of three fractions: α-casein, β-casein, and para k-casein. Unfortunately it is not known how they are arranged or interact with one another. Caseins are amphoteric, that is they react with either an acid or a base. Therefore pH is a determining factor in how casein molecules interact in cheese. As the pH of cheese decreases from pH 5.6 to 5.3, the protein network becomes less rigid and is more flexible (bends but does not break). In a certain pH range (ca. 5.3-5.2), round eyes are formed when gas exerts pressure on the casein network. Without the protein flexibility (certain degree of resistance or elasticity), the network would either resist the pressure or succumb to it resulting in splits rather than round eyes. When heated, cheese melts better at a lower pH than at a higher pH. The stretch and pliability of Mozzarella is also governed by pH. Optimum stretch occurs around pH 5.2, and the pliability of curd improves as the pH is lowered. However at pH ca. 5.0-4.9, the curd becomes brittle and breaks rather than stretches. In direct acidified cheeses the pH at which cheese stretches (5.6) and melts may be higher, but the influence of pH over the properties of the protein is still valid. Studies on direct acidified cheeses (Keller et.al. 1974) indicate that the level of calcium bound to protein has a major role.
in governing the interaction of the casein molecules to each other. Indeed, as the pH is lowered, more calcium is dissociated from the casein. This may offer an explanation as to the impact pH has on casein interaction. However, since the structure, interrelatedness of the caseins, and other factors that influence the protein network are not known with certainty, the explanation as to the role of calcium remains an enigma.

Caseins are large molecular weight molecules whose interaction with other proteins is determined by its integrity. If the protein is broken apart into smaller pieces (called proteolysis), major changes are brought about in the protein network. Proteolysis is due to the activity of enzymes such as the coagulant, plasmin, and proteolytic activity of microorganisms. Since we do not understand the structure of the protein network (interaction of water, protein and possibly calcium), nor know how the casein molecules are interrelated in cheese, it is sometimes difficult to attribute the change in functional properties of cheeses solely to composition, proteolysis, pH and/or calcium without considering the interactions between them. Studies by Bogenrief and Olson (1995) indicate that the melt of cheese, although chiefly influenced by pH and calcium levels, may also be attributable to proteolysis of \( \beta \)-casein by the milk clotting enzyme from \textit{Cryphonectria (Endothia) parasitica}. Enhanced hydrolysis of \( \beta \)-casein in Mozzarella with \textit{C. Parasitica} enzyme was associated with increased meltability (Yun et al. 1993. a,b).

Specific Defects

Based on our yearly questionnaire, the most common defect in cheese, regardless of variety, is soft, pasty, mushy body. This manifests itself in cheese as poor shreddability, poor sliceability and increased fusion of shreds as the product is aged. This defect is due to proteolysis and is especially acute in cheeses with low pH, and high moisture, or low protein concentration, i.e. a combination of high fat and high moisture. The remedy may be obvious, but may also be a financial catastrophe; lower the moisture but lose cheese yield. Unfortunately, proteolysis in cheese is inevitable and the cheese will become smoother (more pasty) with age; however, the process can somewhat be slowed. Since the problem is enzymatic, factors controlling enzyme activity or microbial growth (especially non-starter Lactobacilli such as \textit{L. casei}) may offer the best alternative solution. Low temperature (40°F) storage is one such method. Temperature abuse in retail outlets is a major factor contributing to the problem especially since the cheese already teeters at the brink. High storage temperature coupled with low turnover pushes the cheese beyond its limitations. In Mozzarella, higher mixer/molder water temperature and longer retention times can be used to inactivate the coagulant and lower the number of microorganisms contributing to proteolysis. In non pasta filata cheeses, use of less coagulant or a less proteolytic one may be a solution. Higher pH cheeses (above ca. 5.3), may retard the softening effect since these cheeses are firmer to begin with. However, these cheeses may have lower meltability. Use of salt sensitive cultures may inhibit acid development. Higher salt levels (higher salt to moisture ratio) may impede proteolysis (microbial growth) but may negatively affect flavor.

High protein cheeses, especially those with low moisture to protein ratios, tend to become brittle rather than pasty, yet the cheese still becomes smoother. During proteolysis, for each bond that is broken in the protein molecule, water becomes bound at the site. Water is used in the proteolytic process but more is bound by the ionic groups formed by proteolysis. The amount of moisture taken up through the sites of proteolysis decreases the amount of water imbibed by the protein network. In essence, the "state" or location of the water in relation to the protein has now changed. Water that might have helped soften or
plasticize the cheese becomes bound rather than held like water in a sponge, and the cheese behaves as if it were drier i.e., shorter and more crumbly. Through proteolysis the state of the protein also changes. The protein (now protein fragments) that once held the water may now actually become dissolved in it, contributing to the overall effect of a smoother, pasty body. The more moisture, the softer, more pasty the cheese.

Other common defects that cross varietal boundaries include discoloration, manifested as mottling or bleaching of the naturally occurring or added colorants of the cheese, and acid flavor. These are all attributable to low pH. The question is how the cheese becomes acid. It is not due necessarily to the amount of acid in the cheese but to the pH of the cheese. While it may seem that pH and the amount of acid are the same thing, in chemical terms, they are not. Acid is developed through the fermentation of lactose (and galactose). For each molecule of lactic acid produced there is also an (H+) hydrogen ion formed. pH is the measurement of the concentration (activity) of this free ion. Bound H+ is not measured. Phosphates, amino acids (proteins), and citric acid can, and will bind H+ at a particular pH. These substances can also release the bound H+ if a base is subsequently added to the solution. They are called buffers because they can hold the pH constant by either releasing or binding H+.

In cheese, lactose is fermented to lactic acid, with the concomitant release of H+ ions which are bound by the buffer system (mainly phosphate and amino acids-protein). As more H+ ions are formed, the buffer system is overwhelmed and the pH decreases. It is, therefore, the amount of the buffer system and the amount of acid produced that determines the extent and rate at which the pH drops. If there were no buffer in cheese, the pH would fall rapidly and to a lower point, with much less acid produced. Conversely, the more buffer in cheese the more acid can be produced without the cheese necessarily developing a low pH. Therefore in an acid, or low pH cheese, it follows that the amount of acid produced overwhelms the buffer system. This scenario is common in high moisture cheese in which the lactose has not been diluted or removed prior to pressing. It is also common in cheeses that are drained at low pH (6.1 or less), since some of the buffer is dissolved from the protein and is lost into the whey. The buffer capacity remaining is continually consumed. Some of the incidences of poor stretch but increased melt in Mozzarella can be attributed to the loss of buffer capacity of the cheese, and continued sugar fermentation, leading to a low pH. As the cheese ages, the potential buffer capacity of the protein will be expressed, leading to an increase in pH. The increase in buffering is due to the release of free amino groups or amino acids as the result of proteolysis. If there were no continued sugar fermentation in cheese the pH of the cheese would rise as the buffer system, in time, absorbed the H+ ions present. This explains why bleached areas within a cheese may disappear with time and the acid flavor is mellowed as the cheese ages.

To prevent acid cheeses (cheeses too low in pH), the most commonly applied measure is to either dilute the whey or rinse the curd prior to salting. Another method, although not always advisable, is to slow acid development by the starter. This can be accomplished by increasing the salt level in the cheese to inhibitory levels for culture metabolism or the use of more salt sensitive starters. The inherent danger is that the pH will not drop far enough after salting, the curds may fail to fuse, the cheese may be tough and not melt as well as desired, and residual sugar may be fermented by contaminating bacteria resulting in gas and fruity, or fermented flavors. Another way to prevent low pH is to drain the whey at a higher pH, and/or salt at a higher pH, thereby retaining more of the buffering agents. Also by lowering the moisture content of the cheese the amount of lactose is reduced in the curd. Keep in mind that the more acid the whey as it is expelled from the curd, the greater the loss of buffering capacity of the curd. It follows that the more whey removed at a low pH, the more
buffer is lost. Therefore, if it is possible to decrease moisture during the cook or stir out step without the large drop in pH, the cheese will retain more of the buffer capacity. This method is used in Swiss cheese manufacture (sometimes along with a small water addition).

Poor whey drainage from curd also contributes to cheese discoloration. In stirred curd cheeses, large lumps of matted curd tend to entrap moisture causing uneven moisture and salt distribution. Even subsequent pressing may fail to remove the whey resulting in localized areas with high moisture and high acid. Excessive initial pressure applied to large blocks of high moisture cheese (soft curd) will also entrap whey.

Some of the most common defects have their origin in the growth and metabolism of contaminating bacteria. These include softening of the cheese, flavor defects (particularly unclean and fruity) and gas development which leads to splits or sweet holes, depending on the pH, extent of proteolysis, composition of the cheese and temperature of the cheese at gas formation. The most common culprits producing these defects are bacteria called Lactobacilli. However, they are also thought to be, at least in part, responsible for desirable cheese flavors. Pasteurization will decrease the numbers of lactobacilli to undetectable levels in milk and the lower the initial level of bacteria, the lower the numbers remaining. Post-pasteurization contamination appears to be a major source of Lactobacilli. Lactobacilli are capable of forming a polysaccharide that enables them to adhere to metal, plastic, or wooden surfaces. Aggregates of the bacteria and polysaccharide are called biofilms. The original source of the Lactobacilli (or other bacteria also capable of forming biofilms) may be the few Lactobacilli that survive pasteurization, natural human skin flora, aerosols, raw milk, and improperly sanitized equipment. Once the biofilms have become established, the organisms they contain are less sensitive to sanitizers. Cleaning and effective sanitation help control biofilm formation but it is inevitable that Lactobacilli will be present and grow in cheese. Low temperature storage (40°F) will retard the growth of Lactobacilli. It is important however, that the temperature is lowered before the Lactobacilli have a chance to reach high numbers in the cheese so rapid cooling may help. Another factor related to the adverse affect of Lactobacilli is the fermentation of residual sugars. Heterofermentative Lactobacilli ferment the lactose or galactose to acids, alcohol and gas. As a result of the gas formation, splits or sweet holes form and packaged cheese may become puffy.

References
IT'S 2002: WHAT DO I DO?
Jerry Dryer, The Jerry Dryer Group, Inc., Northbrook, IL

This year's Marschall Italian and Specialty Cheese seminar is very appropriately titled "Proud of the Past – Focused on the Future."

Producers of Italian cheeses and other specialty cheeses truly can be proud of their past. You have produced a wide variety of products with a wide variety of functions. You make them available to your customers bulk, sliced, diced, shredded, and powdered and you put them in a host of packages and package sizes.

That's why Italian cheeses and other specialty cheeses have enjoyed steady and, in most cases, relatively profitable sales and usage growth.

And the very fact that so many of you are here participating in this seminar indicates that you are focused on the future.

Here's a quick look back:

• Mozzarella cheese production has more than doubled since 1984, from just under a billion pounds to more than 2 billion pounds.

• In 1984, 146 mozzarella plants produced an average of 6.5 million pounds. Last year, 128 plants produced an average of 16.1 million pounds.

• Production of other Italian varieties of cheese has increased by more than 50%, from 366 million pounds in 1984 to 554 million pounds in 1994.

• In 1984, 123 plants produced an average of 3 million pounds of other Italian varieties and in 1994, 100 plants produced an average of 5.5 million pounds.

On the specialty cheese side of the ledger, data isn't quite as reliable, but it appears that production increased about 65% between 1984 and 1994 and totalled about 150 million pounds in 1994. The average specialty cheese plant produced 1.1 million pounds of product in 1984 and 1.4 million pounds of product in 1994. While the number of plants producing Mozzarella and other Italian cheeses has continued to decline over this 10-year period, the number of plants producing specialty cheeses has increased from 86 in 1984 to 106 in 1994.

Okay, it's 1995. Where will you and your cheese company be in the year 2002? Two out of every five won't be in business seven years from now. Because you are participating in seminars like this and looking ahead, looking around the comer at the future, your odds might be a little better. But the industry on the whole will lose at least two out of the current five cheese companies doing business.

On the flip-side, we expect to see a number of new entrants in the cheese business. WHY? Because as the big get bigger, opportunities are created for new entrants - people prepared to supply specialty and niche markets.

Let's talk about the pending changes in the cheese business by putting them in perspective relative to three other developments. These are things that will be here in the year 2002.
1. When you go in to purchase a new automobile, instead of walking through a lot of cars ready to buy, you will sit down in front of a computer screen and design the car that you want. The colors, the accessories, the horsepower – you'll be able to make literally hundreds of decisions on the spot.

You say, “But, Jerry, I've done that.” Some of you may have, but did you get your new automobile delivered to you in three days?

2. They discover that you have an artery clogged with cholesterol.

Doctors will determine the extent and precise location of the blockage and inject a microcomputer chip into one of your veins. The computer chip will go to the location, chip away the cholesterol and dissolve into your bloodstream – bloodless surgery.

3. The boys and girls at Hoover Vacuum Cleaners are in for even more of a surprise.

Computer chips programmed to the dimensions of your living room can be sprinkled on your carpet before you go to bed at night and when you wake up in the morning, there will be a little pile of dust over in one corner of your room.

At the Jerry Dryer Group we make a living tracking trends, helping to predict what new products will succeed and fail, what new packages will be required and how delivery systems can be made more efficient.

Tracking trends is considerably more than just pointing to existing trends and saying they will continue or they won't. Yes, in the next decade we expect to see more ethnic foods: Mexican, Italian, Caribbean, Mediterranean, Middle Eastern, Asian. Yes, the aging baby-boomer will continue to play an ever more important role in how food is bought and sold. Yes, consumers will continue to insist on convenience in every product and service they use. And yes, demand for value will continue...intensify. And yes, demand for variety will continue to intensify.

However, we see several other developments that will very significantly impact how you interact with your suppliers, your customers, and the ultimate consumer.

Let's look at the consumer first; after all, that's where it all begins. Everyone in the production chain – the supplier, the manufacturer and your customer (retailers, foodservice operators and other food manufacturers) – must respond to the wants and needs of individual consumers if they are to be in business in the year 2002.

The lines between food and medicine are blurring.

The Holy Grail in the foodbusiness for the past several years has been producing a product that tastes good and is good for you. Not just “not bad for you,” but actually good for you. Low-fat is part of this, but it is just one way this idea is manifesting itself.

Ultimately, what people will want to be able to do is eat as much as they want and not worry about their diets. They want to be able to drink a milkshake and get all the vitamins and minerals they need for the day without any calories. Today, we have fluid milk and yogurt fortified with beneficial acidophilus and bifidus. We have been fortifying milk with vitamin A and vitamin D for years.

Tomorrow it's cheese. What beneficial bacterium can we use to fortify cheese? Can we fortify cheese with fiber to help prevent colon cancer? Certain varieties of cheese might be the multiple vitamins of tomorrow.

By the year 2000, consumers who are over 55 years of age will control more than 75% of this country's wealth. All of the consumer research we look at says these people do not just want to live longer, they want to live better. In other words, they want to prevent disease, not
cure disease. They want to remain physically active, whether it's having the strength to run in a marathon or the strength to shop all day in a megamall.

Food as fun.

With more leisure time (by the year 2002 people will be retiring at age 55 or earlier) there is more time for fun, more time for entertaining. Whether that entertaining is a dinner at home, a meal at a foodservice outlet, tailgating at a sporting event or munching down in the stadium, people want to have fun. Not just those that are retired, but those that are still working. Working harder and playing harder; it all translates into fun and food is a central part of that experience.

That's one of the forces driving the growth in the specialty cheese business today, and it will only be compounded as we move toward the year 2002.

We think that means some dramatic changes, not just in the types of cheese, but in the packaging of cheeses. You'll see more single servings, more shelf-stable products, higher flavor profiles and new flavors.

It means substantial growth in all segments of the market - from ready-to-bake, easy-to-bake bries to finger foods like string cheese and condiments like grated parmesan and crumbled blue cheese to be added to salads, soups, sandwiches and entrees.

Food on the run.

The world keeps moving faster and faster and that has people eating on the run. It has restaurateurs responding by opening up outlets in a wide variety of places. It has retailers responding with ready-to-eat foods at service stations. There are 93,000 convenience stores and 30,000 supermarkets in this country – most of them fully equipped with delis. In the foodservice business, trying to catch consumers on the run has turned into intensive competition. Look at just these few examples:

• Omaha, Nebraska, is only big enough to justify 16 regular Taco Bell outlets, but you can buy Taco Bell tacos in 150 different places in Omaha including a string of kiosks, school lunch programs and many outlets in discount stores.

• McDonald's has plans to open 1,000 satellite stores in Wal-Marts and food courts in other small markets.

• Little Caesar's has created Pizza Station Express Hotel kiosks for use in Holiday Inns.

• Five hundred and sixty units of K-Mart are the home of Little Caesar's.

• Over half of those 93,000 convenience stores offer some form of foodservice.

On the other side of the ledger are supermarkets. At least 20% of the existing 30,000 supermarkets in this country will probably disappear over the next five to seven years. They'll be replaced by the fast food outlets we talked about and the opening of 180,000 square foot supermarket/discount centers like Wal-Mart. Wal-Mart plans to open 100 per year.

Boston Markets, formerly Boston Chicken, now has 850 units; they had just 25 three years ago. What are they selling? Rotisserie chicken, an item invented by supermarkets.

All of this change is in the name of being where the consumer is, when the consumer is hungry. Eating on the run.

Your customers, these supermarkets and fast food outlets, need your help. What ideas can you bring to these operators to help them solve their problems? Are you packaging your
product correctly? Are you distributing your product to them correctly? Is it easy to get your cheese to those 150 outlets in Omaha?

When we talk about your customer, we are talking about retailers and foodservice operators, food manufacturers using your cheese as an ingredient that is ultimately delivered to the consumer, John and Jane Doe walking down the streets of America. The lines between your consumer and your customer are rapidly blurring.

**Consumers become customers.**

In a May 29 article in *Fortune Magazine*, one retailing expert predicts that by the year 2010, 55% of the nation's shopping will be conducted in non-store venues: on-line services, direct mail, catalogs, 800 numbers.

We are already seeing “virtual supermarket” services such as Shopper's Express, Shopping Alternatives and Pea Pod delivery service. They allow people to order food via their computer and have it delivered to their homes at a specific time. Easy to use software lets them browse through the supermarket aisles without ever leaving their kitchen or their desk.

What does this mean to you? For one thing, we think you will be selling a lot more cheese this way, perhaps even direct to the consumer in the next century. Someday soon every company will have its own “web site” or the 21st century's equivalent to that. All consumers will need to do is log on and order their cheese directly from you.

This isn’t as scary as it might seem. Many of you are already doing business this way with some of your institutional – supermarket, foodservice and manufacturing – customers. Once the technology gets a little better, everyone is going to figure out that it make sense to buy food this way.

For specialty cheesemakers this will be a bonanza. If I were a specialty cheesemaker starting up today, one of the first things I'd do is find a way that people could order my products directly – on-line, via 800 number, via fax, the catalogs of tomorrow.

This technology will also affect how you do business with your customers. As a sale is rung up in a grocery store, it will automatically place an order with you to replace the item just sold. Everyday at midnight your supermarket chain customer's computer will talk to your computer dictating it needs X cases of this, Y cases of this and Z cases of that to replace stocks that were removed from the shelf the day before.

**Doing business with your suppliers.**

You need to be a problem solver for your customers and consumers of the future. Likewise, your suppliers are preparing to be problem solvers for you and your business.

We see a sharp increase in the number of strategic alliances developed by suppliers and you, their customers. Many of you already have relationships like this with your customers. That is, your research and development efforts compliment or even support the new product development efforts of your customers.

Suppliers are now rapidly moving toward doing exactly the same thing for you. They will help you do the research and development to help create new products. They’ll tailor their equipment, they’ll tailor their cultures, they’ll tailor their packaging to meet the requirements of your customers. This will all be done on an exclusive proprietary basis. And it will become increasingly important. Many of you will be making 12, 15, 20, 100 different types of cheese. They may all be Mozzarella, but they’ll have 100 different sets of specifications given the needs and requirements of your customers who are responding to the consumer.
On the supplier side, biotechnology and bioengineering will also be an important factor. Cows are already being engineered to produce milk to fight specific diseases and prevent other diseases. In the not-too-distant future, cows will be able to produce milk to your specifications. They will be producing milk with the right protein and fat ratio according to the particular cheese you want to make. You'll be routinely dealing with somatic cell counts of less than 100,000 and antibiotic residues will become less and less of a problem as more and more milk producers improve their management to prevent mastitis and adopt holistic treatment procedures.

**Your customers in other countries.**

We see substantial opportunities to market cheese in other countries. As a result of the NAFTA and GATT negotiations, doors have been opened up around the world for U.S. manufacturers of high quality cheese. Emerging economies have citizens in countries around the world wanting to eat like Americans.

There are several ways to capitalize on this opportunity. Pizza Hut has 2,500 units in 89 countries outside of North America. Domino's says it will have 3,000 international units by the end of the decade. McDonald's has thousands of units overseas. These are opportunities for you to move product overseas for customers you may already be doing business with.

And now, there's a new opportunity to identify and supply customers in other countries via the new United States Dairy Export Council. Dairy farmers, through their check off funds and participation in Dairy Management Inc., have spun-off this subsidiary making it available as a membership organization to cheese marketers like yourselves. By joining this organization, you can help leverage the $4 million dairy farmer investment and leverage your opportunities to market cheese overseas.

**What does this all add up to? Opportunities, Opportunities, Opportunities.**

For instance, consider the snack food business. Because of Frito-Lay's volume requirements, it only makes sense for them to buy cheese from very large suppliers. Likewise, because of the size of some cheese factories, it only makes sense for them to supply very large customers.

It's just not practical for them to run small volumes of product for small consumers. Therefore, a two-tier system of customers and suppliers is developing. Smaller specialty cheesemakers will always have a market, because there will always be smaller specialty snack makers.

There will continue to be large opportunities for lowfat cheeses. In fact, low-fat foods of every description will require low-fat, high flavor cheese. Using new cultures, new technologies and flavor enhancers, reduced-fat cheeses will have very high flavor profiles.

More and more shoppers will continue to buy more and more natural foods. Play to that strength, whether you're a high volume manufacturer or a low volume manufacturer. Consumers will continue to seek out more and more sharper flavors as part of their never-ending search for more variety.

Your customers will continue to look for a high brand profile from some players in the cheese business. That means more opportunities for co-branding your name and the retailer's name; your name and the food manufacturer's name on the package of prepared foods.

The frozen pizza business continues to boom and the next stage of this evolution is more and more hand-held microwaveable sandwiches. Not that the microwave is going to enjoy a resurgence in the home. Nobody uses it there now except to heat some water, but it
will appear in that automobile that you ordered via computer three days earlier. It’ll be there to cook your meals as you drive down the highway.

And don’t overlook markets outside of the cheese business. That brings us to a word about whey. Sports drinks today account for more than $1 billion in annual sales and they are expected to grow at least 10% annually. Athletes require more protein in their diet and whey protein drinks are the way to deliver that. Ditto for sports bars, infant formula, weight gaining and weight loss products, protein fortified fruit juices and a host of other health foods and drinks.

Specialty is becoming a way of life. In just two years, the specialty beer business grew from about $600 million in sales to nearly $1 billion in sales.

Meatless meals are on the rise. In 1992, less than one-third of the consumers believed it was necessary to eat meat everyday. That’s down from 75% who felt meat was essential in the mid-80s.

By the year 2002, almost 30% of the nation’s work force will be working out of their home, or at least not in that central office. That accelerates and amplifies the opportunity for single-servings, speed, convenience, and accessibility.

All and all, it adds up to dozens and dozens – literally hundreds of opportunities for the manufacturers of Italian and specialty cheeses.
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MOZZARELLA CHEESE: IMPACT OF THREE COMMERCIAL CULTURE STRAINS ON COMPOSITION, YIELD, PROTEOLYSIS AND FUNCTIONAL PROPERTIES

David M. Barbano, Ph.D., Youn-ho Hong¹, J. Joseph Yun², Kristie L. Larose³ and Paul S. Kindstedt³
Department of Food Science, Cornell University, Ithaca, NY
¹ Department of Food and Nutrition, Chonnam National University, Kwangju, 500-757, Republic of Korea
² Diversified Research Laboratories Limited, 1047 Yonge Street, Toronto, Ontario, Canada
³ Department of Animal and Food Science, University of Vermont, Burlington, VT

Abstract

The impact of different lactobacillus culture strains on chemical composition, yield, proteolysis, and functional properties of Mozzarella cheese during refrigerated storage was determined. Three vats of cheese were made in 1 day using three culture strains of rods (L. delbrueckii ssp. bulgaricus R110 and R160; L. helveticus R150). Cheese making was replicated on 3 different days. No differences in general cheese composition because of the difference in culture strain were detected. Changes in pH and titratable acidity during cheese making were significantly slower with the R110 than with R150 or R160. Fat loss in whey from cheese made with R110 was lower than for R150 or R160. No differences in fat loss in stretching water or protein loss in whey or stretching water were detected. The R110 showed a higher adjusted yield and cheese yield efficiency than either R150 or R160, however, the differences were not statistically significant. It appears that the shorter the time from rennet addition to draining, the higher the fat loss in the whey and the lower the cheese yield (given constant target pH at draining).

There was a significant effect of culture strain with time of refrigerated storage on cheese pH. No significant differences in the rates of increase in the nitrogen soluble in pH 4.6 acetate buffer or 12% TCA were observed. Storage time had a much greater impact on changes in TPA parameters than culture strain. Meltability increased significantly with time of refrigerated storage for all cheeses. Significant differences in meltability due to an interaction of culture strain and storage time were detected. After 15 days of refrigerated storage, meltability of cheeses were similar, but by 50 days cheeses made using R110 had lower meltability than cheeses made with R150 or R160. On pizza baking at 15 days, the color and blistering were similar for all strains. However, after 50 days of refrigerated storage, cheeses made with R150 and R160 strains showed excessive melting when baked on a pizza and large light blisters. In general, cheese made using the R110 strain maintained desirable melting and blistering characteristics longer than either R150 or R160. Thus, selection of lactobacillus culture strain for use in Mozzarella cheese making can influence functional characteristics of the cheese.

Introduction

The major function of a starter culture during Mozzarella cheese making is the production of lactic acid from lactose. The culture will control the development of nonstarter flora, inhibit pathogens, improve shelf life, and contribute to flavor development, proteolysis and cheese ripening (1). Presently, the relationships between use of different commercial lactobacilli culture strains and Mozzarella cheese composition, yield, proteolysis during
ripening and functional properties are not well understood. The objectives of our study were to determine the influence of three different commercial lactobacilli strains on chemical composition, yield, proteolysis, and functional characteristics during 50 days storage of Mozzarella cheese at 4°C.

MATERIALS AND METHODS

Cheese Making. Low-moisture, part-skim Mozzarella cheese was made on each of three days using a stirred-curd, no-brine cheesemaking method (2). Raw skim milk and raw cream from the Cornell University dairy plant were combined to obtain a target fat on a dry basis (FDB) in cheese of 38%. The standardized milk was pasteurized at 72°C for 16 s, cooled to 4°C, divided into three equal portions (about 200 kg each), and stored overnight at 4°C. The next day, the milk (about 200 kg per vat) was poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI) and heated to 38°C. Details of coagulant and cheese making conditions (except culture) up to the point of stretching and sampling are as described previously (2). The mixer screw speed was 12 rpm and temperature of cheese at stretching was 57°C.

Direct-to-vat frozen starter culture, S. salivarius ssp. thermophilus (Thermococcus C120®) was used for all vats of cheese. The lactobacillus cultures were bulk-set culture (Biolac™ D.S.S.TM, Defined Strain Starter). Two different strains of L. delbrueckii ssp. bulgaricus (R110 and R160) and one strain of L. helveticus (R150) were used. According to the culture supplier, the R110 culture was expected to have the most proteolytic activity and the least peptidase activity, while the R150 was expected to have the least proteolytic and have the most peptidase activity, and the R160 would be intermediate. All cultures were commercial cheese making cultures from the Marschall Products division of Rhône Poulenc, Madison, WI. The total amount of cultures used was 3.85 ml/kg of milk (.35 ml coccus and 3.5 ml rod) for all vats. These amounts gave an approximate 1 to 1 ratio of viable rod to coccus at the point of culture addition to the milk.

Cheese Analyses. Changes in titratable acidity of milk and whey, and curd were monitored during cheese making, and moisture, fat, protein, salt, and calcium contents of fresh cheese were determined as previously described (3). Proteolytic changes (i.e., nitrogen soluble in pH 4.6 acetate buffer and in 12% TCA, and intact $\alpha_1$-CN plus $\alpha_2$-CN and $\beta$-CN) of the cheese were monitored at 3, 15, 29, 50 days of storage at 4°C (3).

Recovery and Yield Calculations. Since fat and protein are the major milk solids in Mozzarella cheese, account for milk fat and protein distribution in the three products of cheese making, namely, whey, stretching water, and cheese. Percentage of fat recovery in each product was calculated as the weight of each product multiplied by its percent fat content and then divided by the total weight of fat present in the original milk and multiplied by 100. The total fat recovery is the sum of the weight of fat in all products divided by the weight of fat in the milk used for cheese making. Total percent fat recovery will not equal exactly 100%, due to cumulative experimental errors in both fat test and weight measurements. However, total fat accountability should be very near 100%. To neutralize the small differences from vat-to-vat in measured total fat recovery, relative (normalized to 100%) fat recoveries in whey, stretching water, and cheese were calculated. Total nitrogen recovery (i.e., protein) and relative (or normalized) percentages of nitrogen recovered in whey, stretching water, and cheese were calculated using the same approach.

Actual cheese yield for each vat of cheese was calculated by dividing the weight of the cheese after cooling by the total weight of milk minus weight of milk samples taken up to the time of rennet addition. Moisture and salt adjusted cheese yield (AJY) was calculated using an equation with a desired cheese moisture of 48.5% and a desired cheese salt content of 1.3%.
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\[
AJY = \frac{\text{Actual yield} [100 - (\text{Actual } \% \text{ moisture} + \% \text{ salt})]}{100 - (\text{Desired } \% \text{ moisture} + \% \text{ salt})}
\]

Theoretical cheese yield (TY) was calculated by the modified Van Slyke cheese yield formula (4).

\[
TY = \frac{[0.85 \times \% \text{ milk fat} + (\% \text{ milk casein} - .1)] 1.13}{1 - \frac{\text{Desired cheese } \% \text{ moisture}}{100}}
\]

The .85 factor in the formula assumes that 85% of the fat in milk will be retained in the cheese. The equation also assumes that .1% casein will not be recovered in the cheese. The 1.13 factor takes into account a constant factor for retention of added salt and the noncasein and nonfat milk solid components retained in the aqueous phase of the cheese. The desired cheese moisture was 48.5%. Cheese yield efficiency is calculated by dividing the composition adjusted yield by the theoretical cheese yield and multiplying by 100.

**Functionality Tests.** Functional properties of the cheese were determined by TPA, a modified Schreiber Test (for meltability), a helical viscometry (for AV), and a centrifugation method (for free oil) as previously described (3). All functionality tests were conducted after 3, 15, 29, and 50 d of storage at 4°C. The cheese was tested on day 15 for browning characteristics during baking using a MacBeth Color-Eye spectrophotometer as described previously (2).

Frozen pizza crusts (12" in diameter) were purchased and stored overnight at refrigeration temperature for thawing. Pizza sauce (150 g) was spread evenly over the surface leaving 1" on the edge without sauce. Mozzarella cheese was shredded using an electric slicer/shredder (model professional Salad Shooter; National Presto Industries, Eau Claire, WI), and the shredded cheese (300 g) was sprinkled over the sauce on pizza crust. The pizza was baked without adding any other toppings at 232°C (450°F) for 5 min using a conveyor oven (model Imprimer II; Lincoln Food Service Products Inc., Ft. Wayne, IN).

After cooling the pizza at room temperature for 30 min, the pizza was placed under 4 photo flood lights (250W each). Photographs were taken using slide film (Ektachrome model 64T; Kodak, Rochester, NY) and a 35 mm camera (model T70, Canon, Tokyo, Japan) with shutter speed of 1/15 of a second and f-stop of 8.

**Experimental Design and Statistical Analysis.** Three vats of cheese, each using a different starter culture strain (i.e., R110, R150, and R160), were made on 1 day from one batch of milk. The cheese making was replicated on 3 different days. On each day, the order of cheese making for the three different starter culture strains was randomized. Changes in proteolysis and functional properties during refrigerated storage were assessed using a split-pilot design with starter culture strain as a wholeplot factor. The statistical significance was determined at \( P < .05 \).

**Results and Discussion**

**pH, Titratable Acidity, and Cheese Making Time.** The change in pH and titratable acidity during cheese making was slower for R110 than for R150 or R160 (Figure 1a,b). The average curd pH at the end of salting was 5.39 for all culture strains. As a result, cheese making time from addition of rennet to stretching was significantly (\( P < .05 \)) longer for the R110 (134 ± 3 min) than for the R150 (117 ± 6 min) and the R160 (108 ± 4 min).
**Chemical Composition.** The fat, protein, and casein contents of milks used for cheese making were (mean ± SD) 2.30 ± .08, 3.03 ± .04, and 2.34 ± .03%, respectively. No detectable differences occurred in cheese composition because of the difference in culture strain (Table 1). The mean moisture, fat, protein, and salt contents of the cheese made using different culture strain were within the specification for low moisture, part-skim Mozzarella (5). The initial pH of the cheeses made using 3 different culture strains were similar (Table 1) at day 2. However, there was a significant interaction effect of culture strain and time of refrigerated storage cheese on pH. The pH of the cheeses made with the R150 and R160 strains decreased significantly with time of refrigerated storage, whereas the pH of R110 cheese remained higher and constant during 50 d of refrigerated storage (Figure 2a). The titratable acidity of the cheese made with the strain of R110 was lower than those of R150 and R160 and almost constant during 50 d of refrigerated storage, which is consistent with the higher cheese pH for R110 (Figure 2b).

**Fat Recovery.** Milk fat, a major component in milk, is trapped in the milk protein matrix during cheese making. When recovery of other milk components is constant, the higher the fat recovery in the cheese, the higher the Mozzarella cheese yield. Total actual fat recovery in cheese, whey, and stretching water for cheese made with R110, R150, and R160 were 99.95, 98.32, and 99.27%, respectively. Total fat accountabilities of less than 100% total were caused by small, but expected, losses due to adherence of fat on surfaces of cheese making equipment (6). To make an unbiased comparison of the relative fat recoveries in cheese, whey and stretching water, the data were normalized to give a total of 100% for the treatments (Table 2). There was a significant difference in fat loss in whey among the three culture strains, but no detectable difference in fat loss in stretching water was observed. Fat recovery in cheese made using R110 was higher than that made with R150 or R160. Thus, different culture strains had an effect on the retention of fat in the curd and loss of fat in whey, with R110 having the lowest loss in the whey.

**Protein Recovery.** Similar to milk fat, protein is a major component that plays an important role in cheese yield. Protein recovery will reflect protein distribution in various products of cheese making. The measured total protein recoveries for cheese making with R110, R150, and R160 were 101.44, 102.15, and 101.61%, respectively. Mass balance type accountabilities for milk protein were close to 100% and were converted to normalized data, as was done for fat recovery. Normalized protein recovery in whey, stretching water, and cheese shows no differences due to culture strain (Table 3). Thus, the differences in protease and peptidase activities among these commercial culture strains was not large enough to cause differences in protein recovery.

**Cheese Yield.** Actual cheese yield, moisture and salt adjusted yields, theoretical yields, and cheese yield efficiencies are given in Table 4. Actual yields for cheeses made with R110, R150, and R160 were 8.95, 8.75, and 8.83 kg/100 kg milk, respectively. Cheese yield performance is easiest to compare using yield efficiencies, which are cheese yields that have been adjusted to the same moisture and salt content and then expressed as percentage of the theoretical cheese yield (7). The composition adjusted yield and cheese yield efficiency of cheese made with R110 was higher than that of R150 or R160, but no statistically differences were detected.

It is often easier to detect significant differences in losses of fat and protein in whey than it is to detect a significant difference in cheese yield because the measurement of composition adjusted yield and cheese yield efficiency accumulates the errors from many more measurements than does the determination of loss of fat or protein in whey (8). Detection of a significant difference between treatments in loss of fat or protein in whey on a
mass balance basis provides information on which one can expect a difference in cheese yield even though differences in cheese yield efficiency are not significant in that same cheese making trial. This is the case in this study. The culture strain caused a significantly higher loss of fat in whey and the observed differences in cheese yield efficiency were consistent with this observation but are not statistically significant. Despite the fact that the differences in cheese yield efficiency are not significant, there probably is a difference in cheese yield between the R110 culture versus the R150 and R160, under the conditions used in this study. With the small number of replicates in this limited trial, the LSD for cheese yield efficiency is relatively high and is a much less sensitive index of yield difference than fat or protein loss in whey.

Thus, the R110 culture would produce a higher yield of cheese due to better fat retention. However, is the lower fat loss at draining the whey for the R110 culture a direct impact of the culture strain or is it an impact of the difference in the rate of acid production and pH change during the time from set to draining of the whey? The authors propose that the latter is the cause of the difference in fat loss in the whey among the culture strains. Thus, if this is the case, then decreasing the inoculation amount of R150 or R160 (or decreasing their ripening time to achieve the same pH change with time during cheese making as experienced with the R110) would be expected to make the fat loss among the culture strains similar. Therefore, we feel that the most important point is that increasing the rate of acid production (i.e., shorter make time) will increase fat loss in whey, regardless of culture strain, if all other factors are equal. Further work is needed to confirm this hypothesis.

Proteolysis. No significant differences in the rates of increase in the amounts of nitrogen soluble in pH 4.6 acetate buffer or 12% TCA due to the differences of culture strain during 50 days of refrigerated storage were detected (Figure 3a, b). The soluble nitrogen contents increased significantly with time of refrigerated storage for all cheeses as reported previously (3).

The evaluation proteolysis using electrophoresis gave results consistent with data from soluble nitrogen analysis. The αs-caseins were broken down with time of storage but were not affected significantly by culture strain (Figure 4a). The β-casein decreased slightly with storage, however cheese made with R110 started out with a significantly higher concentration of intact β-casein and then maintained slightly higher amount of β-casein during storage than cheese made with R150 or R160 (Figure 4b). No reason for the difference in β-casein (other than culture strain) is apparent from the data.

Unmelted Cheese Functionality. No statistically significant effects of different culture strain on any of TPA parameter of the cheese were detected. Overall, storage time had a much greater impact on TPA parameters than culture strain. The TPA hardness and TPA springiness for all cheeses decreased with age (Figure 5a,b), indicating a progressive softening. The TPA cohesiveness was variable (Figure 5c).

Melted Cheese Functionality. Melting characteristics of all cheeses changed significantly during refrigerated storage (Figure 6). Meltability increased significantly with time of refrigerated storage for all cheese, and significant differences in meltability due to an interaction of culture strain and time of refrigerated storage were detected. At 50 days of refrigerated storage, the cheese made with R110 had lower meltability than cheeses made with R150 or R160. Apparent viscosity decreased significantly during refrigerated storage for all cheeses, but was not influenced by culture strain (data not shown). Free oil increased significantly with refrigerated storage for all cheeses, but no significant differences in free oil formation because of differing culture strain were detected (data not shown).
Cheeses in this study showed typical patterns of change during refrigerated storage with respect to unmelted texture and melting characteristics (3, 9, 10, 11). The difference of culture strain had only minor impact on unmelted cheese texture and melting characteristics during storage. The L-value, a-value, and b-value for baked Mozzarella cheese at 15 days of refrigerated storage were significantly influenced by culture strain (L-values: 73.20, 73.96, and 71.85; a-values: -0.21, -1.35, and -0.27; b-values 26.22, 23.43, and 23.95) for R110, R150, and R160, respectively. The cheese made with R110 had more yellow/brown color, but the differences were relatively small. On pizza baking at day 15, the cheese made with R110 showed more intensive brown color and a larger number of smaller-size blisters than other cheeses made with R150 and R160 strains. At day 50, the meltability and flow of the cheese made with R150 and R160 were much greater than R110. This caused a large difference in blister forming characteristics. Thus, cheese made with R110 retained the ability to form small blisters for a longer time during storage, while the other cheeses did not form small blisters and therefore did not have the typical blistering and browning characteristics. These differences were more pronounced on day 50 than on day 15.

Conclusions

No significant effects of different culture strains used in this study on cheese composition, unmelted TPA parameter, and free oil formation were detected. The cheese made using R110 had significantly more intact β-casein in the cheese initially and throughout storage. The cheese made with R110 culture strain had lower meltability than cheeses made with R150 or R160 strains. Compared with the other culture strains, the R110 culture strain showed more browning intensity and smaller blisters on pizza and maintained these characteristics for a longer time of refrigerated storage. Thus, lactobacillus culture strain selection for use in Mozzarella cheese making can influence functional characteristics of the cheese.

Make time for cheese made with R110 (134 ± 3 min) was significantly (P < .05) longer than the R150 (117 ± 6 min) or the R160 (108 ± 4 min). There was a significantly lower loss of fat in the whey with the R110 culture, but no differences in loss of protein in the whey among the different cultures were detected. Although a significant difference in cheese yield efficiency was not detected among the three culture strains, the differences in observed cheese yield efficiencies were consistent with the differences in fat loss. The lower fat loss in whey with the R110 culture may not be a direct effect of the culture, but only a result of the difference in the rate of pH change that occurred from set to draining. It appears that the shorter the time from set to draining, given a constant target whey pH at draining, the higher the fat loss in the whey.

Acknowledgments

The authors thank Maureen Chapman, George Houghton, Sung-Guk Kim, Shirley Koslowski, Laura Landolf, Aditya Sapru, and Pat Wood for their technical assistance. Financial support was provided by Northeast Dairy Foods Research Center. Youn-Ho Hong thanks the Korean Ministry of Education for financial support of his sabbatical leave to conduct this research.
Table 1. Average (n=3) initial chemical composition of Mozzarella cheeses made with three different culture strains.

<table>
<thead>
<tr>
<th>Component</th>
<th>Culture Strain</th>
<th>SEM</th>
<th>LSD$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R110</td>
<td>R150</td>
<td>R160</td>
</tr>
<tr>
<td>pH</td>
<td>5.19</td>
<td>5.07</td>
<td>5.12</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>47.38</td>
<td>46.95</td>
<td>47.74</td>
</tr>
<tr>
<td>Fat, %</td>
<td>21.64</td>
<td>21.02</td>
<td>21.06</td>
</tr>
<tr>
<td>FDB, %</td>
<td>41.13</td>
<td>39.61</td>
<td>40.30</td>
</tr>
<tr>
<td>Protein, %</td>
<td>25.78</td>
<td>26.46</td>
<td>26.20</td>
</tr>
<tr>
<td>Salt, %</td>
<td>1.34</td>
<td>1.44</td>
<td>1.27</td>
</tr>
<tr>
<td>S in M,$^3$ %</td>
<td>2.83</td>
<td>3.07</td>
<td>2.66</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>.741</td>
<td>.748</td>
<td>.752</td>
</tr>
<tr>
<td>Ca (% of P),$^4$ %</td>
<td>2.88</td>
<td>2.83</td>
<td>2.87</td>
</tr>
</tbody>
</table>

$^1$P < .05.
$^2$Fat content on a dry weight basis.
$^3$Salt concentration in water phase of the cheese.
$^4$Calcium as a percentage of protein content of the cheese.

Table 2. Average (n=3) normalized fat recovery in whey, stretching water, and Mozzarella cheeses made with three different culture strains.

<table>
<thead>
<tr>
<th>Culture strain</th>
<th>Fat Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
</tr>
<tr>
<td>R110</td>
<td>9.58$^a$</td>
</tr>
<tr>
<td>R150</td>
<td>11.26$^a$, $^b$</td>
</tr>
<tr>
<td>R160</td>
<td>12.46$^b$</td>
</tr>
<tr>
<td>SEM</td>
<td>.40</td>
</tr>
<tr>
<td>LSD</td>
<td>2.43</td>
</tr>
</tbody>
</table>

$a,b$Means in the same column not sharing common superscript are different (P<.05).
Table 3. Average (n=3) normalized protein recovery in whey, stretching water, and Mozzarella cheeses made with three different culture strains.

<table>
<thead>
<tr>
<th>Culture strain</th>
<th>Protein Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whey</td>
</tr>
<tr>
<td>R110</td>
<td>25.22</td>
</tr>
<tr>
<td>R150</td>
<td>25.40</td>
</tr>
<tr>
<td>R160</td>
<td>25.33</td>
</tr>
<tr>
<td>SEM</td>
<td>.15</td>
</tr>
<tr>
<td>LSD</td>
<td>.93</td>
</tr>
</tbody>
</table>

Table 4. Average (n=3) yield of Mozzarella cheeses made with three different culture strains.

<table>
<thead>
<tr>
<th>Culture strain</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual</td>
</tr>
<tr>
<td>R110</td>
<td>8.95</td>
</tr>
<tr>
<td>R150</td>
<td>8.75</td>
</tr>
<tr>
<td>R160</td>
<td>8.83</td>
</tr>
<tr>
<td>SEM</td>
<td>.06</td>
</tr>
<tr>
<td>LSD</td>
<td>.34</td>
</tr>
</tbody>
</table>
Changes in pH during Mozzarella Making (Impact of Culture Strain)

- R110
- R150
- R160

Changes in TA during Mozzarella Making (Impact of Culture Strain)

- R110
- R150
- R160
Figure 2a.

**pH of Mozzarella Cheese**
*(Impact of Culture Strain)*

![Graph showing pH of Mozzarella Cheese over storage time for different culture strains. Each strain is represented by a different marker and line style.]

Figure 2b.

**TA of Mozzarella Cheese**
*(Impact of Culture Strain)*

![Graph showing titratable acidity of Mozzarella Cheese over storage time for different culture strains. Each strain is represented by a different marker and line style.]

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Figure 3a.

**pH 4.6 Soluble Nitrogen**
*(Impact of Culture Strain)*

Figure 3b.

**12% TCA Soluble Nitrogen**
*(Impact of Culture Strain)*
Figure 4a.

\( \alpha_s \)-Casein in Mozzarella

(Impact of Culture Strain)

\[ \begin{array}{c}
\alpha_s \text{-Casein} (\%)
\end{array} \]

Storage Time (d)

---

R110
R150
R160

Figure 4b.

\( \beta \)-Casein in Mozzarella

(Impact of Culture Strain)

\[ \begin{array}{c}
\beta \text{-Casein} (\%)
\end{array} \]

Storage Time (d)
Figure 5c.

TPA Cohesiveness
(Impact of Culture Strain)

Figure 6.

Meltability
(Impact of Culture Strain)
References


EFFECT OF SCREW SPEED AND RESIDENCE TIME AT HIGH STRETCHING TEMPERATURE ON COMPOSITION, PROTEOLYSIS, FUNCTIONAL PROPERTIES AND THE WATER PHASE OF MOZZARELLA CHEESE

Paul S. Kindstedt, Ph.D., M.R. Guo, W.H. Viotto1, J.J. Yun2, D.M. Barbano2
Northeast Dairy Foods Research Center, Department of Animal and Food Sciences, University of Vermont, Burlington, VT
1Faculdade de Engenharia de Alimentos
Universidade Estadual de Campinas, Brazil
2Department of Food Science
Cornell University, Ithaca, NY

Abstract

The impact of mixer screw speeds at high stretching temperature on chemical composition, proteolysis, functional properties, and the water phase of Mozzarella cheese during storage at 4°C was determined. Three vats of cheese with three different screw speeds (slow = 5, medium = 12, and fast = 19 rpm) were made in one day using a stirred-curd, no-brine method. Cheese making was replicated on three different days as a randomized block design. Residence time during stretching was 19, 14, and 12 min. and average cheese temperature during stretching was 66 °C (150°F), 64 °C (147°F), and 62 °C (145°F) for slow, medium, and fast screw speed, respectively. Chemical composition of cheeses was not affected, except that slower screw speed produced cheeses with higher pH and lower titratable acidity. Nitrogen soluble in pH 4.6 acetate buffer and 12% TCA increased for all cheeses with age, but the rates of increase were slower in cheese stretched at slow screw speed. This indicated that the coagulant and starter culture were heat inactivated during stretching at the slowest screw speed. Screw speed did not significantly affect changes in functional properties during aging. However, in general, the rate of changes in functionality was slower in cheeses stretched at slower screw speed. The amount of expressible serum obtained on centrifugation at 12,500 x g for 75 min at 25°C decreased during aging, corresponding to an increase in water-binding properties. However, the rate of decrease was much lower in cheese stretched at slow screw speed, which indicated the persistence of poor water-binding properties. Crude and pH 4.6 soluble protein, and calcium and zinc levels in the expressible serum increased during aging, but the rates of increase were slower at slow screw speed. This suggests that a complex dynamic relationship exists between the casein matrix and the water phase of the cheese, which may be related to functional properties, and which may be affected by the thermal conditions during stretching.

Introduction

The heating and stretching of cheese curd in hot water is a key operation in the manufacture of pasta filata cheeses such as Mozzarella (9). During stretching, the amorphous three-dimensional protein matrix of the curd is aligned into a network of parallel protein fibers, interspersed with open columns that are occupied by water and fat droplets (10). This unique microstructure contributes strongly to the functional properties of the cheese.

Traditionally, batches of Mozzarella curd were stretched in hot water by hand. In the U.S., batch processing has been replaced by continuous single and twin screw mechanical mixers, coupled with steam injection systems. A wide range of stretching conditions are used...
in the industry, which are determined by a number of parameters such as the temperature of the stretching water, the screw speed, and the specific configuration of the mixer.

It is important to recognize that the Mozzarella mixer influences at least three important aspects of the cheese. First, cheese composition (particularly fat and moisture contents) and cheese yield can be altered by changing the mixer conditions, as was discussed at the Marschall ISCS last year (2). In addition, the microstructural aspects of the cheese, such as the dispersion of fat within the curd matrix, are affected by stretching conditions (15). Finally, the thermal treatment (time and temperature) that the curd receives during stretching will determine whether the starter culture and coagulant are partly or completely heat-inactivated during stretching. Active starter culture and coagulant both play important roles in the development of functional characteristics during aging (1,13).

This paper presents the results from one in a series of studies on the relationships between mixer operating conditions and the chemical and functional characteristics of Mozzarella cheese. Two previous studies looked at the effect of different mixer temperatures at a constant intermediate screw speed (15), and the effect of different screw speeds at a constant low mixer temperature (11).

In the previous study on screw speed, changing the screw speed at a constant low mixer temperature did not affect cheese proteolysis during aging. However, screw speed did affect cheese composition. Specifically, cheeses containing lower moisture content and lower fat content on a dry basis (FDB) were produced at higher screw speed (2).

In the earlier study on stretching temperature, it was shown that both starter culture and coagulant were substantially inactivated at high stretching temperature, which resulted in cheese with higher pH, less proteolysis, and altered functional changes during aging. Stretching at high mixer temperature also resulted in cheese with unusually poor water binding characteristics, as was evidenced by the separation of free water from the melted cheese even after 50 days of refrigerated aging. When we reviewed the literature on water-binding in cheese, it became evident that very little is known about the state of the water phase in Mozzarella and its relationship to functional characteristics. In follow-up studies, we investigated the use of high speed centrifugation to separate the expressible serum (i.e., the aqueous phase obtained on centrifugation) from Mozzarella cheese as means to evaluate water-binding properties and to study the composition of the water phase (5). These studies revealed that complex changes occur in the water phase of Mozzarella during the first weeks of aging, which may play a role in the development of functional properties.

The objective of the present study was to determine the effect of screw speed at a constant high mixer temperature on cheese composition, proteolysis, and functional properties of Mozzarella. In addition, changes in the water phase of the cheese during aging were evaluated using the newly developed method for expressible serum. In contrast to the previous study on screw speed, where the mixer temperature was low enough to preclude heat inactivation of coagulant and starter culture, the high mixer temperature employed in this study meant that there was a strong potential for their thermal inactivation.

MATERIALS AND METHODS

Cheese Making

Low-moisture, part-skim Mozzarella cheese was made on each of three days using a "stirred-curd, no-brine" cheese-making method (3). Raw skim milk and raw cream from the Cornell University dairy plant were combined to obtain a target FDB in cheese of 38%.
standardized milk was pasteurized at 72°C for 16 s, cooled to 39°F (4°C), divided into three equal portions (about 200 kg each), and stored overnight at 39°F (4°C).

The next day, the milk (about 200 kg per vat) was poured into a cheese vat (model 4MX; Kusel Equipment Co., Watertown, WI) and heated to 38°C. Details of culture, coagulant, and cheese making conditions up to the point of stretching are as described previously (3). Three different mixer screw speeds were used for stretching: 19 rpm, fast; 12 rpm, medium (ca. 50% of mixer full speed); 5 rpm, slow. Three vats of cheese were made per day. One vat was stretched at 19 rpm, one at 12 rpm, and one at 5 rpm for each day. The mixer was emptied and cleaned between each treatment vat.

A twin-screw, pilot-scale Mozzarella mixer (model 640; Stainless Steel Fabricating Co., Columbus, WI) containing the circulating salt water (6% salt wt/wt) at 165°F (74°C) was used to stretch the curd. Salted curd was fed into the pilot scale mixer at a rate of about 2 kg/min. The temperature of the circulating salt water and the mixer jacket temperature were maintained at 165°F (74°C) during stretching.

Nine 1.4-kg cylinders of cheese (7.5 cm in diameter x 30 cm long) were made per vat. After 60 min of cooling, the cheese was removed from the tube and vacuum packaged in a barrier bag (model B150; Cryovac, Duncan, SC) and stored at 4°C.

Cylinder numbers 3 and 4 were used for the analyses of composition, proteolysis, texture, and meltability. Cylinder numbers 2 and 5 were used for tests of calcium content, apparent viscosity (AV) and free oil formation. Cylinder number six was used for analysis of expressible serum (ES).

CHEESE ANALYSES

Chemical Composition

Analyses of the cheese for fat (quadruplicate), moisture (quadruplicate), total nitrogen (triplicate), salt (duplicate), calcium (duplicate), and pH and titratable acidity were conducted as described previously (12).

Proteolysis

Amounts of nitrogen soluble in pH 4.6 acetate buffer and in 12% TCA were determined to measure the extent and depth of proteolysis (4), respectively, after 3, 28, 56, and 112 d of storage at 4°C. Results were expressed as a percentage of total nitrogen content of cheese.

Functional Properties

Texture Profile Analysis (TPA) and meltability were determined as described previously (14) after 3, 28, 56, and 112 d of storage at 4°C. The AV and free oil were measured as described previously (14) after 3, 15, 28, 43, 56, and 112 d of storage at 4°C.

Expressible serum

Cheeses were evaluated for expressible serum (ES) after 2, 4, 6, 8, 10 and 12 d of storage at 4°C. The ES was prepared by centrifuging 160 g of finely ground cheese at 12,500 x g for 75 min at 25°C (5). Crude protein (6) and pH 4.6 soluble protein (7) in the ES were determined in duplicate. Protein and peptide profile in the ES was evaluated by a urea polyacrylamide gel
electrophoresis method (urea-PAGE) (5). Concentrations of calcium, zinc, and potassium in the ES were determined by inductively coupled plasma atomic emission spectrometry (5).

Experimental Design and Statistical Analysis

Three vats of cheese, each using a different mixer screw speed, were made on 1 d from one batch of milk. The cheese making was replicated on three different days. On each day, the order of cheese making was randomized for the three different mixer screw speeds. The data for initial chemical composition were analyzed using PROC ANOVA of SAS (SAS Institute Inc., Cary, NC). Changes in proteolysis and functional properties of the cheese, and amount and composition of the ES, during refrigerated storage were assessed using a split-plot design with mixer screw speed as a whole plot factor. PROC GLM of SAS was used.

Results and Discussion

The effects of screw speed on the residence time of the cheese in the mixer and the temperature of the cheese near the exit of the mixer are shown in Table 1. Slower screw speed resulted in a longer residence time and higher cheese temperature. Therefore, the total heat treatment of the cheese during stretching was much more severe at the slowest screw speed (5 rpm) due to the cumulative time/temperature effect.

Chemical Composition

The initial chemical compositions of the cheeses made using the three different mixer screw speeds are shown in Table 2. Screw speed did not have a significant effect on any of the parameters of composition, except for pH. Cheeses stretched at the slowest mixer speed (5 rpm) had the highest pH throughout aging (Figure 1); they also had the lowest values for titratable acidity (Figure 2). This strongly suggests that the starter culture was inactivated during stretching at the slowest screw speed due to the severe heat treatment (Table 1). Consequently, the starter was unable to ferment the residual lactose in the cheese after stretching to lactic acid, resulting in lower titratable acidity levels and a higher cheese pH.

Proteolysis

Mixer screw speed had a significant effect on the levels of pH 4.6 soluble nitrogen in cheeses during aging. The formation of pH 4.6 soluble nitrogen is a good indicator of proteolytic activity caused by the coagulant. Levels increased in all cheeses during aging, but the rate of increase was substantially lower in the cheese stretched at the slowest screw speed (Figure 3). This indicates that the coagulant was substantially inactivated during stretching at the slowest screw speed due to the severe heat treatment. This is consistent with earlier studies that showed that chymosin at pH 5.2 (the approximate pH of Mozzarella cheese during stretching) is progressively inactivated at temperatures of 65° C (148° F) and higher (8).

Similar results were obtained for 12o/o TCA soluble nitrogen during aging (Figure 4). The formation of 12% TCA soluble nitrogen is a good indicator of proteolytic activity caused by the starter culture. Levels increased in all cheeses during aging, but the rate of increase was much lower in the cheese stretched at the slowest screw speed (Figure 4). These results are consistent with the data presented above for pH and titratable acidity of the cheeses (Figures 1 and 2), and provide further evidence that the starter culture was destroyed and the starter proteolytic enzymes were substantially inactivated during stretching at slow screw speed.

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Functional Properties

Changes in TPA hardness, TPA springiness, meltability, and apparent viscosity during aging are shown in Figures 5 through 8, respectively. Although mixer screw speed did not have a statistically significant effect on any of these functional parameters, a common pattern is evident with respect to the slowest screw speed (5 rpm). Specifically, cheeses stretched at the slowest screw speed had, on the average, consistently higher values for hardness, springiness, and apparent viscosity, and consistently lower values for meltability throughout aging. These data, along with the proteolysis results, indicate that the slowest screw speed caused a slower rate of breakdown and aging of the cheese during refrigerated storage.

Changes in free oil formation during aging are shown in Figure 9. During the first few weeks of aging, free oil was similar for the three screw speeds. However, there was a sharp divergence in free oil levels during the very late stages of aging. The cause of this divergence and its practical significance are not known at this time.

Expressible Serum

Cheese samples were centrifuged to obtain expressible serum (ES). The amount of ES provides an index of the water-binding properties of the cheese. A large quantity of ES indicates that the cheese has relatively poor water-binding properties. In addition, analysis of the composition of the ES can provide insight into changes that occur in the water phase of the cheese during aging.

The percentage of ES obtained from cheeses (i.e., g ES/100 g cheese) on day 2 through day 12 of aging are shown in Figure 10. ES from cheeses stretched at the fastest screw speed (19 rpm) decreased sharply during the first two weeks of aging, indicating a large increase in water-binding properties. This is the normal pattern for Mozzarella cheese during the early stages of aging (5) and is important for the development of good shredding and melting properties. In contrast, cheese stretched at the slowest screw speed (5 rpm) showed little change in ES over the same period, indicating the persistence of poor water binding properties. Such cheese may be difficult to shred because of the presence of free moisture, and may show separation of water when melted.

The concentrations of crude protein and pH 4.6 soluble protein in the ES are shown in Figure 11. There are several noteworthy observations. First, both crude protein and pH 4.6 soluble protein in the serum increased during aging, and crude protein increased at a faster rate than soluble protein. The increases in pH 4.6 soluble protein indicate that proteolytic breakdown products (e.g., peptides and amino acids) accumulated in the serum (water phase) of the cheese. The much larger increases in crude protein indicate that not only proteolytic breakdown products (which are soluble at pH 4.6) but also intact caseins (which are insoluble at pH 4.6) accumulated in the cheese serum. The accumulation of intact αs-casein and especially, β-casein in the ES was confirmed by electrophoresis (Figure 12). Thus, the relationship between the casein matrix and the water phase of the cheese during aging appears to be quite complex, involving the release of casein breakdown products into the water phase, as well as the release of intact caseins (which are the building blocks of the casein matrix) into the water phase.

It is also evident that both crude protein and pH 4.6 soluble protein levels increased at slower rates with slower screw speed. Thus, the accumulation of both casein breakdown products and intact caseins in the serum occurred more slowly in cheeses stretched at slower screw speed.
Similar patterns were obtained for levels of calcium (Figure 13) and Zinc (Figure 14) in ES during aging. Both Ca and Zn are strongly associated with casein micelles and thus are partly located within the casein matrix of cheese. The data in Figures 12 and 13 suggest that Ca and Zn (along with intact caseins) migrated from the casein matrix to the water phase of the cheese during aging, and that the rate of migration was strongly influenced by screw speed.

Conclusion

The temperature and time exposure of the cheese during stretching is strongly influenced by the mixer screw speed. In the present study, the combination of high stretching water temperature and slow screw speed (longer residence time) resulted in substantial thermal inactivation of the coagulant and destruction of the starter culture. Consequently, proteolysis was slower, cheese pH was higher, and functional changes generally proceeded more slowly during aging. In addition, poor waterbinding properties persisted during aging in cheeses stretched at slow screw speed. Changes in the composition of the expressible serum during aging suggest that a complex dynamic relationship exists between the casein matrix and the water phase of the cheese, which may play a role in the development of functional properties, and which may be affected by thermal conditions during stretching.
Table 1. Residence time and curd temperature during stretching at three different screw speeds. Stretching water temperature was 74°C (165°F).

<table>
<thead>
<tr>
<th>Screw Speed</th>
<th>19 rpm</th>
<th>12 rpm</th>
<th>5 rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence time</td>
<td>12 min</td>
<td>14 min</td>
<td>19 min</td>
</tr>
<tr>
<td>Curd temperature</td>
<td>62°C (145°F)</td>
<td>64°C (147°F)</td>
<td>66°C (150°F)</td>
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</tbody>
</table>

Table 2. Initial chemical composition of Mozzarella cheese made with three different screw speeds. Stretching water temperature was 74°C (165°F).

<table>
<thead>
<tr>
<th>Screw Speed</th>
<th>19 rpm</th>
<th>12 rpm</th>
<th>5 rpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.32&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>5.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture, %</td>
<td>48.92</td>
<td>48.84</td>
<td>48.48</td>
</tr>
<tr>
<td>Fat, %</td>
<td>21.22</td>
<td>21.35</td>
<td>21.37</td>
</tr>
<tr>
<td>FDB, %</td>
<td>41.54</td>
<td>41.74</td>
<td>41.48</td>
</tr>
<tr>
<td>Protein, %</td>
<td>24.43</td>
<td>24.25</td>
<td>25.05</td>
</tr>
<tr>
<td>Salt, %</td>
<td>1.28</td>
<td>1.28</td>
<td>1.23</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>.71</td>
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Figure 1.- Changes in pH in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.
Figure 2. - Changes in titratable acidity in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

Figure 3. - Changes in pH 4.6 soluble nitrogen in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.
Figure 4. - Changes in 12% TCA soluble nitrogen in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

![12% TCA Soluble Nitrogen (Impact of Screw Speed)](image)

Figure 5. - Changes in TPA hardness in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

![TPA Hardness (Impact of Screw Speed)](image)
Figure 6. - Changes in TPA springiness in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

Figure 7. - Changes in meltability in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.
Figure 8. - Changes in apparent viscosity in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

Apparent Viscosity
(Impact of Screw Speed)

Figure 9. - Changes in free oil formation in Mozzarella cheese made with three different screw speeds during 112 days of storage at 4°C.

Free Oil Formation
(Impact of Screw Speed)
Figure 10. - Changes in amount of expressible serum obtained from Mozzarella cheese made with three different screw speeds during storage at 4°C.
Figure 11. Changes in the contents of crude protein (solid lines) and pH 4.6 soluble protein (broken lines) in the expressible serum obtained from Mozzarella cheese made with three different screw speeds during storage at 4°C.
Figure 12. Changes in the electrophoretic profile (urea-PAGE) of the expressible serum obtained from Mozzarella cheese made with three different screw speeds during storage for 2, 6, 8, and 10 days at 4°C. (F = fast, 19rpm; M = medium, 12 rpm; S = slow, 5 rpm). Lane 1 is a β-casein standard.
Figure 13. Changes in the content of calcium in the expressible serum obtained from Mozzarella cheese made with three different screw speeds during storage at 4°C.
Figure 14. - Changes in the content of zinc in the expressible serum obtained from Mozzarella cheese made with three different screw speeds during storage at 4°C.
REFERENCES


1996 marks the 90th year for the Marschall business. As depicted by this logo and highlighted yesterday by Norm Wood, we are proud of our innovations and the quality products that have served this industry for the past 90 years.

As proud as we are of our past, we are equally focused on just what it will take to remain competitive in the future. Business models from the 70's and 80's no longer are sufficient to ensure success in the 90's. No longer can one just focus on making the best products, waiting to take orders and ship from inventory. Successful business today is gauged more by how effective you are in helping your customers solve their problems which in turn makes you more competitive as their partner.

Successful businesses today focus on delivering the best value to their customers. And value is not just the lowest price, but the best value is determined by the best quality and service at the lowest prices.

In our efforts to make things simpler with automation and computerization, it often appears that life is becoming more complex. And everything happens so much faster. When was the last time you conducted a major business negotiation using only the traditional mail system. Remember when you used to exchange information by type written letters. Today it's more likely a fax, followed with several voice mails or E-mails, a last minute overnight FedEx, and capped off with a transatlantic or transpacific video conference. We are now literally accessible by anyone at anytime from virtually anywhere on the planet. We arrive to work in the morning to find communications at our desk requiring same day, if not immediate action. Andy Grove, the CEO of Intel, describes E-mail as "turning days into minutes." The only thing missing in this fast paced world of instant communication is time to think about what you need to communicate.

Business practices are rapidly changing, and the one technology that is driving this rate of change to faster and faster speeds is that of information processing by computers. The cost of computing power drops roughly 30% every year, and microchips are doubling in performance power every 18 months. A recent article in the Wall Street Journal predicted that by the year 2000, $1800 will buy a standard desktop computer with a 600 MHz processor (compared to an average 60 MHz today), 64 megabytes of RAM memory (compared to an average 8 megabytes today), 8000 megabytes or 8 gigabytes of hard drive data storage (compared to an average 420 megabytes today), and outside data transfer links at 100 million bits per second (compared to an average 14,400 bps today), among other features. What this all means is not only faster downloads of bigger documents (which we don't have time enough today to read), but the ability to send and receive full video to explain what's in those documents that we don't have time enough to read. In addition, the long promised voice recognition and voice-to-text transcription will likely become reality. A more recent article in PC Magazine described some computer technology still in the development pipeline at Lehigh University that ten years from now might result in a computer running at a clock speed of 10 GHz (or 10,000 MHz), packing a gigabyte of RAM memory and 100 gigabytes of storage, weighing only four ounces and capable of running for weeks on battery power.

At this rate of acceleration of data processing, it is easy to come to the conclusion that it will not be the computer that will be the bottleneck in our operations in the future, but rather how we manage this constant stream of information. It seems overwhelming at times when you stop and think about how much information crosses our desks and how rapidly
everything seems to be changing. At the current rate of change, the information available to us doubles every five years.

Predictions, according to The Food Marketing Institute, include an increasing number of supply chain partnerships, in order to reduce duplication and non value-added activities. FMI foresees more free flow of information among suppliers, processors and retailers to effectively meet the individualized needs of customers by 2005. Consumers equipped with computer technology will be able to shop for much of their food requirements from the comfort of their homes, and home delivery of food will surely increase to the extent that "mobile supermarkets" and restaurant fare prepared on the road may become a significant part of the food supply chain of the year 2005.

But change is only relative. It's like walking on a treadmill; as long as you keep up with the pace, things around you remain in focus and are manageable. But once you stop walking, you rapidly lose control and eventually fall off the back end flat on your back.

Our successors will look back someday (reading our computer backup disks of archived E-mails) and say, "they wrote a lot about change back in 1995, how fast-paced and chaotic the workplace was becoming; but they seemed to contradict themselves as they wrote often about these strange things called "jobs" where the same people came to work at the same time everyday, at the same place, did virtually the same tasks everyday, and got paid the same whether or not the business made a profit or lost money." Jobs, at least as we know them, developed for the Industrial Age, may someday prove not to be the most effective way to get work done in the so-called Information Age.

At Rhône-Poulenc Dairy Ingredients, we see and feel change all around us. Our market is becoming more global, our customers more multinational. Our customers are demanding better value...higher quality products with expert technical assistance when and where they need it, all at lower prices. In the remaining minutes, I would like to highlight a few of the changes that RPDI will be making in 1996 that hopefully will improve our ability to deliver the best value to our customers.

As most businesses today, we have focused our efforts on satisfying our customers, finding out what they want, need and desire; and then determining how to go about exceeding those expectations. Our business strategies center around the core objectives illustrated in this slide.

CUSTOMER VALUE is the pinnacle of our efforts, delivering quality and service at the lowest prices. The three previous speakers from RPOI highlighted some of the product INNOVATIONS that we are bringing to our customers. In addition, INNOVATION is increasingly being directed beyond just "product" and more towards "process" to learn how to make things work better in faster times at cheaper costs. All this results in higher levels of PRODUCTIVITY, leading to lower costs and strong profitability that enables continued investment in this business. And finally, at the heart of our business is our people, the only true asset that is irreplaceable. COMMUNITY collectively describes the environment that we are attempting to build at RPDI that will ensure we continue to retain and attract the best people in this industry to serve our customers.

In 1996, RPDI will be moving its "problem-solving capability closer to the customer." Taking advantage of wireless communications, notebook computers and electronically accessible information, RPDI will network its team of field experts into an integrated customer resource. This decentralization of our technical service should reduce the bottleneck caused by centralized decision-making and improve our overall responsiveness to customer problems. Made possible by a few personnel relocations, we hope we will be in a better position to service a greater portion of the U.S. market.
Increasingly we see our largest customers getting bigger and bigger. As a result, products we have designed for general market use are no longer sufficient. In fact, some of these larger customers are essentially becoming their own market, or what I refer to as a “market of one.” Internally we are challenged to take advantage of the scale of economy of mass production to attain the lowest costs, yet customize for individual customer needs. We have successfully mass customized our line of freeze dried cultures for cheese, yogurt and buttermilk or sour cream. And as Kevin Gillies alluded to in his presentation earlier, we continue to explore opportunities that will allow better delivery of our expertise in lactic acid bacterial biomass production to some of our customers.

And finally, we have recognized that all our customers are not the same; their needs are different depending on the styles of cheese they produce, the size of their operation and the customers they serve. To better serve these sometimes divergent customer needs in 1996, RPDI will dedicate an internal group, to become known as Marschall Specialty Ingredients, to serve the smaller operations that more frequently specialize in specialty cheese manufacture. Their problems are often different than those of the large processors who focus on large scale commodity cheese production. More information regarding Marschall Specialty Ingredients will follow as we get closer to 1996.

Forecasting the future is sort of a two-edged sword. It is impossible to predict what is going to happen in the coming years; but then again, at this point in time no one can say with absolute confidence that what you predict today will not eventually occur.

Looking a few years into the future, as the business director for RPDI, I predict that the roles of suppliers and customers will continue to move closer together as efforts are made by both parties to eliminate non value-adding activities in the food supply chain. Success in the future will come from “true” partnerships where mutual benefit is delivered to all the parties involved. In other words, more win-win and fewer win-lose relationships.

More specifically for RPDI, I foresee that the utility of lactic acid bacteria will go beyond that of just stable acid production. It will expand into a role of delivery vehicles for a variety of functional proteins to add new value to the finished food product. In addition, our expertise in producing lactic acid bacterial biomass could move beyond that of only producing products that we sell to you to a more direct supporting role in some form of on-site managed starter programs. Once again, the word partnership best describes the integrated relationship between RPDI and its customers as we work together to improve our processes to gain improved quality, reliable supply and responsive service.

Many changes have occurred in the first 90 years of Marschall history, and considering the accelerating pace of change today, I fully expect to see many more changes to our business and the dairy industry in the coming years. We figure that we must have done some things right during the first 90 years to still be around, and we are looking forward to the next 90 years of successful partnerships with our dairy customers.

Thank you.