9-11-1991

Proceedings from the 28th Annual Marschall Italian Seminar

Various Authors

Follow this and additional works at: https://digitalcommons.usu.edu/wdc_conference

Part of the Food Science Commons

Recommended Citation
https://digitalcommons.usu.edu/wdc_conference/18

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Cheese Industry Conference by an authorized administrator of DigitalCommons@USU. For more information, please contact digitalcommons@usu.edu.
PRESENTED BY
MARSCHALL PRODUCTS
A BUSINESS UNIT OF RHÔNE-POULENC
MADISON, WISCONSIN
IN CONJUNCTION WITH
SEMINAR EXHIBITORS
AND NON-EXHIBITING HOSTS
SEPTEMBER 11 & 12, 1991

THE FORUM
DANE COUNTY EXPOSITION CENTER
FAIRGROUNDS DRIVE
MADISON, WISCONSIN
PROCEEDINGS from the 28th ANNUAL MARSCHALL ITALIAN CHEESE SEMINAR
September 11 & 12, 1991

Sponsored by:
Rhône-Poulenc Marschall Products
P.O. Box 592
Madison, WI 53701
<table>
<thead>
<tr>
<th>Paper No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991-1</td>
<td>“Positioning Wisconsin-made Italian Cheeses in the Marketplace”</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>by Ms. Andrea Neu, Vice President of Marketing Services, Wisconsin Milk Marketing Board</td>
<td></td>
</tr>
<tr>
<td>1991-2</td>
<td>“Evaluating Performance of Thermophillic Coccus-Rod Cultures”</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>by Dr. William Sandine, Oregon State University</td>
<td></td>
</tr>
<tr>
<td>1991-3</td>
<td>“Modifying Stretch, Melt, and Cook Color in Mozzarella Cheese”</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>by Dr. Craig Oberg, Weber State University, School of Microbiology</td>
<td></td>
</tr>
<tr>
<td>1991-4</td>
<td>“Mozzarella Cheesemaking in Italy”</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>by Mr. Giovanni Stefanini, Marketing Manager, Lacto-Labo Products, Rhône-Poulenc Italia S.p.A.</td>
<td></td>
</tr>
<tr>
<td>1991-5</td>
<td>“Care and Maintenance of Salt Brines”</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>by Dr. William Wendorff, Asst. Professor, University of Wisconsin-Extension, Dept. of Food Science</td>
<td></td>
</tr>
<tr>
<td>1991-6</td>
<td>“Antibiotic Residues in Milk; How It Happens and What’s Being Done To Prevent It”</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>by Dr. Alan Bringe, University of Wisconsin, Madison, WI</td>
<td></td>
</tr>
<tr>
<td>1991-7</td>
<td>“Relationship between Mozzarella Manufacturing Parameters, Cheese Composition, and Functional Characteristics: Development of a System for Controlled Research Studies”</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>by Dr. David Barbano, Cornell University, Dept. of Food Science</td>
<td></td>
</tr>
<tr>
<td></td>
<td>by Dr. Paul Kindstedt, The University of Vermont</td>
<td></td>
</tr>
<tr>
<td>1991-9</td>
<td>“Manufacturing Reduced Fat Cheese with SImplesse®”</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>by Ms. Rene Snook, Account Manager, Cheese/Fats/Oils, The NutraSweet Company</td>
<td></td>
</tr>
</tbody>
</table>
POSITIONING WISCONSIN-MADE ITALIAN AND SPECIALTY CHEESES IN THE MARKETPLACE

by Andrea Neu

ABSTRACT

Since 1983, the Wisconsin Milk Marketing Board, funded by the 33,000 dairy farmers, has formed a unique partnership with the Wisconsin cheese manufacturers in their efforts to market the products they make with advertising, promotion, education and research programs.

These marketing programs have focused on Wisconsin's cheese quality and cheesemaking expertise as the foundation for the strategic direction that WMMB has set for our programs and services. Communications strategy was developed to capitalize on the equity in the name "Wisconsin" which has been confirmed through consumer and foodservice market research.

This presentation will give an overview of WMMB's programs and services delivered during the past six years and describe some of the tactics that have specifically helped to position Wisconsin-made specialty and Italian-type cheeses in the U.S. marketplace.

Since July 1983, the Wisconsin Milk Marketing Board (WMMB), funded by the state's dairy farmers, has formed a unique partnership with the Wisconsin cheese manufacturers, marketers and our marketing partners within the channels of distribution.

WMMB's marketing programs and services are designed to support our partners in the retail, foodservice and food processing markets. We have targeted these distribution channels because, it is estimated that the 1.9 billion pounds of cheese produced in Wisconsin each year is divided nearly equally between the three markets. (34% retail/consumer market; 34% foodservice; 32% industrial/food processing.)

The presentation today will focus primarily on WMMB's efforts to reach the consumers or end-users of cheese within the retail and foodservice markets. You will see examples of how we have capitalized on the equity in the name WISCONSIN and have used the equity as our marketing hook and point of differentiation for Wisconsin-made cheeses. The program will focus on Italian-type and specialty cheeses since both of these categories have been extremely important to the growth of the U.S. cheese industry, especially for Wisconsin.
In 1984, total Italian cheese production in the U.S. was 1.3 billion pounds, compared to 2.2 billion in 1990. During that same time period, Wisconsin’s production grew from 441 million pounds to 693 million — and within the “All Italian” category, Italian specialties or “Other Italian” produced in Wisconsin grew nearly 16%, from 116 million in 1984 to 134 million pounds in 1990. However, even more indicative of the importance of these Italian cheese varieties to the Wisconsin industry is the fact that Wisconsin has experienced a 613% growth in this total category from 1964 to 1990.

The second cheese category that has also been extremely important for the Wisconsin cheese industry is the specialty cheese or “Other” types, as identified and tracked by USDA and Wisconsin Dairy Statistics. Wisconsin production in this category grew from 193 million pounds in 1984 to 263 million in 1990 — a 36% increase.

The increased production in this specialty category has been important to WMMB and its marketing partners because more value-added cheese products have become available for buyers who are already sold on Wisconsin and its cheese quality. As a result, Wisconsin specialty cheeses have become the cornerstone for WMMB’s positioning of “Wisconsin cheese ... top quality, over 200 varieties and great taste.” These three key messages provide the focus for our communications strategy. They provide strong marketing advantages and points of differentiation for Wisconsin cheese. WMMB has used these key messages in several different ways during the past six years to set the strategic direction for our Wisconsin cheese marketing campaigns.

To give you the historical marketing perspective, WMMB made the decision to build on Wisconsin’s reputation for quality cheese and the well-established equity in the “Wisconsin” name after completing consumer research with MARKET FACTS in 1985. This study was conducted in ten major U.S. markets and confirmed that positive consumer perceptions of Wisconsin cheese were high. Seventy-two percent of the consumers interviewed believe “Wisconsin makes the best quality cheese” and 82 percent would “prefer to buy cheese from Wisconsin.” Consumer attitude research also continues to show that taste and quality are key factors in the cheese purchase decision and Wisconsin cheese delivers on both of those purchase criteria.

This consumer research was used as the basis to position Wisconsin cheese, and Italian cheese specifically, as part of WMMB’s first advertising campaign in 1985 — “Mmm Wisconsin... the Cheese More People Choose.” These “Taste our Claim to Fame” TV commercials were created to reinforce the consumer preference for Wisconsin-made cheeses and to increase awareness of the quality, variety and taste messages. The 30-second spot showed “the Italian cheeses of Wisconsin” in the most popular and appetite appealing dishes — pizza, pasta and snacks —
which demonstrated the characteristic melts, stretch and grating applications for the Italian varieties.

[Play :30 Italian TV Spot]

America's growing love affair with ethnic foods — especially Italian dishes — has had a significant impact on the growth of the entire domestic cheese industry during the past two decades. Pizza, in its many forms and styles, is the all-time favorite. The following trends and sales data illustrate just how important Italian foods are for Wisconsin's cheese industry:

- Frozen pizza is a $1 billion market today registering a 7 percent annual growth for the past two years;\(^4\)

- Chicago is the No. 1 frozen pizza market with $69 million in sales and New York is No. 2 with $57 million;

- However, total retail sales of frozen pizza have declined since 1984, from a 73 percent share in '84 to a 54 percent share in '91.\(^4\)

- Meanwhile, freshly prepared pizza purchased from supermarket deli departments has increased its share from 21 percent in 1984 to 40 percent in 1990.\(^5\)

- Pizzerias increased in number from nearly 36 thousand in 1985 to over 51,000 in 1990.\(^6\) These pizza places, particularly suited to satisfy both the in-home and in-restaurant demands of the era, lead all categories of restaurants with significant unit growth from 1982 to 1989.\(^5\)

- Italian restaurants serving a broader menu of Italian entrees grew 70 percent from 1987 to 1990.\(^5\)

- The categories of pizza and Italian restaurants together also grew significantly in customer traffic from 1982 to 1989, with pizzerias increasing 75 percent and Italian eateries increasing 50 percent.\(^6\)

- Since 1984, the average U.S. household is spending 50 percent more on pizza and eating it a third more often.\(^4\)

- Gourmet pizzas and pasta are hot trends in the foodservice and retail markets.\(^6\)
According to the International Dairy-Deli-Bakery Association (IDDA) 1991 “What’s in Store” Trends Analysis, the most important trends in cheese growth today are:

- Cheese as an ingredient in prepared and take-home entrees and the continuing popularity of pizza;
- Cheese as a convenience food;
- Growth of low-fat, low-sodium or low-cholesterol cheeses;
- Healthfulness of cheese as a protein and calcium source and perceived as “something good” for you;
- Growth of natural and specialty cheeses;
- Cheese as a “status food”.

TRANSLATING TRENDS TO MARKETING STRATEGY

Now, I would like to share some examples of how WMMB has developed marketing programs and services which take advantage of these cheese growth trends and help position Wisconsin-made Italian and specialty cheeses in the market place.

Our second Wisconsin cheese campaign introduced in 1987 positioned all varieties of Wisconsin’s award winning cheeses as specialty cheeses — or “status foods” — in one of the 30-second TV spots called “Winners” or “America’s Best-Tasting Cheeses.”

[Play :30 “Winners” TV Spot]

This campaign strategy encouraged Wisconsin manufacturers to label their cheese products as made in “Wisconsin” and told buyers to look for the word “Wisconsin” (or the Real Wisconsin Cheese symbol) on the package. The “award winning” message was included in point-of-sale (POS) materials, educational materials and print ads. It was also extended into our co-op advertising program with retailers, which resulted, through the efforts of WMMB’s five Regional Marketing Managers, in blue ribbon ads like this.
Foodservice print advertising also focused on the award-winning cheeses and cheesemaker expertise for Italian and specialty cheeses. Wisconsin cheese varieties most often sold in the deli or in upscale restaurants have been positioned against imported varieties as "very special cheeses imported from a very special place."

WMMB has been a leader in cheese promotion programs for the foodservice market, by developing innovative promotional and educational materials and programs for foodservice operators and distributors. One example is the "Profiles" series, which offers basic information on how to select and handle the many Wisconsin cheese varieties and ideas on how operators can incorporate those varieties as part of their menus.

WMMB's most recent campaign also offered foodservice operators more specific menu enhancement ideas. This "Improve the State of Your Menu with Wisconsin Cheese" promotion will be extended this year to expand the quality message and to position Wisconsin as the source for a broad variety of specialty cheeses that can be incorporated into existing menus as signature items. This will add value and strong customer appeal as the operators feature Wisconsin cheese varieties as a quality statement on their menus.

WMMB's foodservice marketing strategy also includes a strong distributor program, which targets 40 of the top 50 full-line distributors and 7 of the top 9 distributor buying groups in the U.S. This comprehensive foodservice program includes print materials for use as leave-behinds by DSRs. Depending on the extent of the contracts with the distributor, many of the programs and materials are customized. The programs may include co-op advertising, education and training services, special events, tours and incentive programs. Many times Italian cheese varieties are specifically included in these market plans.

Co-op advertising programs with targeted multi-chain operators have also been a significant part of WMMB's foodservice program during the past three years. And several cooperative efforts have included pizza chains like Pizza Pit, Rocky Rococo and Pizza Inn. Tombstone Pizza has also featured Real Wisconsin Cheese in their marketing programs as a value-added quality statement.

[Play 3 TV Commercials - Foodservice]
Trade shows and conferences are also important communication vehicles that are used to showcase Wisconsin cheese varieties, our manufacturers and WMMB programs and services.

One trade show is especially important for Italian cheese manufacturers. WMMB has played an active role in the Pizza Expo for the past three years, offering educational seminars, and last year sponsoring a booth to highlight the Wisconsin Cheese Pizza Blends test kitchen study conducted by WMMB in 1990. Pizza Expo will continue to be a priority show for WMMB in ’92, as our staff will again be part of the educational seminars.

The National Restaurant Association Show and the International Dairy-Deli-Bakery Association’s Expo & Seminar are also key shows that enable WMMB to position Wisconsin-made Italian and specialty cheeses as the best. These shows give our staff an opportunity to showcase Wisconsin cheese products through education in our booths as well as through special events, seminars and training.

Over the years, WMMB has been recognized for its expertise in cheese merchandising and educational materials and promotional programs. An important part of WMMB’s strategy for our national communications program has been to be an innovator and a trend setter, putting this organization on the leading edge with promotional ideas to reach food opinion leaders in the retail, foodservice and consumer markets.

WMMB tactics that have built on the cheese growth trends summarized earlier from the IDDA Trends Analysis include the areas of 1) “cheese as an ingredient” and a 2) “convenience food” to fit with today’s consumer lifestyles. Here are three examples of promotions conducted by WMMB during the past few years that directly impact the Wisconsin-made Italian and specialty cheese varieties. They include 1) the Chef Showcase™ sponsored annually, 2) food page publicity in national consumer, retail and foodservice publications and 3) the successful launch of Wisconsin-Style HavartiR, which was developed and offered to the Wisconsin cheese industry through WMMB’s research and business development program.

The Chef Showcase™, held in April 1990, focused on supermarket deli/foodservice chefs. This event resulted in extensive media coverage for Wisconsin cheese and garnered important positioning and visibility for Italian cheeses. The winning recipe, “Rigatoni Con Quattro Fromaggi”, prepared by Nancy Lazara of Larry’s Markets, Seattle, Washington featured three Italian varieties — Parmesan, Romano and Mozzarella. Additionally, three other Italian varieties were featured in the published recipes. This competition for the supermarket chefs was a “first” for the new and rapidly expanding deli/foodservice industry. The WMMB event was recognized by food opinion leaders as a leading edge promotion and was greatly appreciated by the supermarket industry.
The Pizza Chef Showcase™ completed in April of this year in Chicago, was a tribute to the importance of pizza and its role in the growth of Wisconsin cheese production. Thirteen trend-setting pizza chefs from across the nation were invited to compete in the three categories of (1) Traditional Pizza, (2) New Wave Pizza and (3) Supermarket Pizza. Their signature pizza recipes used 16 varieties of premium Wisconsin Italian and specialty cheeses with creative entries ranging from a breakfast pizza to a two-course pizza for two. Publicity results included coverage in the foodservice and retail trade publications as well as consumer media coverage both nationally and regionally. During the past three years, national publicity for Wisconsin's Italian and specialty cheeses has hit the food sections and front pages of major national newspapers and consumer magazines. Examples include feature stories in Redbook, Country Living, the Washington Post, and the Chicago Tribune.

In addition to editor tours and special events, WMMB prepares full-color ROP food pages that generate at least five million circulation per release in major U.S. newspapers. A recent release featured the “Wisconsin Cheese...Your Grand Finale” dessert booklet. This beautiful booklet puts Wisconsin Asiago, Gorgonzola, Fontina, Ricotta and Mascarpone in starring roles. The specialty cheese educational piece also promotes Wisconsin Muenster, Aged Cheddar, Swiss, Brie, Edam, Gouda and Cold Pack. This promotion strategy builds on two of the IDDA cheese growth trends, 1) “cheese as a status food” and 2) “growth of natural and specialty cheeses”.

Another example of WMMB's marketing strategy which takes advantage of the growth trends in the specialty cheese market is the launching of the technologies, make-procedures and promotional support for new varieties like Wisconsin-Style Havarti®. This project was the first phase of WMMB's research program to expand and promote the development, production and marketing of new specialty cheeses by Wisconsin cheesemakers and marketers.

A final example of WMMB marketing strategies designed to capitalize on the IDDA cheese growth trends summarized in the '91 Trends Analysis is in the area of (1) “reduced fat and sodium cheeses” and the reinforcement of the (2) “healthfulness of cheese as an essential nutrient source” and a “something good for you” food. One of WMMB's newest educational efforts focuses on the 16-page booklet, “A Slice of Lifestyle...A Guide To your Healthy Love of Cheese.” This booklet offers options for cheese lovers as they plan their food choices. It helps them understand the role that cheese can, and should, play in their diets — even if they are trying to follow the new Dietary Guidelines. The “Lifestyles” brochure will be a integral part of our communications strategy as we head to New York this fall to visit with major food editors from the top 25 consumer and foodservice magazines. Positioning Italian and specialty cheeses from Wisconsin, in particular Mozzarella, String and the hard grating cheeses is an important part of the "Lifestyles" message that cheese fits in naturally! The reduced fat cheese category, which has become a significant part of
the Wisconsin specialty cheese message, is strongly communicated in the booklet. The new cheese category highlights the expertise and know-how of the Wisconsin cheesemaker.

As WMMB heads into its seventh year of marketing programs dedicated to the promotion of Wisconsin-made cheeses, you will see even more emphasis on Wisconsin cheesemaker expertise, Wisconsin food traditions and the imagery of America's Dairyland and its natural environment for dairy product production.

The strategy behind the current advertising campaign entitled, “When the Meal Comes from Your Heart, Shouldn't the Cheese Come from Wisconsin?™” is to build an emotional equity for Wisconsin Cheese.

(Play :30 "Meals" Tv Spot]

The promotional elements of the campaign are designed to answer the question — "What is a Wisconsin?"

WMMB will continue to build on the consumer/buyer franchise the Wisconsin industry already has with the equity that's been built into the name "Wisconsin." The campaign includes promotions like the "Wisconsin Country Cafe" which is currently available to supermarkets across America. The holiday promotion, scheduled for November and December, is entitled "Take Home the Holiday Traditions of Wisconsin Cheesemakers" — an extension of the tone and image established with the County Cafe promotion.

This plan should continue to help WMMB and all of our marketing partners position Wisconsin-made Italian and specialty cheeses as the ultimate in cheeses available in today's market place.

In closing, I want to congratulate the Italian cheese industry here in Wisconsin and throughout the U.S. for their foresight, hard work and craftsmanship in making Italian cheese varieties the very first domestically-produced specialty cheeses. You have a lot to be proud of. And you have a lot to look forward to as the growth potential for Italian and specialty cheeses continues to point upward.

WMMB also thanks Rhone-Poulenc/Marschall Products for their vision 28 years ago in creating a forum for the exchange of ideas and information that directly benefits the Italian cheese industry by keeping everyone abreast of new technologies, new equipment and new marketing ideas.
1 National Dairy Board Estimates: 1990
2 Wisconsin Dairy Statistics 1964-1990
4 Pizza Study: December 24, 1990
5 RE-COUNT data presented at COEX '91
6 CREST data presented at COEX '91

* Based on data from MRCA, Data Development Corp., Foodservice Operators Study and USDA Commercial Disappearance.
EVALUATING PERFORMANCE OF THERMOPHILIC COCCUS-ROD CULTURES

W. E. Whitehead, M. E. Matalon, J. W. Ayres* and W. E. Sandine
Department of Microbiology and School of Pharmacy
Oregon State University

Introduction

Demand for cheeses produced using thermophilic coccus-rod (C/R) cultures has increased tremendously during the past 2 decades. Noteworthy in this regard has been increased per capita consumption of Mozzarella cheese used in pizza and in a variety of home-cooked dishes. Number of factories producing Mozzarella cheese in the United States, about 200, has not changed significantly over this time, creating production pressures, especially where thermophilic starter cultures are concerned. Since 1979 we have had an active research program designed to improve bulk starter growth media. Recently we have turned our attention to C/R culture media for growing Streptococcus salivarius subsp. thermophilus (S. thermophilus - St) and Lactobacillus delbrueckii subsp. bulgaricus (L. bulgaricus - Lb). Herein we report some of our findings.

Optimum Temperature

Early on we wanted to determine optimum milk growth temperatures for representative St and Lb strains isolated from commercial cultures. It was felt that this would enable us to test the often stated theory that growing C/R cultures at higher temperatures (108-112°F) would favor rod growth while growing them at lower temperatures (89-95°F) would favor coccus growth. We examined 9 strains each of rods and cocci from commercial cultures. In general the cocci grew optimally at 101°F while rods showed optimum growth at 112°F. Optimum temperatures for acid production averaged 110°F and 112°F for cocci and rods, respectively. Table 1 shows results for individual strains and the data suggest that cocci produce acid better at the optimum growth temperature for rods rather than at their own optimum growth temperature.

Next we combined cocci and rods with divergent optimum temperatures and grew them at each of their respective optimum temperatures to see what influence this would have on C/R ratios. In so doing we could not influence the C/R ratios. It seems, therefore, that the synergism between strains in C/R cultures is more of a deciding factor in strain balance, or lack thereof, than growth temperature and it is well known that mixtures of cocci and rods grow better than single strain cultures of either. This is due to stimulation of the cocci by amino acids liberated from milk protein by the rods and stimulation of the rods by formate and carbon dioxide.
produced by the cocci. More details on the influence of temperature on associative
growth of C/R cultures can be found in the paper by Radke-Mitchell and Sandine (6).

**Proteolysis**

Another factor which influences C/R ratios is the amount of proteolysis carried out
by individual strains. Table 2 shows data from Rajagopal and Sandine (8). It may be
seen that cocci are weakly proteolytic, and liberate only 2 to 15 micrograms of
tyrosine per ml from milk proteins. The rods, on the other hand, liberate from 60 to
150 micrograms per ml. Oberg et al. (5) have made similar findings for rods and
noted further that Mozzarella cheese made with proteinase deficient strains of St
and Lb produced cheese with poor melting characteristics (4).

**Determining C/R Ratios**

There appears to be no standardized method for determining C/R ratios in frozen
concentrated bulk set cultures, bulk starter or cheese. In cheese factories this is
done microscopically and therefore one cannot distinguish between live and dead
cells. Rods may be dead, especially in frozen bulk sets or holdover starter. In fresh
bulk starter and cheese, stained smears likely present mostly live cells but, even so,
reporting the C/R ratio is problematic. How does one count cocci and rods to
determine the ratio? For it to correlate with viable counts one must estimate the
number of cell groupings, such as chains or clumps, which likely will generate a
colony for plate count determination. Another way is to estimate the relative density
of cocci and rods as they appear in the stained smear. Figure 1 presents examples
of ratios so reported. It seems useless to count individual coccus and rod
cells to
determine the ratio since this would be quite tedious and cells likely do not function
in the cheese equally as single entities.

For fresh bulk cultures and frozen concentrates, starter may be diluted 1:10,
blended (or not, as practices vary) 30 seconds and a uniform aliquot (.01 ml) taken
and spread uniformly on the glass slide. Methylene blue commonly is used as the
stain by flooding the heat-fixed smear for about 2 minutes. For cheese, the sample
needs to be blended or stomached at a 1:1 dilution with an effective cell-releasing
emulsifier such as warm (110°F) 2% sodium citrate. Smears on glass slides then are
prepared as usual but need to be defatted using xylene.

A satisfactory method of determining viable C/R ratios is use of the hydrophobic grid
membrane filter (HGMF) procedure described by Millard et al. (3). The HGMF is a
0.45 micron pore diameter size filter with water-repelling grid lines dividing the
membrane into 1600 individual compartments. The diluted cheese or culture sample
is filtered through the HGMF apparatus impinging cells on the filter within the
separated squares. The filter then is placed on the surface of an MRS agar plate
containing 0.02% erioglaucine. After incubation at 37°C for 72 hours, St colonies grow out as small light blue colonies while Lb colonies are larger and dark blue. Bacterial numbers are determined by the MPN method according to the AOAC procedure (1) using the formula MPN = \[ \log_{e} \left( \frac{N}{(N-X)} \right) \] where N = 1600 and X = number of squares occupied.

The desirable or acceptable C/R ratio in Mozzarella cheese starters ranges from 1:1 to 5:1 and total counts on the starter should be \( 10^8 \) to \( 10^9 \) cfu/ml. For a bulk culture with \( 6 \times 10^8 \) total cells per ml, a C/R ratio ranging from 1:1 to 5:1 would have, for example, counts of \( 3 \times 10^8 : 3 \times 10^8 \) to \( 5 \times 10^8 : 1 \times 10^8 \) cfu/ml, respectively. Notice that the counts of both rods and cocci are logarithmically the same (10^8), even though there are 5 times more cocci than rods at the 5:1 ratio. In actual numbers this is 500,000,000 cocci and 100,000,000 rods which are the same or similar numbers considering variations in counting procedures for bacteria by either microscopic or plating procedures. So ranges in C/R ratios of 1:1 to 5:1 provide reasonable assurance that both types are present in sufficient numbers to contribute to acceptable cheese quality where texture is concerned. An excess of rods (e.g. C/R = 1:10 or less) can result in soft cheese and an excess of cocci (e.g. C/R = 10:1 or greater) can result in “short” cheese with poor melting properties.

**Thermophilic Starter Media**

Much less has been written on this subject than for mesophilic starter media, though a few recent papers have appeared (2, 9, 11, 12). In the ideal bulk tank starter fermentation, cocci begin growth, providing carbon dioxide and formate which then stimulates rod growth which enables cocci to keep growing for reasons pointed out above. Such does not always occur, especially if bacteriophages, antibiotics or other inhibitors are present. St is very sensitive to antibiotics. Also, some drugs used to treat mastitis are sufficiently heat resistant to be present in dry milk or whey used to blend with other ingredients to produce starter media. Furthermore, inhibitory levels of acetate may be present in dry whey if it originated in a factory producing Mozzarella by the direct acid procedure. Residual detergents also can be inhibitions for St and Lb. So slow starters can be caused by a number of factors other than bacteriophages, though phages are easily detected in whey from Mozzarella cheese factories (7). Phages for cocci are most common but rod phages appear also (Table 3).

Our experience indicates that rod phages are much less specific for cation use in replication. Media containing sufficient phosphate and/or citrate to inhibit C/R phages do not support good culture growth (13). This places a high premium on sanitation in Italian cheese factories. We have reported successful inhibition of laboratory maintained coccus and rod phages in a C/R starter medium (2) but field experience with this medium was disappointing. A revised formulation is much improved but still not absolute in controlling phage replication.
Several Italian starter media are now on the market and are prepared differently as to solids level, and type of neutralization. Reddy and Richardson (10) suggested the use of externally neutralized whey starter medium but no mixed C/R culture performance data were or have since been presented. Other media neutralization systems have been described by Khosravi et al., (2) and include use of various neutralizers (KOH, NH₄OH and Cul-Sure Activator) used once or more during fermentation to achieve various pH levels. One new medium on the market requires no neutralization.

We recently compared these media for growth, activity of cells generated, C/R ratios and phage inhibition (11). Six different media were compared, A-G, and Figures 2 through 4 present the data generated following manufacturer's guidelines in starter preparation and growth.

Figure 2 shows the fate of phages specific for cocci and rods when added to the commercial media at 10² plaque-forming units per ml. None inhibited the rod phages and only medium D prevented replication of the coccus phage. Figure 3 shows the effect of the added phages on the proportion of cocci and rods present in the mature culture. For the particular C/R phages used in this experiment, the rods were more susceptible than the cocci except in medium B. Another set of phages and hosts likely would have generated different data and again we can see that phages replicated in these media. Figure 4 compares the activity of cells generated in the different starter media as measured by pH change caused by a 1% inoculum grown in 9% nonfat milk for 2.5 hours at 108°F. All media except B had reduced activities in presence of the specific coccus and rod phages. Except for medium B, inhibition resulted in all media.

Coccus-Rod Ratios in Frozen Concentrates:

Most bulk starters for Italian cheese are made by inoculation of the medium with frozen concentrated bulk set cultures. Since both cocci and rods are expected to grow so as to provide a desired amount of each in the mature starter, we thought it worthwhile to compare microscopic and viable counts of such commercially prepared starters. Microscopic counts of stained smears were estimated by counting as one each chain or clump of cells expected to produce a colony. Freshly grown cultures offer good agreement between viable colony counts and microscopic counts when handled in this manner.

Figure 5 shows data for different cans from three different lots of frozen starter from manufacturer A. It is clear that some rods seen microscopically are dead. This led us to compare viable counts of cocci and rods from other manufacturers and percent live rods in their cultures are shown in Figures 6 through 6. These data are not alarming but do reveal that certain cans of certain cultures are very deficient in rods.
Activity of Stored C/R Cultures:

This topic has been studied to determine if starter media could be developed which would allow Italian bulk set cultures to maintain acid-producing activity for desired time periods when stored frozen (-40°F), refrigerated (35°F) or at room temperature (77°F) in the growth medium. Tables 4 through 6 present the data. Tables 4 and 5 for two different C/R cultures show that those grown in an internally neutralized medium with 6% added malt extract maintain acid-producing activity for at least 8 weeks when frozen and stored at -40°F. Cultures grown in nonfat milk and frozen therein show activity loss by only one week. Table 6 shows that C/R culture R6 grown in the internally neutralized medium maintained activity for 12 days when refrigerated (35°F) and about 5 days at room temperature (77°F). Similar data have been found for other C/R cultures. Therefore improved storeability of C/R cultures can be achieved at all temperatures by using internally neutralized media but possible different effects of such storage on cocci versus rods needs to be assessed.

Conclusion:

From the days when whey or milk grown starters were used exclusively in Italian cheese plants, considerable progress in starter media development has been made. While phage inhibitory starter media for mesophilic starters now are readily available, such is not the case yet for thermophilic starters. Some are better than others in this regard, however. Currently available high performance media are an asset to Italian cheesemakers but continued vigil to minimize bacteriophage infection of starters is essential. Sanitation, air flow control and culture rotation are important in this regard. Hopefully the progress now being made to genetically construct phage resistant mesophilic starter strains will soon also be possible for thermophiles.
References


Table 1: Optimum temperatures for growth and acid production by coccus and rod cultures isolated from commercial thermophilic cultures

<table>
<thead>
<tr>
<th>Coccus</th>
<th>Growth</th>
<th>Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>108</td>
<td>118</td>
</tr>
<tr>
<td>14</td>
<td>100</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>106</td>
<td>111</td>
</tr>
<tr>
<td>5</td>
<td>95</td>
<td>111</td>
</tr>
<tr>
<td>39</td>
<td>100</td>
<td>104</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>109</td>
</tr>
<tr>
<td>Y</td>
<td>104</td>
<td>109</td>
</tr>
<tr>
<td>15</td>
<td>100</td>
<td>111</td>
</tr>
<tr>
<td>OP</td>
<td>100</td>
<td>109</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rod</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>113</td>
<td>113</td>
</tr>
<tr>
<td>14</td>
<td>111</td>
<td>115</td>
</tr>
<tr>
<td>2</td>
<td>111</td>
<td>102</td>
</tr>
<tr>
<td>5</td>
<td>109</td>
<td>120</td>
</tr>
<tr>
<td>39</td>
<td>111</td>
<td>102</td>
</tr>
<tr>
<td>1</td>
<td>115</td>
<td>117</td>
</tr>
<tr>
<td>Y</td>
<td>111</td>
<td>115</td>
</tr>
<tr>
<td>15</td>
<td>115</td>
<td>118</td>
</tr>
<tr>
<td>OP</td>
<td>109</td>
<td>109</td>
</tr>
</tbody>
</table>
Table 2. Proteolytic activity of coccus and rod cultures incubated in nonfat milk at 108°F for 4 hours

<table>
<thead>
<tr>
<th>Strain</th>
<th>Coccus</th>
<th>Rod</th>
<th>Mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8.2</td>
<td>68</td>
<td>350</td>
</tr>
<tr>
<td>14</td>
<td>9.3</td>
<td>126</td>
<td>151</td>
</tr>
<tr>
<td>15</td>
<td>2.1</td>
<td>--</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>98</td>
<td>294</td>
</tr>
<tr>
<td>2</td>
<td>10.8</td>
<td>92</td>
<td>310</td>
</tr>
<tr>
<td>404G</td>
<td>2.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>39</td>
<td>11.3</td>
<td>88</td>
<td>278</td>
</tr>
<tr>
<td>OP</td>
<td>14.8</td>
<td>145</td>
<td>187</td>
</tr>
<tr>
<td>1</td>
<td>5.3</td>
<td>80</td>
<td>257</td>
</tr>
<tr>
<td>oly</td>
<td>--</td>
<td>100</td>
<td>--</td>
</tr>
<tr>
<td>Y</td>
<td>--</td>
<td>59</td>
<td>--</td>
</tr>
</tbody>
</table>

μg tyrosine liberated per ml
Table 3. Presence of bacteriophages in whey from Mozzarella cheese factories as detected by lysis (+) of coccus or rod cultures

<table>
<thead>
<tr>
<th>Coccus Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>39</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>404G</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rod Strain</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>OLY</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Rajagopal and Sandine (7)
Table 4: Acid producing activity (pH) in nonfat milk of coccus-rod culture CR12 grown in internally neutralized (IN) and other media and stored at -40°F

<table>
<thead>
<tr>
<th>Weeks at -40°F</th>
<th>IN</th>
<th>IN + ME†</th>
<th>NFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.4</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td>1</td>
<td>4.5</td>
<td>4.5</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>4.3</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>4</td>
<td>4.3</td>
<td>4.2</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>4.3</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>4.4</td>
<td>4.2</td>
<td>4.6</td>
</tr>
<tr>
<td>8</td>
<td>4.4</td>
<td>4.1</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Ave of Triplicates; x control pH=6.7, n=9
† 6% malt extract

Table 5: Acid producing activity (pH) in nonfat milk of coccus-rod culture AS grown in internally neutralized (IN) and other media and stored at -40°F

<table>
<thead>
<tr>
<th>Weeks at -40°F</th>
<th>IN</th>
<th>IN + ME†</th>
<th>NFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.1</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
<td>4.2</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>4.5</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>3</td>
<td>4.5</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>4.6</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>5</td>
<td>4.8</td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td>6</td>
<td>4.9</td>
<td>4.4</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>4.9</td>
<td>4.3</td>
<td>4.6</td>
</tr>
<tr>
<td>8</td>
<td>4.8</td>
<td>4.3</td>
<td>4.6</td>
</tr>
</tbody>
</table>

* Ave of Triplicates; x control pH=6.7, n=9
† 6% malt extract
Table 6: Acid producing activity (pH) in nonfat milk of coccus-rod culture R6 grown in internally neutralized (IN) or nonfat milk and stored at 77°F or 35°F

<table>
<thead>
<tr>
<th>Days Stored</th>
<th>77°F</th>
<th>35°F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFM</td>
<td>IN</td>
</tr>
<tr>
<td>0</td>
<td>4.1</td>
<td>4.1</td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>7</td>
<td>6.0</td>
<td>4.4</td>
</tr>
<tr>
<td>12</td>
<td>6.1</td>
<td>5.5</td>
</tr>
<tr>
<td>30</td>
<td>6.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

pH from 1% inoculum: 108°F, 5.5h*

* Average of triplicates; x control pH = 6.75, n = 84
Table 1: Microscopic appearance of coccus-rod (CR) bulk starter cultures showing CR ratios as estimated by relative densities of each.
Figure 2: Fate of phages added (10^2 PFU/ml) to 7 thermophilic starter media inoculated with _S. thermophilus_ and _L. bulgaricus_, each being susceptible to their respective phages.
Figure 3: Effect of addition of $10^2$ PFU/ml coccus and rod phages specific for S. thermophilus and L. bulgaricus, respectively, on percentage of rods present in 7 different mature starter media. Percent cocci in the control and phage contaminated starters is 100 minus the percent rods.
Figure 4: Acid producing activity of mature coccus–rod starters prepared in 7 different media in the absence (control) and presence of $10^2$ PFU/ml phages specific for *S. thermophilus* and *L. bulgaricus*. Activity was measured by the pH change from a 1% inoculum of the control and phage infected cultures into 9% nonfat milk incubated at 108°F for 2.5 hours.
Figure 5: Percent viable rods in different cans from three different lots of frozen concentrated cultures prepared by commercial manufacturer A as determined by the hydrophobic grid membrane procedure and microscopically.
Figure 6: Percent viable rods in frozen concentrated cultures prepared by commercial manufacturer B as determined by the hydrophobic grid membrane procedure.
Figure 7: Percent viable rods in frozen concentrated cultures prepared by commercial manufacturer C as determined by the hydrophobic grid membrane procedure.

Figure 8: Percent viable rods in frozen concentrated cultures prepared by commercial manufacturer D as determined by the hydrophobic grid membrane procedure.
FACTORS AFFECTING STRETCH, MELT, AND COOK COLOR IN MOZZARELLA CHEESE

Craig J. Oberg, Department of Microbiology
Weber State University, Ogden, Utah, 84408-2506

ABSTRACT

Physical properties of Mozzarella cheese, particularly stretch, melt, and cook color, determine cheese quality. These properties are affected by microbiological and chemical interactions that occur during and after cheese manufacture. Changes in proteolytic activity of starter cultures can modify all three properties. Use of proteinase positive paired cultures can result in increased cheese melt over paired proteinase deficient cultures. Replacement of Lactobacillus delbrueckii ssp. bulgaricus with Lactobacillus helveticus cultures greatly reduces cook color, since L. helveticus cultures are galactose positive. In direct acid Mozzarella, changes in the type of milk coagulating enzyme affects both stretch and melt. Freezing, thawing, and shredding also affect stretch and melt. Mozzarella cheese frozen at -196°C, shredded, and held for at least 21 days stretched most, while cheese cut in blocks, frozen at -20°C, and stored for only 7 days melted most. In all experiments an inverse correlation between stretch and melt was found. As stretch decreased, melt increased. Changes in cultures, milk coagulating enzymes, storage time, and storage methods influence stretch, melt and cook color of Mozzarella cheese.

INTRODUCTION

Per capita consumption of Mozzarella cheese in the U.S. has increased over tenfold since 1960(1). Consumption of Italian-type cheese has soared 9.5 percent per year since 1982, with Mozzarella and Ricotta cheeses leading in this market segment. Production of Mozzarella cheese now ranks second to Cheddar cheese and this trend has been predicted to continue at least through the year 2000 (19). Major purchasers of Mozzarella or pizza cheese are concerned about deterioration in physical properties that can occur as early as the first two weeks of storage (1). In a nationwide survey, only 92% of Mozzarella cheese examined was of acceptable quality, and there was large variation in melting quality (13). A recent survey of Mozzarella cheese quality in Vermont pizza restaurants indicated over 50% of the cheese had quality problems including poor shredding properties, blistering, and soupy melt (22). Some buyers require that cheese be graded and frozen quickly to stabilize stretch, blistering, melt, oiling off, and browning during cooking. Studies have shown that physical properties of Mozzarella cheese vary greatly based on cheese age, pH, salt content, and starter cultures used.
Several chemical factors have been found to affect the physical properties of Mozzarella cheese including salt content and cheese pH. Cervantes et al. (3) found that increasing the percent salt in Mozzarella cheese resulted in increased firmness and decreased cohesiveness. They also noted that NaCl concentrations between 1.0-2.4% had no significant effect on texture up to 24 days of aging. Cervantes et al. (3) noted that longer storage times following thawing of Mozzarella cheese resulted in softening of the body. Kindstedt (9) noted that low concentrations of NaCl in the cheese resulted in it being very soft and gelatinous, while high concentrations of NaCl caused the cheese to be extremely tough and elastic. Masi and Addeo (11) showed that Mozzarella cheese becomes softer and less shreddable with increasing moisture and FDB (fat on dry basis). Acid drive of the starter cultures has also been found to play an important role, because it affects the final pH of the cheese (23). Proper acid production during manufacture allows the curd to reach a pH of 5.2 where curd demineralization can occur. At a pH of 5.1 to 5.4, casein is converted from the dicalcium form to the monocalcium form, reducing calcium bridging, and thus allowing the fibers to stretch. If acid production in the vat is slow, increases in time at the elevated cooking temperatures of Mozzarella production can drive out moisture. Kindstedt (9) found a significant association between moisture and apparent viscometry.

TESTS FOR PHYSICAL PROPERTIES

Few studies concern physical properties of Mozzarella cheese, possibly because there is a lack of objective methods to measure these properties. Nilson and LaClair (13) measured melt by placing 5 mm thick, 6.2 cm³ discs of cheese on filter paper, heating and then comparing the change in area. Instron Universal Testing Machines have also been used to measure physical changes in Mozzarella. Park and Rosenau (21) found the Arnott test more accurately measured melt in Mozzarella cheese than the Schreiber test. In the Arnott test, a cylinder of cheese approximately 17 x 17mm is placed on a glass tray and heated at 100°C for 15 min. Kindstedt and Rippe (10) found the helical viscometer most accurately measures apparent viscosity of Mozzarella cheese.

In our work, the helical viscometer method of Kindstedt et al. (10) was used with modifications to measure stretch (16). We use the term “stretch” as it is used empirically in the Mozzarella industry to describe the combination of rheological properties measured by helical viscometry. A modification of the method used by Olson and Price (20) was used to measure meltability (16). Test tubes of grated cheese used in the stretch test were put in a boiling water bath (96°C) for 60 min. Color differences were measured with a Minolta Chroma Meter CR-100 (16).
CULTURE PROTEOLYSIS

A major area of investigation is the proteolysis of the thermolactic starter cultures, particularly since numerous studies have shown a relationship between proteolysis and changes in body and texture of cheese (4, 5, 15). A recent review on the proteinases and peptidases of lactobacilli carefully describes what is known about these enzyme systems, but no correlations between these characteristics and the development of physical properties of Mozzarella cheese are mentioned (8). The proteolytic activity in \textit{L. delbrueckii} \textit{spp. bulgaricus} varies more than in other lactic organisms (14). Certainly, an understanding of the relationship between culture proteolysis and development of stretch, melt, and cook color properties in Mozzarella cheese is important.

The o-phthaldialdehyde (OPA) test and amino acid analysis were used to characterize proteolysis of milk proteins during growth of \textit{Lactobacillus delbrueckii} \textit{spp. bulgaricus} (14). Each strain had a distinct pattern of individual amino acid concentrations. Amino acid profiles provided information about the proteolytic activity of these strains that was not available from the OPA test. Cluster analysis, based on amino acid profiles of each strain, was then used to differentiate the seven strains beyond what is possible by visually comparing the amino acid analysis results. These three methods represent a succession of increasing ability to assess proteolysis associated with culture strains. Differences among amino acid profiles reflect differences in proteinase, peptidase, and transport activities of lactic culture strains. A specific amino acid profile might correlate with a particular type of enzymic activity. Since \textit{L. delbrueckii} \textit{spp. bulgaricus} is used in the manufacture of Mozzarella cheese, wide variation in total proteolysis among strains of this organism has major implications for physical properties of Mozzarella cheese. Profiling cultures by statistical analysis of amino acid analysis data can show which strains will give the most desirable characteristics in Mozzarella cheese.

EFFECTS OF CULTURE PROTEOLYSIS ON PHYSICAL PROPERTIES

Strains of \textit{L. delbrueckii} \textit{spp. bulgaricus} and \textit{L. helveticus} were chosen for Mozzarella cheese manufacture based on their proteolytic activity as measured by the OPA and AAA tests, as previously mentioned. Mozzarella cheese was made from single strains of \textit{L. delbrueckii} \textit{spp. bulgaricus} or \textit{L. helveticus}. Mozzarella cheese was also made from paired cultures containing a single strain of \textit{L. delbrueckii} \textit{spp. bulgaricus} or \textit{L. helveticus} and a single strain of \textit{S. salivarius} \textit{spp. thermophilus}. Paired strains contained either a set of strongly proteolytic or a set of weakly proteolytic organisms. Mozzarella cheese made by direct acidification used the method of Breene et al. (2). When cheese was made with paired strains of \textit{L.
delbrueckii ssp. bulgaricus and S. salivarius ssp. thermophilus the curd pH dropped more rapidly than with only single strains of L. delbrueckii ssp. bulgaricus. Manufacturing times decreased by 2 h when compared to cheese made with single strains. Proteinase-positive pairs produced more acid than proteinase-deficient pairs.

In Mozzarella cheese manufactured with only strains of either proteinase-positive or proteinase-deficient L. delbrueckii ssp. bulgaricus, significant differences in stretch were noted (Figure 1) (16). Cheese made with proteinase-deficient strains rapidly lost its ability to stretch after 7 d. As time increased, stretch for all cheeses decreased. Cheese made with proteinase-deficient strains melted more easily than cheese made with proteinase-positive strains (Figure 2). Proteinase-deficient cheese showed a rapid increase in melt by day-seven, but by day-twenty eight the differences were less dramatic. Direct acid cheese melted more easily at day-one than cultured cheeses, but its melting properties remained about same throughout the 28 d. Cheese made with proteinase-positive single strains of L. delbrueckii ssp. bulgaricus was darker after cooking than either cheese made with proteinase-deficient strains or by direct acid (Figure 3). A result of more amino acids available to react with galactose in the heat mediated browning reaction. Direct acid cheese remained much lighter after cooking throughout the testing period than did the cultured cheeses.

No differences between proteinase-positive pairs containing one strain of L. delbrueckii ssp. bulgaricus and one of S. salivarius ssp. thermophilus and proteinase-deficient paired strains for stretch were found. Cheese made with proteinase-positive pairs showed better melting characteristics than cheese made from proteinase-deficient cultures (Figure 4) (16). Direct acid cheese had much less meltability for the entire period. This was due to the lack of proteolysis usually supplied by the proteolytic enzymes of the starter culture. Cheese made from proteinase-deficient pairs showed less browning after cooking than proteinase-positive cheeses (Figure 5).

Mozzarella cheese was manufactured with an 80:20 ratio of rods to cocci or a 20:80 ratio of rods to cocci (12). For each ratio either a pair of highly proteolytic cultures or a pair of weakly proteolytic cultures were used. No differences in physical properties were observed between the various ratios, but differences were found between the highly proteolytic cultures and the proteinase-deficient cultures as previously mentioned.

EFFECTS OF USING L. helveticus CULTURES

Cheese made with L. helveticus cultures, either single strains or paired with a S. salivarius ssp. thermophilus also showed decrease in stretch over time (Figure 6) (18). Cheese made with the proteinase-positive pair retained the greatest stretch from day 14 through day 28. Cheese made with single strains of L. helveticus
showed less stretch at days 14 and 28. All cheeses showed a very rapid rise in melt by day 7 (Figure 7). Three of the four cheeses made with *L. helveticus* showed a decrease in cook color over time (Figure 8). A positive correlation between cook color and cheese pH was found. The decrease in cook color over time is due to the fact that *L. helveticus* cultures are galactose-positive, while *L. delbrueckii* spp. *bulgaricus* cultures do not ferment galactose. Johnson and Olson (7), using a Hunterlab colorimeter, found a positive correlation between galactose concentration and brown color intensity in Mozzarella cheese during cooking. Our results confirm that as residual galactose is removed from the cheese by bacterial metabolism, cook color problems decrease.

**EFFECTS OF MILK-COAGULATING ENZYMES**

Direct acid Mozzarella cheese was produced with a variety of milk-coagulating enzymes and had normal characteristics for this cheese type. No correlations were found between either pH or moisture when compared to the development of physical properties for cheese made with each enzyme type. Cook color was not affected by choice of enzyme. All cheese manufactured remained essentially white during the entire storage period. Melt was affected by choice of enzyme and also by storage time (Figure 9). Cheese made with porcine pepsin showed the least amount of melt over time. Stretch was also significantly affected by the type of enzyme used and by storage. The most rapid decrease in stretch for all cheese types occurred during the first seven days with a gradual decline following for the next 21 days (Figure 10). Cheese made with calf chymosin showed the least amount of stretch over the storage period, while cheese made with either porcine pepsin or bovine pepsin showed the most stretch over the testing period.

**EFFECTS OF FREEZING, SHREDDING, AND THAWING**

Instability of key physical properties, such as stretch and melt, has led producers to freeze Mozzarella just after manufacture. Mozzarella cheese is often shredded prior to freezing to decrease freezing time and facilitate use when thawed. In our experiment, commercially manufactured low moisture, part-skim Mozzarella cheese (2.25 kg loaves) was either shredded or cut into 5 x 10 x 7 cm blocks. Shredded cheese and blocks were either frozen at -196°C and stored at -70°C or frozen and stored at -20°C. Cheese was then thawed at either 4.4, 12.8, or 25°C.

Stretch was significantly affected by shredding and by storage time, but not by freezing temperature or by thawing temperature. Frozen cheese showed more stretch over time than did refrigerated control cheese (Figure 11). Frozen shredded cheese showed more stretch than frozen non-shredded cheese. The longer the storage time, the greater the difference in stretch between shredded and non-shredded frozen cheese. Freezing temperature, -196°C versus -20°C, did not affect stretch.
Melt was significantly affected by shredding, freezing temperature, and storage time. Frozen cheese melted less than refrigerated control cheese over the entire storage period (Figure 12). Shredded frozen cheese melted significantly less than non-shredded frozen cheese. The temperature at which cheese was frozen also affected melt over time (Figure 13). After 14 d, cheese frozen at -20°C had more melt than cheese frozen at -196°C.

The inverse relationship between stretch and melt of Mozzarella cheese seen in other studies (8,14,15) also occurred in this study. Mozzarella cheese with the greatest stretch had the least melt. Shredded frozen cheese had more stretch than non-shredded frozen cheese. The opposite was true for melt. Frozen cheese stretched more than non-frozen control cheese, but non-frozen control cheese melted more than frozen cheese. Cohesiveness in curd protein structure is required for stretch, but has the opposite effect on melt. Protein breakdown in cheese reduced cohesiveness and softened the body, thus increasing melt. Tempering of thawed Mozzarella cheese for 2 to 3 wk to improve melt probably allows proteolysis to break down cheese body (4), which was consistent with the melt of control cheese in this study being greater than melt of the frozen cheeses.

Cook color was not affected by freezing temperature, storage time, shredding, or thawing temperature. Cook color for non-frozen control cheese and frozen cheese were similar throughout the 42 d testing period.

CONCLUSIONS

Cheese made with single strains of *L. delbrueckii* spp. *bulgaricus* melted less and stretched more when compared to cheese made with paired strains. Cheese manufactured with single strains of proteinase-positive *L. delbrueckii* spp. *bulgaricus* retained more stretch over the entire testing period when compared with all other types of cheese. Cheese made with either single strains or paired cultures of *L. helveticus* stretched as much as cheese made with paired strains of *L. delbrueckii* spp. *bulgaricus* and *S. salivarius* spp. *thermophilus*, but not as well as cheese made with proteinase-positive strains of *L. delbrueckii* spp. *bulgaricus*. Cheese made with either pairs or single strains of *L. helveticus* showed the same melt as cheese made with paired strains *L. delbrueckii* spp. *bulgaricus* and *S. salivarius* spp. *thermophilus*, while cheese made with single strains of *L. delbrueckii* spp. *bulgaricus* showed the least melt over time. Cheese made with paired cultures had better melting properties than cheese made with single strains of either proteinase-positive or deficient *L. delbrueckii* spp. *bulgaricus*. The proteinase-deficient strains all reduced the browning effect during cooking by limiting production of available amino acids to react with galactose. *L. helveticus* cultured cheese showed a decrease in cook color over time and the browning was considerably less than for cheese made with *L. delbrueckii* spp. *bulgaricus* cultures due to galactose metabolism by *L. helveticus*. Mozzarella cheese made by the direct acid method melted poorly and
had almost no browning. Use of different milk-coagulating enzymes could modify the physical properties of direct acid Mozzarella cheese.

The temperature and speed with which Mozzarella cheese is frozen plays an important role in the melt properties of thawed cheese. Cheese frozen at -20°C melted more than cheese frozen at -196°C, which suggests that slower freezing (-20°C versus -196°C) results in large ice crystals and greater breakdown of cheese. The form in which Mozzarella cheese is frozen (block or shredded) also affected the stretch and melt properties of the thawed cheese. The melt and stretch of shredded cheese differed from non-shredded cheese. Stretch was greatest in shredded frozen cheese, apparently because rapid freezing limited the size of ice crystals. The fact that the internal structure was not extensively altered allowed the cheese to retain its maximum cohesiveness, which was manifested as increased stretch and decreased melt. Frozen shredded cheese melted less and stretched more than frozen non-shredded cheese. So, both low temperature and shredding increase the rate of freezing, minimize damage to the cheese, and reduce the rate of melting.

Freezing, storing, and shredding significantly affect the stretch and melt of Mozzarella cheese, but do not affect cook color. Stretch of frozen stored Mozzarella cheese was greatest when it was shredded, frozen as quickly as possible (-196°C in our study), and held for at least 21 d. For greatest melt, it should be left in block form, not frozen or frozen as slowly as possible (-20°C in our study), and stored for as short a time as possible. Manufacturers must balance stretch and melt, because freezing and storing frozen affect stretch and melt oppositely.
REFERENCES

1. Alvarez, R. J. 1986. Expectations of Italian cheese in the pizza industry. 23rd Annual Marschall Invitational Italian Cheese Seminar, Madison, WI.


LIST OF FIGURES

Figure 1  Stretch measurements (relative units) of Mozzarella cheese made with either proteinase-positive or deficient single strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 2  Melt measurements of Mozzarella cheese made with either proteinase-positive or deficient single strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 3  Cook color measurements of Mozzarella cheese made with either proteinase-positive or deficient single strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 4  Melt measurements of Mozzarella cheese made with single strain pairs of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 5  Cook color measurements of Mozzarella cheese made with single strain pairs of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 6  Stretch measurements (relative units) of Mozzarella cheese made with either single strains or mixed single strain pairs of *Lactobacillus helveticus* and *Streptococcus salivarius* ssp. *thermophilus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 7  Melt measurements of Mozzarella cheese made with either single strains or mixed of single strain pairs of *Lactobacillus helveticus* and *Streptococcus salivarius* ssp. *thermophilus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)

Figure 8  Cook color measurements of Mozzarella cheese made with either single strains or mixed of single strain pairs of *Lactobacillus helveticus* and *Streptococcus salivarius* ssp. *thermophilus*. (Solid symbols - proteinase positive cultures; open symbols - proteinase deficient cultures)
Figure 9  Melt measurements of Mozzarella cheese made by direct acidification using different coagulants

Figure 10  Stretch measurements (relative units) of Mozzarella cheese made by direct acidification using different coagulants.

Figure 11  Stretch measurements (relative peak area) for frozen shredded and non-shredded Mozzarella cheese, and for non-shredded and shredded refrigerated Mozzarella cheese (frozen, n = 3; control, n = 1).

Figure 12  Melt measurements of shredded and non-shredded Mozzarella either stored frozen (-20°C or -70°C) or refrigerated (4.4°C) (frozen, n = 3; control, n = 1).

Figure 13  Melt measurements of Mozzarella cheese frozen at either -196°C and stored at -70°C or frozen and stored at -20°C (n = 3).
Figure 4
Figure 6

Stretch (total peak area) vs. Time (days) for different conditions:
- Lh.Prt+
- Lh.Prt-
- Lh/St.Prt+
- Lh/St.Prt-
Calf chymosin
Mucor miehei
Bovine pepsin
Porcine pepsin

Figure 9
Figure 12

Melt (cm)

Time (d)

- Shredded Control
- Non-shredded Control
- Non-shredded Frozen
- Shredded Frozen
MOZZARELLA CHEESEMAKING IN ITALY

Mr. Giovanni Stefanini, Marketing Manager, Lacto-Labo Products, Rhône-Poulenc Italia S.p.A, Via G.G. Winckelmann, 2 20146 Milano Italy

FIRST OF ALL I'D LIKE TO THANK YOU ALL FOR ATTENDING, TODAY, THE I.C.S., BECAUSE IT IS YOUR PRESENCE WHICH CONFERS SUCCESS UPON THIS ANNUAL MEETING AS AN INTERNATIONAL CONFERENCE.... AND SPECIAL THANKS ALSO TO MARSCHALL U.S. FOR INVITING ME TO SPEAK ABOUT MOZZARELLA CHEESE RIGHT HERE, IN WISCONSIN, WHERE THE LARGEST MOZZARELLA MANUFACTURERS ARE LOCATED!

TODAY WE WILL SHOW A SHORT VIDEOMOVIE CONCERNING THE PRODUCTION OF ITALIAN MOZZARELLA, TRYING TO UNDERSTAND HOW THE DIFFERENT OPERATIONS WHICH TRANSFORM THE MILK INTO MOZZARELLA MIGHT INFLUENCE THE FINAL PRODUCT AS FAR AS TEXTURE, TASTE, SHELF LIFE ETC. ARE CONCERNED.

BEFORE STARTING, JUST SOME INFOS ABOUT ITALIAN MOZZARELLA WHICH IS, AS YOU MIGHT KNOW, SOMEWHAT DIFFERENT FROM WHAT YOU CALL MOZZARELLA CHEESE IN THE U.S.– WITH REFERENCE TO:

- SHAPE
- WEIGHT (SEE TABLE N. 1)
- COMPOSITION
- PACKAGING
**Table N. 1**
**Italian Mozzarella**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape</td>
<td>Rounded Pieces</td>
</tr>
<tr>
<td>Weight</td>
<td>From 1 oz. (30 grams) to 1 lb each piece (450 grams)</td>
</tr>
</tbody>
</table>
| Composition     | Water 55 – 62%  
|                 | Fat in dry matter 18 – 21%  
|                 | Proteins in dry matter 18 – 21%  
|                 | Ash 1 – 3% |
| Packaging       | Sealed plastic/paper pouches, containing a special liquid (water + salts) for maintaining the original moisture |
| Shelf Life      | From a few hours to 30 days (depending on the technology) |

As you see, this is the typical "ready to eat" cheese.

Actually most of it is eaten just as it is, with or without salads, during lunch or dinner, while as an ingredient for pizzas the pizza-makers prefer to use a cylindrical shaped processed cheese (which is quite similar to the U.S. "Mozzarella cheese").

In Italy there are about 3,000 dairies (taking into consideration that an "Italian dairy" is often a family company – father, mother and sons!) among those manufacturers, about 1,000 produce Mozzarella.

Most of them are located in the south of Italy, where this cheese was invented hundreds of years ago as the only way to transform acid milk into a good cheese ... and it is right in the South of Italy that the traditional way of manufacturing Mozzarella has remained, while in the north the producers are usually bigger in size and use modern and up-to-date technologies.
THE MOZZARELLA PRODUCED ACCORDING TO THE “SOUTHERN” TECHNOLOGY IS ACTUALLY RECOGNIZED AS MORE HIGHLY FLAVORED AND TASTY, WHILE ON THE OTHER SIDE ITS SHELF LIFE IS OFTEN ONLY A FEW DAYS (EVEN FEW HOURS !!!).

MANY REASONS MADE AND CONTINUE TO MAKE THIS CHEESE VERY SUCCESSFUL IN OUR COUNTRY.

IT IS FRESH AND TASTY FOR THE CONSUMERS AND SPECIALLY VERY INTERESTING FOR THE MANUFACTURERS. ACTUALLY NOT ONLY IS THE YIELD HIGH, BUT ALSO SECOND CHOICE MILK (I.E. NOT SUITABLE FOR PRODUCING AGED CHEESE) CAN BE USED TOO: AND THIS IS IMPORTANT AS, FOR EXAMPLE, MILK STANDARDIZATION IS NOT USED NOR ALLOWED.

TABLE N. 2

| COST OF MILK | 370 - 570 U.S. $ / TONN |
| F A T | 3,5 - 4,0 % |
| PROTEINS | 3,1 - 3,3 % |
| YIELD | 11 - 15 % |
| MOZZARELLA | 2,3 - 3,8 $ / LB |

SELLING PRICE: EX-WORKS

IN THE SOUTH OF ITALY MILK IS MORE EXPENSIVE BUT YIELD IS HIGHER AS WELL AS SELLING PRICES.
As far as the manufacturing processes are concerned, nothing can be taken as a general rule: of course there are certain parameters (pH, T° etc.) but the differences from one producer's technology to another are sometimes very relevant.

Generally speaking, the milk acidification is the "core" of the manufacturing process.

There are 5 methods of acidifying the milk:

- By natural fermentation of unpasteurized milk
- By adding selected cultures (bulk starter system)
- By adding selected cultures (direct vat inoculation)
- By adding citric acid
- By adding citric acid + selected cultures

Mozzarella taste, flavor, body, texture and shelf life depend, of course, on how the acidification process has been carried out. Generally speaking, a good shelf life and a certain standardization of the taste are obtained by using selected cultures, while the cheese produced by citric acid is usually relatively lacking in flavor and, finally, the "natural cultures system" might give top quality today and bad tomorrow, depending on many many factors, very difficult to enumerate and to control.

Today we will not get too deeply into technical details, the discussion will be very "practical", and we will watch together how a typical mid-size manufacturer in the south of Italy "creates" his particular type of mozzarella.

Before starting, some general information about the company involved in the movie.

- It is located in mountainous countryside, between Naples and Bari, in a region called "Boianese", renowned for the excellence of its mozzarella;
- The average quantity of milk processed is 20,000 liters/day;

- The available equipment (vats, stretching machine and so on) allows an industrial production, even if many operations are still "hand-made", in accordance with long-standing tradition.

Here is the record of the manufacture we are going to watch (see Table N.3/4)

All the cutting operations are operated to allow:

1) A firm curd giving a limited loss of fat in the whey

2) A correct acidification of the paste to stretch (between pH 5.2 to 5.0) to prevent loss of fat and protein during the stretching

3) A correct syneresis upon which depends the final structure of mozzarella

A surprising fact is that our friend, as most of our "casari" (production managers) do, relies upon and counts more on his fingers than on his pH meter!!

In conclusion, in Italy we have not only large manufacturers using most up-to-date technologies, but also many small producers still taking real care (personally) of their mozzarella: and in spite of a very strong tradition, little by little new technologies will take over from the age-old processes (as it has happened to our friend using now Ezal), specially when, as the DVI, not only allow to work faster and better but increase taste and shelf-life too.

We foresee that direct vat inoculation - with specially developed, well-specified strains of typical mozzarella type bacteria - will gradually be accepted by almost all producers.

This change will improve manufacturing efficiency, cheese quality, profitability and most importantly, customer acceptance and increased demand.

Thank you for your kind attention and all success to you American mozzarella producers.
### TABLE N. 3

| MILK PROTEIN | 3,1 – 3,4 % |
| MILK FAT | 3,5 – 4,0 % |
| PASTEURIZATION | 158° F 20 SEC. |
| VAT VOLUME | 7,700 LB |
| STARTER | STR. THERMOPHILUS EZAL LYOPH. TA 057 |

### TABLE N. 4 A

<table>
<thead>
<tr>
<th>OPERATION</th>
<th>TIME HR. MIN.</th>
<th>TEMP. °F</th>
<th>pH</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADD STARTER</td>
<td>00:00</td>
<td>99</td>
<td>6,50</td>
<td>40 UNITS = ABT 40 GR. = 1,5 OZ FOR 7,700 LB</td>
</tr>
<tr>
<td>ADD RENNET</td>
<td>00:30</td>
<td>99</td>
<td>6,50</td>
<td>MARZYMIE 15 3 OZ/100 LB MILK</td>
</tr>
<tr>
<td>CUT CURD</td>
<td>1) 01:00</td>
<td>99</td>
<td>6,45</td>
<td>FIRST VERY SOFT, ONLY ON SURFACE</td>
</tr>
<tr>
<td></td>
<td>2) 01:10</td>
<td>98</td>
<td>6,45</td>
<td>MECHANICALLY SLOW: HOLDING</td>
</tr>
<tr>
<td></td>
<td>3) 01:20</td>
<td>98</td>
<td>6,40</td>
<td>MECHANICALLY FAST, NUT SIZE: HOLDING</td>
</tr>
<tr>
<td>DRAIN WHEY</td>
<td>03:20</td>
<td>97</td>
<td>5,30</td>
<td>UNLOAD WHEY FROM BOTTOM OF VAT</td>
</tr>
</tbody>
</table>
## TABLE N. 4 B

<table>
<thead>
<tr>
<th>OPERATION</th>
<th>TIME</th>
<th>TEMP. °F</th>
<th>pH</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNLOAD CURD</td>
<td>03:35</td>
<td>97</td>
<td>5.25</td>
<td>UNLOAD OVER A TABLE: HOLDING</td>
</tr>
<tr>
<td>CUT/TURN PASTE 1)</td>
<td>03:50</td>
<td>95</td>
<td></td>
<td>CUT IN LARGE CUBES HOLDING</td>
</tr>
<tr>
<td></td>
<td>04:00</td>
<td>93</td>
<td></td>
<td>CUT IN LARGE CUBES HOLDING</td>
</tr>
<tr>
<td>MILLING</td>
<td>04:10</td>
<td>86</td>
<td>5.15</td>
<td>MILLING, IF PASTE IS TOO ACID</td>
</tr>
<tr>
<td>STRETCHING/HOLDING</td>
<td>04:15</td>
<td>176-194</td>
<td></td>
<td>HAND MADE BY STRETCHING MACHINE</td>
</tr>
</tbody>
</table>

## TABLE N. 4 C

<table>
<thead>
<tr>
<th>OPERATION</th>
<th>TIME</th>
<th>TEMP. °F</th>
<th>pH</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>COOLING 1)</td>
<td>04:25</td>
<td>60</td>
<td></td>
<td>NOT TOO COOL TO AVOID &quot;PEELING&quot;</td>
</tr>
<tr>
<td></td>
<td>04:40</td>
<td>50</td>
<td></td>
<td>A LITTLE COLDER TO HARDEN</td>
</tr>
<tr>
<td>RINING</td>
<td>04:55</td>
<td>50</td>
<td></td>
<td>SOLUTION NaCl 20%</td>
</tr>
<tr>
<td>PACKAGING</td>
<td>05:05</td>
<td>50</td>
<td></td>
<td>IN SEALED BAGS (WATER + 0.5 - 1% SALT)</td>
</tr>
</tbody>
</table>
CARE AND MAINTENANCE OF SALT BRINES

By Bill Wendorff, Ph.D.
Dept. of Food Science
and
Mark Johnson, Ph.D.
Center for Dairy Research
University of Wisconsin
Madison, WI 53706

ABSTRACT

Salting is an important phase in the cheesemaking process and provides the following functions: 1) controls the growth of starter bacteria and non-starter bacteria, 2) aids in whey drainage and formation of the rind and 3) imparts its characteristic taste and enhances the flavor of the cheese. The key factors affecting the absorption of salt from brines include salt concentration, acidity or pH, calcium content and brine temperature. Control of these factors are necessary to avoid problems in brined cheeses such as rind rot, slippery cheese, tough rind and hornlike rind. Brines are also subject to microbial contamination which may lead to quality problems in the cheese. The discussion will cover proper control and maintenance of salt brines from preparation of new brines to maintenance of old brines.

Salting or brining is an important phase of the Italian cheese-making process. A number of critical changes are taking place in the cheese during this brining phase. Salt is being absorbed into the cheese while whey is being expelled from the cheese. This expulsion of whey is critical for proper moisture control in the final cheese. The salt which is absorbed into the cheese significantly affects the textural, physical and flavor characteristics of the cheese. Therefore, it is essential that you have good control over the brine and the brining operation to ensure that you can produce consistent product from day to day to meet your customer's critical specifications.

Salt provides several key functions in Italian type cheeses. First, it aids in whey drainage which is critical for proper moisture control in the final cheese. Without sufficient expulsion of whey, high moisture cheese would be produced which is more susceptible to spoilage and excess acid production. Secondly, it controls the growth of starter bacteria and non-starter bacteria during the ageing or ripening process. In the case of Parmesan, Romano or aged Provolone, the proper salt level is essential to favor the growth of those organisms that produce the characteristic pungent
odors and sharp flavors that characterize those aged cheeses. In the case of unripened cheeses, e.g., Mozzarella and mild Provolone, the salt concentration must be maintained at a level that effectively controls non-starter and proteolytic bacteria to reduce the breakdown of the casein which eventually affects the texture of the cheese. Thirdly, the salt level in the cheese will determine the body and texture of the cheese. Generally, the higher the level of salt in the cheese the firmer the body will be. Fourthly, the salt will enhance the flavor of the cheese. Saltiness will mask some of the tartness of the lactic acid and will accentuate the desirable aromatic flavors of ripened cheeses.

Since salt does affect various properties of the final cheese, careful consideration should be given to the factors which affect salt absorption by cheese in the brine. These factors include: 1) salt concentration, 2) cheese size and shape, 3) salting time, 4) curd pH, 5) temperature of curd and brine and 6) curd moisture. It is generally felt that an increase in salt concentration in the brine results in higher rates of salt absorption in the cheese, however, the rate of salt uptake increases at a slower rate with increasing salt concentration in the brine.

Salt absorption is linearly related to the surface area to volume ratio of the cheese. Normally you would expect that smaller cheeses would have a higher salt content than larger ones after brining for an equal period of time. However, this is only true if the shape and relative size are the same. The number of directions of salt penetration from the brine and the ratio of planar to curved surface areas of the cheese will also affect the salt absorption.

The concentration of salt absorbed in the cheese increases with brining time; however, the rate of salt absorption will decrease with time due to the reduced salt differential between the cheese moisture and the brine. Guinee and Fox report that the quantity of salt absorbed by the cheese is proportional to the square root of brining time, (4).

As for curd pH, cheese with a high curd pH will retain more salt than cheese with low pH (4). Lawrence and Gilles (6) suggest that curd is more soluble at higher pH values and that will result in higher salt retention by the curd structure per se.

Breene et. al. (1) found that curd tempered to 90°F absorbed less salt than curd at lower or higher temperatures. This was due to a layer of exuded fat on the surface of the curd which restricted salt absorption. At lower temperatures there was not as much free fat at the surface and at higher temperatures the fat was liquid and dispersed in the brine. Guerts et. al. (3) reported that increasing brine temperatures
resulted in increased salt absorption in the cheese. The temperature of the cheese going into the brine should be as close to the brine temperature as possible. If there are major differences between the curd temperature and brine temperature, significant problems can arise. These problems will be discussed more thoroughly in the brining problems section.

Salt absorption is increased as moisture content of the curd increases. With increased moisture in the cheese, the pore sizes in the protein matrix are larger, thus reducing the frictional effect of inward-diffusing salt molecules (3).

These six factors affecting salt absorption are the key to a successful brining process for your Italian cheeses. Good control of your brining system is necessary to provide for uniformity of product from day to day. Unfortunately problems do arise from time to time. Therefore, we need to review some of the causes of those problems so we can establish a good brine maintenance program to try to prevent those from happening.

The five most critical parameters to watch in your brining process are: 1) salt concentration, 2) brine pH, 3) mineral concentration in the brine, 4) brine temperature, and 5) microbial contamination. As we indicated earlier, increased salt concentrations in the brine will result in increased salt absorption in the cheese. Therefore, you need to ensure that you have a constant level of salt in your brine to have uniform levels of salt in your cheese. When making up new brines or monitoring old brines, a salometer or Baume hydrometer may be used to determine the salt level in the brine. However, keep in mind that as new brines are used, they will retain other dissolved solids from the exuded whey or curd particles and an increase in solids determined by the hydrometer doesn't necessarily represent an increase in salt. For this reason, we recommend measuring the sodium chloride content in the brine periodically with a sodium-selective glass electrode or measure the chloride concentration with a chloride-selective solid-matter membrane electrode. The other problem that can arise with salt concentration in the brine is local areas of dilution around cheese as whey is being expelled, if the brine tank is crowded with cheese. For that reason, we recommend good agitation of the brine to maintain saturation around the cheeses in the brine tank.

Brine pH should be the same as the pH of the cheese as it is introduced into the brine. If the brine pH is lower than the cheese, calcium ions will be pulled from the cheese resulting in a hard rind with a short body in the cheese. On the other hand, if brine pH is higher than cheese pH, then the brine will produce a greasy or slippery cheese. For this reason, we recommend that when making up new brines, you add acetic acid or lactic acid to the brine to bring the pH down to that of your cheese.
The mineral concentration of the brine, and especially calcium, is the primary factor leading to most failures with new brines. The calcium content of the brine must be similar to that of the cheese to be placed in that brine. In a newly prepared brine, calcium will be leached from the surface of the cheese and casein solubilized thereby yielding a cheese with a soft rind. This will continue until the calcium level of the brine eventually reaches that of the cheese being placed in the brine.

For that reason, we recommend the addition of calcium chloride to new brines to eliminate the problem of soft rind in Italian cheeses. The calcium content of the brine will depend on the pH value of the cheese being brined. Low pH cheeses will give off more calcium to the brine than higher pH cheeses. Kinstedt (5) recommends the addition of 0.06% CaCl₂ to brines for Mozzarella cheese while Walstra (2) reports that .45-.60% CaCl₂ would be typical for semi-hard cheeses. Too high a calcium level in the brine will yield cheese with a firm, dry horn-like rind.

As you can see the composition of the brine should be very similar to that of the cheese being placed in the brine. For that reason, many cheesemakers will add a portion of whey to their new brines to reduce the potential for soft rind or slippery-surfaced cheeses. By doing so, they are adding some soluble calcium and some acid to the brine. However, it is much more controllable to add the exact level of calcium chloride and food grade acid to the new brine as is needed to match the cheese.

Temperature of the cheese and temperature of the brine can be used to control the final moisture in the cheese and the rate of salt absorption into the cheese. Earlier we pointed out that the cheese temperature should be the same as the brine temperature to avoid major problems during brining. If warm cheese is placed into cold brine, salt will diffuse into the cheese but expulsion of whey will be slowed. The high salt at the surface will draw more water to it thus producing a soft surface to the cheese. By cooling the cheese well before going into the brine and keeping the brine cool, you can retain more moisture in the cheese and reduce the salt level in the cheese. On the other hand, since salt absorption and whey expulsion increase with brine temperature, you may be able to get a lower moisture and higher salt in the cheese by raising your brine temperature slightly. However, if brine temperatures get above 55°F, you may get protein swelling and growth of undesirable organisms. If cheese is not cooled quickly enough, *Lactobacillus casei* may grow and cause the cheese to have a soft body.
Up to this point we have been concerned with the factors responsible for salt absorption and moisture loss in cheese as controlled by the brining process. However, you must also be concerned about the potential contamination of brines and its effect on your cheeses. In the brining process, salt is absorbed and whey is expelled from the cheese. That whey will contain dissolved solids along with some curd particles, microorganisms and fat. The microbial contamination may be from starter organisms, thermoduric organisms from the milk or post-pasteurization contamination that was picked up by the cheese somewhere in the cheesemaking process. The protein and other constituents from the whey may form a type of sludge in the brine which if left unchecked could deposit on finished cheeses coming from the brine tank. These particles may lead to downgraded cheese if they appear as contaminating particles on the surface of the cheese. Brines must be routinely filtered to reduce this type of contamination.

The major microbial contamination in brines involve salt-tolerant yeasts. However, as we have seen this year in Wisconsin, listeria and other pathogens may also be a problem in brine tanks in Italian cheese plants. These organisms may not grow in the brine, but they tolerate the brine conditions long enough until the cheese is pulled from the brine and growth conditions on the surface of the cheese become more favorable for the growth of the organism. In this case, the brine solution just serves as a vehicle to transfer contamination from one area of the plant to the whole lot of cheese.

To control microbial contamination in the brine, the first step is to ensure that the cheese we are introducing into the brine is good quality cheese made from properly pasteurized milk. All potentials for post process contamination should be minimized to reduce the load going into the brine tank. The brine should be routinely filtered to remove particulate matter and the sodium chloride level should be properly maintained. If yeasts or bacteria are becoming a potential problem in the brine, the brine could be vat pasteurized to eliminate potential pathogens.

In the past, if salt brines became heavily contaminated with solids or microbial contamination, cheese plants would dispose of those by landspreading or discharge to treatment systems. However, with recent changes in environmental regulations concerning ground water contamination, disposal of old salt brines is becoming a problem. Therefore, we must maintain those brines as long as we can and still produce quality Italian cheese.
About one and a half years ago, a new system of brine maintenance was introduced which should help cheesemakers maintain quality brines for extended periods of time. This system involves membrane filtration designed to remove fats, proteins and suspended matter from the brine, but leaves the salt balance intact so the brine can be recycled and reused indefinitely. The system also removes essentially all microorganisms from the brine solution.

There are several companies that currently manufacture these brine purification systems and are represented at the Marschall Italian Cheese seminar today. The basic design of the systems are fairly similar, but each uses a slightly different type of membrane filter in the system. Important considerations when purchasing a system are: size, product compatibility, initial filter costs, life of the filter, surface area of the filter, flux rate of the filter and cleanability.

We hear stories of century-old brines in European cheese factories and I am not sure if that is true or not. However, with proper maintenance of your salt brines, you certainly should be able to maintain your quality brines for several years and produce top quality Italian cheeses with uniform salt levels.
REFERENCES


The survey was developed for the purpose of obtaining information concerning the characteristics of dairy farms identified as having antibiotic residues in bulk tank milk at the milk plant. Cooperation of the dairy plant field representative was requested to complete a question form on the farm when investigating the conditions resulting in an antibiotic positive milk test on bulk tank milk. The data form does not identify the farm, only the county where the farm is located.

This report only includes data collected from December 1990 to July 1991. One hundred reports were received from milk plants. The dairy farms are from 35 counties in Wisconsin. Not all data requested was reported by the field representative visiting the farm. This is identified as NR. Conditions concerning the residue make some questions not applicable. These are identified as NA in the tables.

Table I. Milk Plant Test Used to Find the Antibiotic Residue

<table>
<thead>
<tr>
<th>Test Used to Find the Antibiotic Residue</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus stearothermophilus Disk Assay</td>
<td>31</td>
</tr>
<tr>
<td>Disk Assay</td>
<td></td>
</tr>
<tr>
<td>Plus Charm</td>
<td>12</td>
</tr>
<tr>
<td>Plus Penzyme</td>
<td>13</td>
</tr>
<tr>
<td>Plus Probe</td>
<td>1</td>
</tr>
<tr>
<td>Penzyme</td>
<td>26</td>
</tr>
<tr>
<td>Charm Plus Penzyme</td>
<td>2</td>
</tr>
<tr>
<td>Charm</td>
<td>4</td>
</tr>
<tr>
<td>Delvo</td>
<td>1</td>
</tr>
<tr>
<td>Probe- (2) Penicillin (1) Sulfamethazine</td>
<td>3</td>
</tr>
<tr>
<td>NR</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. Reported Drug or Family of Drugs

<table>
<thead>
<tr>
<th>Reported Drug or Family of Drugs</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-Lactams</td>
<td>24</td>
</tr>
<tr>
<td>Plus Penicillins</td>
<td>46</td>
</tr>
<tr>
<td>Plus Cephalosporins</td>
<td>11</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2</td>
</tr>
<tr>
<td>Sulfa-compds</td>
<td>2</td>
</tr>
<tr>
<td>NR</td>
<td>16</td>
</tr>
</tbody>
</table>

1 Survey financial support by Midwest Region of Food and Drug Administration and the Committee on Milk Quality and Milk Residues of Wisconsin Dairy Products Association is acknowledged.
Table 3. What Disease Was Treated?

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastitis</td>
<td>82</td>
</tr>
<tr>
<td>Leg Injury</td>
<td>2</td>
</tr>
<tr>
<td>Teat Injury</td>
<td>3</td>
</tr>
<tr>
<td>Parturition</td>
<td>1</td>
</tr>
<tr>
<td>Mastitis Dry Treatment</td>
<td>1</td>
</tr>
<tr>
<td>High Temperature</td>
<td>1</td>
</tr>
<tr>
<td>Uterine</td>
<td>1</td>
</tr>
<tr>
<td>NA</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 4. Who Administered the Treatment?

<table>
<thead>
<tr>
<th>WHO ADMINISTERED</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owner</td>
<td>70</td>
</tr>
<tr>
<td>Hired Milker</td>
<td>5</td>
</tr>
<tr>
<td>Veterinarian</td>
<td>8</td>
</tr>
<tr>
<td>Spouse</td>
<td>3</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
</tr>
<tr>
<td>NR &amp; NA</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 5. Was a Sample tested by Milk Plant before Milk in Tank?

<table>
<thead>
<tr>
<th>TESTED BY MILK PLANT</th>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>29</td>
</tr>
<tr>
<td>Yes-But treatment mislabeled</td>
<td>1</td>
</tr>
<tr>
<td>NA &amp; NR</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 6. What Was The Number of Treatments? 7 NA & NR

<table>
<thead>
<tr>
<th>NUMBER</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Time</td>
</tr>
<tr>
<td>Twice</td>
</tr>
<tr>
<td>Three Times</td>
</tr>
<tr>
<td>Four Times</td>
</tr>
<tr>
<td>Six Times</td>
</tr>
<tr>
<td>Eight Times</td>
</tr>
<tr>
<td>Treatment every 12 hours by 11 farms of the 36.</td>
</tr>
</tbody>
</table>

Table 7. If obtained from a Vet., was the vet the herd vet?

<table>
<thead>
<tr>
<th>YES</th>
<th>NO</th>
<th>FROM A STORE</th>
<th>NA</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>3</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>From a store</td>
<td></td>
</tr>
</tbody>
</table>
Table 8. What Was the Treatment Dosage?

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Tube</td>
<td>49</td>
</tr>
<tr>
<td>2 - 8 tubes</td>
<td>22</td>
</tr>
<tr>
<td>10 cc</td>
<td>5</td>
</tr>
<tr>
<td>20 cc</td>
<td>1</td>
</tr>
<tr>
<td>One-Half Tube</td>
<td>1</td>
</tr>
<tr>
<td>No Treatment</td>
<td>1</td>
</tr>
<tr>
<td>NR &amp; NA</td>
<td>21</td>
</tr>
</tbody>
</table>

Table 9. Where Was the Treatment Location?

<table>
<thead>
<tr>
<th>Location</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quarter</td>
<td>77</td>
</tr>
<tr>
<td>Plus IM</td>
<td>3</td>
</tr>
<tr>
<td>Plus IV</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
</tr>
<tr>
<td>IM</td>
<td>5</td>
</tr>
<tr>
<td>Uterus</td>
<td>1</td>
</tr>
<tr>
<td>NA &amp; NR</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 10. Are Lactation and Dry Treatment Drugs Stored Separately?

<table>
<thead>
<tr>
<th>Storage</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>82</td>
</tr>
<tr>
<td>NO</td>
<td>8</td>
</tr>
<tr>
<td>NA</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 11. How Long Was Milk Withheld From Quarters After Last Treatment?

<table>
<thead>
<tr>
<th>Hours</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>36</td>
<td>1</td>
</tr>
<tr>
<td>48</td>
<td>4</td>
</tr>
<tr>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>92</td>
<td>1</td>
</tr>
<tr>
<td>96</td>
<td>5</td>
</tr>
<tr>
<td>Until test Negative *</td>
<td>1</td>
</tr>
</tbody>
</table>

*Failed because incomplete identification on the label of all antibiotics in Vet. Clinic preparation.

Table 12. Was the Treated Cow Positively Identified With a Fail Safe Marking System?

<table>
<thead>
<tr>
<th>Identification</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>48</td>
</tr>
<tr>
<td>NO</td>
<td>38</td>
</tr>
<tr>
<td>NA &amp; NR</td>
<td>14</td>
</tr>
</tbody>
</table>
Table 13. What Does the Producer Think Caused the Residue?<br><br>**NUMBER**<br><br|Milked Treated Cow | 59  
|-------------------|-----  
|Beyond withholding time | 7  
|Cow not marked  
  |No chalk available to mark cow |  
  |Forgot to mark and wife was milking |  
  |Milk samples mixed up on farm |  
|Cow marked  
  |Kids were milking |  
  |Milker not responding to marking |  
  |Treated cow lost legband |  
  |Lack of concentration & milked marked cow |  
|Withheld from marked cow but Vet Clinic Label not include all antibiotics so farm test for label OK, but not at plant where another antibiotic was identified present. |  
|Not withholding 4 quarters when treat one |  
|Milked cows purchased at sale and did not know were treated. | 4  
|Entered fresh cow too soon in bulk tank | 6  
|Milked dry cow that was dry cow treated | 10  
|Wrong cow treated with dry cow treatment | 3  
|Wrong cow marked | 1  
|Uterine treatment | 1  
|???? Do not know, No treatments, Residue in milk buckets, | 9  

Table 14. How Many Times Was Your Vet on Your Farm Last Year concerning Mastitis?<br><br>|FARMS| NUMBER OF TIMES|  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>20 or more</td>
</tr>
<tr>
<td>24</td>
<td>10 to 20</td>
</tr>
<tr>
<td>10</td>
<td>5 to 10</td>
</tr>
<tr>
<td>11</td>
<td>less than 5</td>
</tr>
<tr>
<td>NA &amp; NR</td>
<td>44</td>
</tr>
</tbody>
</table>
Table 15. What Was the Herd Somatic Cell Count?

<table>
<thead>
<tr>
<th>NUMBER OF HERDS</th>
<th>BULK TANK SCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Under 100,000</td>
</tr>
<tr>
<td>25</td>
<td>101,000-200,000</td>
</tr>
<tr>
<td>36</td>
<td>201,000-400,000</td>
</tr>
<tr>
<td>27</td>
<td>401,000-999,000</td>
</tr>
<tr>
<td>8</td>
<td>NR</td>
</tr>
</tbody>
</table>

Average herd SCC in this survey is 350,011

Table 16. Milking Herd Size.

<table>
<thead>
<tr>
<th>NUMBER OF HERDS</th>
<th>HERD SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>100 or greater</td>
</tr>
<tr>
<td>33</td>
<td>50-99</td>
</tr>
<tr>
<td>47</td>
<td>49 or less</td>
</tr>
<tr>
<td>6</td>
<td>NR</td>
</tr>
</tbody>
</table>

SUMMARY

1. This residue survey data indicates value to dairy farms for a valid relationship with a veterinarian. Implementation of treatment records and a fail safe marking system of treated cow needs to occur.

2. The reasons for treatment and herd SCC show the need for dairies to implement effective mastitis prevention programs. Maximum efforts will decrease the need for the number of mastitis treatment exposure and risk of error.

3. It is important that the label list all active antibiotics and chemicals. On the farm tests can be limited by depending on an incomplete label. Dairymen must insist the vet provide complete label information.

4. Before adding milk to bulk tank from recently purchased cows or heifers test for residues. Producers can place pressure on dairy cattle sales for assurance of non treated animals. Require that if sale animals are treated that the information goes with the animal.

5. Separate dry treated cows from the milking herd.

6. The decision to treat or not needs better analysis by both research, vets and dairy producers. The majority of treatments represented in this data likely was of minimum value to the dairy cow but still contaminated a large volume of milk.
TEN POINT RESIDUE AVOIDANCE GUIDELINES

1. Preventive herd health management including sanitation, nutrition, reproduction, vaccination and parasite control, disease prevention, and mastitis control and monitoring.

2. Establish a valid veterinarian/client/patient relationship.

3. Use only FDA approved over the counter or prescription drugs, including those used extra-label (deviates from label claims and recommendations).

4. All drug labels and directions need to comply with Grade "A" milk control labeling requirements.

5. All drugs must be stored in compliance with Grade "A" milk control requirements.

6. All drugs must be administered properly and treated cows must be positively and distinctly identified or isolated from the milking herd.

7. Cow treatment records are properly maintained and treated animals adequately identified.

8. Proper drug residue testing capabilities readily available on and off farm.

9. All farm employees involved in milking must demonstrate an awareness and knowledge of proper use of treatments and testing methods to avoid marketing adulterated products.

10. This quality assurance check list should be completed annually.
was used to find the drug residue?

or family of drugs was found?

ing interviewed? Owner ____ Milker ____ Other ____

s the producer think caused the residue?

ple tested by milk plant before milk placed in bulk tank? Yes ____ No ____ N/A ____

s the cows? Owner ____ Spouse ____ Children ____ Hired ____ Other ____
mistered the treatment(s)? Owner ____ Spouse ____ Hired ____ Veterinarian ____ Other ____
ase was treated?

d frequency of treatment? _______ every _____ hours for _______

he treatment (s) given? Teat/Quarter ____ IM ____ IV ____ Oral ____ Uterine ____
s drug(s) obtained? Veterinarian ____ Store ____ Mail order ____ Door to door sale ____
obtained from veterinarian, is the veterinary your herd veterinarian? Yes ____ No ____
teated cow positively identified with a fail safe marking system? Yes ____ No ____ Describe!
(hours) was milk withheld from all four quarters after the last treatment?

mally withheld from all quarters of drug treated cow? Yes ____ No ____
on and dry treatment drugs stored separately? Yes ____ No ____
he number of cows in the herd? Milking _______ Dry _______

current herd SCC?

times was your veterinarian on your farm last year concerning mastitis?
Relationship between Mozzarella Manufacturing Parameters, Cheese Composition and Functional Characteristics: Development of a System for Controlled Research Studies.

By D.M. Barbano, J.J. Yun, L.J. Kiely, & P.S. Kindstedt
Northeast Dairy Foods Research Center
Cornell University and University of Vermont

ABSTRACT

A major research focus at the Northeast Dairy Foods Research Center is to characterize systematically the relationships between individual parameters in the Mozzarella cheese making process and cheese characteristics. The goal is to provide industry with a better understanding of how each parameter in the manufacturing process influences the chemical characteristics and functionality of the cheese, so that a more uniform, higher quality, product can be produced. The nonuniformity of Mozzarella cheese composition (i.e., salt and moisture gradients) within each loaf of cheese causes large systematic variations in composition and functionality that makes it very difficult to conduct controlled research. To eliminate this problem a Mozzarella cheese making procedure was developed for use in our research that incorporates salt into the cheese prior to the mixer and produces cheese with uniform salt and moisture content. No brine salting is used. For each experiment, 3 vats of cheese (400 lbs milk/vat) are made in one day from one batch of standardized milk. One manufacturing variable can be varied to three different levels (e.g., milk at pH 5.10, 5.25, & 5.40) on the same day and then the experiment can be repeated on several days. Cheese is evaluated periodically during 50 days of refrigerated storage. Results for the influence of milk pH on the the chemical and functional characteristics of the cheese during refrigerated storage will be reported.

PRELIMINARY WORK AND BACKGROUND:

Mozzarella cheese research is a major focus area for the Northeast Dairy Foods Research Center. Dave Barbano and Paul Kindstedt, and their graduate students and staff, are working together to systematically characterize the influence of various cheese manufacturing factors on the chemical and functional characteristics of Mozzarella cheese. Ideally, a study should focus on one manufacturing factor at a time, vary that factor, and characterize the impact on the chemical composition and functional properties of the cheese. We have initiated a series of studies to do this.

Work by Farkye et. al. (1) characterized the moisture and salt gradients that occur within each piece of Mozzarella cheese when cheese is brine salted. There are large gradients of salt and moisture within each piece of brine salted cheese. The concentration of salt in the outside layers of the cheese can be between 2 and 3%,
while the center of the piece of cheese may only have a concentration of .3% salt. In general, the concentration of salt is high on the outside and low in the center. The moisture was lowest at the center and the outside and highest in the intermediate zone between the outside and center of the piece of cheese. These gradients will gradually even out, but there is still substantial nonuniformity of moisture and salt after 2 to 3 weeks of refrigerated storage. Differences in salt and moisture concentrations may influence both proteolysis during refrigerated storage and the functional properties of the cheese. The nonuniformity in moisture and salt within each piece of brine salted Mozzarella makes it very difficult to get a representative sample for both chemical and functionality testing when one is trying to conduct controlled research studies. Therefore, we decided to develop a Mozzarella cheese making procedure that would produce pieces of Mozzarella that had uniform salt and moisture content. To do this we had to develop a Mozzarella cheese making procedure that did not use brine salting.

After several months of preliminary work we developed a procedure using dry salting after milling. The curd is dry salted in two applications. The dry salted curd is added directly to a small scale twin screw mixer. The water in the mixer contains between 7 and 9% salt when the curd is added. The mixer water is 135°F and the water jacket on the barrel of the mixer is also set at 135°F. The cheese is molded into cylinders (3 inch diameter by 12 inches long) as it comes out of the mixer, cooled immediately in ice water (about 1 h), and then vacuum packaged. There is very little variation in moisture or salt content within each piece or from piece to piece within the same vat when this process is used. The low mixer temperature minimizes fat loss. We standardize the milk to about 2.3% fat and end up with a cheese that has 38 to 40% fat on a dry basis using this process. Therefore, fat loss during cheese making is quite low. This process allows us to produce cheese for controlled studies of the influence of various manufacturing factors on the chemical and functional characteristics of Mozzarella cheese.

Several people from industry have expressed interest in this process for possible use in industry. We are currently working to refine the process so it could be scaled up for industrial use. There are three key problems that remain to be solved. First, the moisture content of the cheese is too low (i.e., 44 to 45% moisture). We are currently working on modifications of the make procedure to increase the moisture. Second, the current design of Mozzarella mixers used in plants is not adequate to make this process work well. We are working to identify the key design changes that need to be made to produce a mixer that would work well with this process. Finally, a new approach to obtain more efficient and rapid cooling of the cheese may be possible with this process and this is under investigation.
SYSTEMATIC STUDY OF CHEESE MAKING VARIABLES - MILL pH:

The process described above was used for cheese making. One batch of milk (ca. 1500 lbs) was standardized to 2.3% fat and split into three portions. Three vats of cheese were made on the same day. This was repeated on two additional days to achieve a 3 x 3 Latin Square design for the experiment. In the first trial in our research, mill pH was the variable studied. Three different milling pH's were used each day (i.e., 5.4, 5.25, and 5.1). The order in which they were done was different on each of the three cheese making days.

One batch of a direct to the vat frozen starter culture (Thermococcus and Thermorod, Marschall Products) was used for all vats of cheese within the same trial. Culture was added at 96°F and ripening time was 60 min. Calf rennet was used as the coagulant, cook temperature was 106°F, and draw pH was 6.40. The salting and mixer conditions were as described above.

Cheese composition is shown in Table 1. The differences in mill pH resulted in significant differences (P < .05) in fresh cheese pH. The final cheese pH's were lower than the mill pH and the differences in final cheese pH between treatments was not as great as the differences in mill pH between treatments. The curd temperature is 106°F during the salting step and during salting (i.e., after mill) the pH continues to decrease. The decrease in pH is larger for the curd milled at pH 5.4 than the other two treatments because the buffering capacity of the curd is not as high at this pH as it is at the lower pH's. As a result the pH of the curd milled at pH 5.4 decreased to 5.22, while the curd milled at pH 5.10 only decreased to 5.09. The differences in pH observed in the cheese at day 3 were maintained through 50 days of refrigerated storage (Figure 1).

The remainder of the cheese composition characteristics were very similar among treatments (Table 1). The moisture content of the curd was low (43.8 to 44.7%). Despite the low moisture content, we feel that the comparison of results across treatments within this experiment will yield a proper evaluation of the impact of mill pH on chemical characteristics of the cheese. Since this experiment was conducted, we have been able to increase the average moisture content of the cheese to between 46 to 47% by modifying the cheese making procedure.

Proteolysis occurs during refrigerated storage of Mozzarella cheese and is probably the main factor responsible for the time dependent changes in cheese functionality that occur. Proteolysis was monitored by measuring the nitrogen soluble in pH 4.6 acetate buffer and in 12% trichloroacetic acid (TCA). The amount of nitrogen soluble in these solutions is expressed as a percentage of the total nitrogen content of the
cheese and shown in Figure 2. There was no impact of mill pH on the soluble nitrogen content or rate of change in soluble nitrogen content of the cheese. However, as can be seen from Figure 2, the soluble nitrogen content of all cheeses increased significantly with time of refrigerated storage at 4°C.

Functional properties (e.g., firmness, melting, and stretching) are important characteristics of Mozzarella cheese. To evaluate the functional characteristics of unmelted Mozzarella cheese a double compression test using an Instron was conducted. Cheese hardness should be related to the shredding characteristics of the cheese and the force required for the first compression of the cheese is shown in Figure 3. Cheeses made with a milling pH of 5.4 and 5.1 both had slightly higher hardness values than the cheese made with a mill pH of 5.25. However, the hardness of cheese from all three treatments decreased at about the same rate during refrigerated storage. The decrease with time was much larger than any differences that may have been due to differences in mill pH.

Meltability was measured using a modified Schreiber test. There was an increase in meltability with time of refrigerated storage for all cheeses, but there was no apparent difference in meltability due to differences in mill pH (Figure 4).

Helical viscometry was used to measure the apparent viscosity of the melted cheese (2). The apparent viscosity of the cheese decreases dramatically during refrigerated storage and the changes observed in the cheeses made in this trial are similar to those observed in commercial cheeses (Figure 5). Compared to the changes in apparent viscosity with time, the differences between mill pH treatments appear to be small. However, if a cheese manufacturer needs a cheese that meets a specific apparent viscosity target value (e.g., 30), then the difference (between mill pH treatments) in time (days) to achieve this value may be important and can be estimated from Figure 5. More work is needed to assess the practical meaning of these differences in apparent viscosity.

Release of some free oil by the cheese is desirable, however excessive free oil release is considered a defect. Free oil formation was measured by the method of Kindstedt and Rippe (3) and is shown in Figure 6. Free oil release by the cheese increased significantly with time of refrigerated storage. Again the time dependent changes were much larger than any small variations between different mill pH treatments.

CONCLUSIONS:

Differences in mill pH resulted in differences in cheese pH that were maintained throughout 50 days of refrigerated storage. Differences in mill pH did not significantly affect moisture, fat, protein, or salt content of the cheese or the indices
of proteolysis and meltability. Cheese produced using a higher mill pH may have a slightly firmer body initially, but the large decreases in firmness with time in refrigerated storage probably makes these initial differences relatively unimportant. There were large time dependent increases in proteolysis and free oil formation and large time dependent decreases in apparent viscosity for cheeses in all treatments.

The practical significance of these results for the cheese maker is that mill pH can probably vary over a range of .2 pH units without significantly affecting the chemical composition or functional properties of low moisture part skim Mozzarella cheese. In fact, this difference probably exists from the beginning to end of most large vats of Mozzarella cheese because of the time it takes to mill a large vat of Mozzarella cheese. The pH of the cheese will have some influence on the growth of undesirable nonstarter bacteria in the cheese. As cheese pH increases there is increased risk for the growth of S. aureus. Because differences in mill pH are reflected in final cheese pH, it is probably best to keep cheese pH low to avoid growth of undesirable nonstarter bacteria and use other cheese making parameters to control the firmness and functional properties of the cheese. Our future work will study other cheese making parameters and determine their impact on cheese composition and functionality.

ACKNOWLEDGMENT:

The authors thank the National Dairy Board and the Northeast Dairy Foods Research Center for financial support of this research. Special thanks are given to Maureen Chapman, Kathy Chu, Joe Davidson, Pat Nelson, and Bob Rasmussen for their technical assistance.

REFERENCES:


<table>
<thead>
<tr>
<th></th>
<th>MILL pH 5.40</th>
<th>MILL pH 5.25</th>
<th>MILL pH 5.10</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHEESE pH</td>
<td>5.22</td>
<td>5.16</td>
<td>5.09</td>
</tr>
<tr>
<td>% MOISTURE</td>
<td>44.74</td>
<td>44.61</td>
<td>43.84</td>
</tr>
<tr>
<td>% FAT</td>
<td>21.67</td>
<td>21.25</td>
<td>21.96</td>
</tr>
<tr>
<td>% FDB</td>
<td>39.20</td>
<td>38.33</td>
<td>39.10</td>
</tr>
<tr>
<td>% TOTAL N</td>
<td>27.84</td>
<td>27.99</td>
<td>28.53</td>
</tr>
<tr>
<td>% SALT</td>
<td>1.44</td>
<td>1.45</td>
<td>1.43</td>
</tr>
<tr>
<td>% SALT/H₂O</td>
<td>3.22</td>
<td>3.26</td>
<td>3.26</td>
</tr>
</tbody>
</table>
FIGURE 1  CHANGES IN CHEESE pH
IMPACT OF MILL pH

FIGURE 2  CHANGES IN SOLUBLE PROTEIN
IMPACT OF MILL pH
FIGURE 3  CHANGES IN HARDNESS 1
IMPACT OF MILL pH

FIGURE 4  CHANGES IN MELTABILITY
IMPACT OF MILL pH
FIGURE 5  CHANGES IN APPARENT VISCOSITY
IMPACT OF MILL pH

FIGURE 6  CHANGES IN FREE OIL FORMATION
IMPACT OF MILL pH
RELATIONSHIP BETWEEN MOZZARELLA MANUFACTURING PARAMETERS, CHEESE COMPOSITION, AND FUNCTIONAL PROPERTIES: IMPACT OF COAGULANT

P.S. Kindstedt, L.J. Kiely, University of Vermont, Burlington
and
J.J. Yun, D.M. Barbano, Cornell University, Ithaca, NY

INTRODUCTION

Today's cheesemaker is faced with a wide choice of commercially available coagulants. Calf rennet, extracted from the stomachs of young calves, was the original coagulant and is still the standard against which all others are compared. Later, coagulants from various animal (e.g., bovine and porcine pepsin) and microbial (e.g., Mucor miehei, Mucor pusillus, and Endothia Parasitica) sources were developed and then refined. Most recently, advanced enzyme purification techniques and recombinant DNA technology have enabled the commercial production of coagulants containing 100% chymosin, the predominant proteolytic enzyme in calf rennet.

All of the coagulants used in cheese making consist of proteolytic enzymes (i.e., enzymes that break down protein). The principal function of the coagulant is to cleave Kappa casein molecules at the surface of casein micelles in milk, thereby initiating the process of milk coagulation.

During the manufacture of most cheeses, a small percentage of active coagulant is retained in the cheese curd which, depending on cheese type, may contribute to texture and flavor development during aging (3). Coagulants differ greatly in the rate and manner in which they breakdown milk proteins (2,5). As a consequence, some coagulants produce better quality aged cheese than other coagulants. For example, it is widely accepted that calf rennet gives better texture and flavor in aged Cheddar cheese than the microbial coagulants.

Thus, when choosing a coagulant the cheesemaker must take into consideration its impact on cheese quality (and yield) as well as its price and availability. This paper will focus on the impact of coagulant on the functional properties of Mozzarella cheese. Because Mozzarella cheese is used primarily as an ingredient for pizza, the functional properties of the cheese, both melted and unmelted, are primary determinants of quality. Are the functional properties of Mozzarella cheese affected by the type of coagulant used in manufacture? A common view within the research community is that coagulant is mostly or completely inactivated during cooking and mixing-stretching, and consequently has little influence on the development of functional properties in aging Mozzarella (1,3,4). However, the experience of
cheesemakers seems to contradict that view. For example, anecdotal reports from the industry suggest that the rate of softening in Mozzarella cheese can vary substantially depending on coagulant. Few definitive studies have been undertaken to determine whether active residual coagulant is retained in Mozzarella cheese and whether it influences the development of functional properties during aging. Hence, our specific objective was to conduct a comprehensive comparison of protein breakdown and functional development in Mozzarella cheeses made from three commercially available coagulants.

MATERIALS AND METHODS

Three 170 kg vats of cultured low-moisture part-skim Mozzarella cheese were made at Cornell University on the same day using the same milk and starter but three different coagulants: fermentation produced chymosin, Endothia parasitica, and Mucor miehei proteases. Cheese making was replicated on three different days using a new experimental cheese making procedure that eliminates brining and offers a high degree of control over manufacturing conditions (see accompanying paper). Coagulants were used at a rate to achieve an equal number of milk clotting units as determined by a modified Berridge assay. The starter culture was a direct set rod:cooccus type consisting of Lactobacillus delbrueckii ssp. bulgaricus and streptococcus salivarius ssp. thermophilus. Direct set thermophilic cultures are slow acid producers during the early stages of cheese making, and this resulted in a relatively high pH at drain. The whey was drained at pH 6.4 and the cheese making cook temperature was 41ºC. The mixer temperature was 57ºC, and milk pH was 5.25.

Cheeses were analyzed for fat by a modified Babcock method, total solids by drying in a forced draft oven at 100ºC for 24 h, salt by the Volhard method, total protein by Kjeldahl, and calcium by complexometric titration. Changes in pH, texture profile (Instron), meltability (modified Schreiber test), and soluble protein content (12% TCA and pH 4.6) were measured at 3, 8, 15, 21, 29, and 50 days of storage at 4ºC. In addition, samples of all cheeses were shipped on ice via overnight express mail to the University of Vermont for analysis. Changes in apparent viscosity (helical viscometry) and free oil formation (modified Babcock test) were measured at 3, 8, 15, 21, 29, and 57 days of storage at 4ºC. Water soluble nitrogen analyses and discontinuous urea polyacrylamide gel electrophoresis were also conducted at 3, 21, and 57 days of storage.

Data were analyzed for statistical significance by analysis of variance using the SAS Statistical Software Package. Treatment effects exceeding the .05 level of probability were deemed nonsignificant.
RESULTS

Composition
Average composition of cheeses made with the three coagulants is shown in Table 1. Moisture, fat, total protein, calcium, and pH were not affected significantly by coagulant, and composition in general was very uniform among the treatment cheeses. Significant differences did occur in salt content, with Mucor cheeses having the lowest and Endothia cheeses having the highest salt concentrations. It is not known why coagulant affected salt levels. However, the differences observed, although statistically significant, were small (i.e. < 0.1%) and probably of little practical importance. Thus, for all intents and purposes cheese composition was virtually identical for all three coagulants.

It is important to note that all of the cheeses were slightly below the legal minimum of 45% for moisture content. As discussed in the accompanying paper, this was due to the novel method used to incorporate salt into the cheeses. Lower moisture tends to slow down proteolytic activity during aging, therefore proteolytic (and functional) changes in our experimental cheeses probably occurred at a somewhat slower rate than would be expected in normal commercial low-moisture part-skim Mozzarella containing 47 to 48% moisture.

Proteolysis During Storage
Four different methods were used to assess the degradation of cheese proteins during refrigerated storage: TCA (12%) soluble nitrogen, pH 4.6 soluble nitrogen, water soluble nitrogen, and discontinuous polyacrylamide gel electrophoresis. TCA soluble nitrogen, which measures the level of free amino acids and small peptides in the cheese, is shown in Figure 1. TCA soluble nitrogen increased in all cheeses during storage, regardless of coagulant; however, the rate of increase differed significantly among coagulants. Levels increased most rapidly in cheeses made with Endothia, while the slowest rate occurred with Mucor.

A similar but more pronounced pattern was observed for pH 4.6 soluble nitrogen (figure 2). This fractionation method is less selective than TCA soluble nitrogen and includes large peptides and some proteins as well as small peptides and amino acids. Again, significant differences occurred in the rate of soluble nitrogen formation among coagulants, Endothia having the highest and Mucor having the lowest rate.

The formation of water soluble nitrogen (WSN) is shown in Figure 3. Analyses of this fraction, which is similar in makeup to pH 4.6 soluble nitrogen, were limited to days 3, 21, and 57. It is evident that WSN followed the same pattern of change with respect to coagulant as TCA soluble and pH 4.6 soluble nitrogen. In summary, proteolysis as measured by the formation of three different soluble nitrogen fractions occurred in stored cheeses at different rates depending on coagulant, with Endothia showing the greatest proteolysis and Mucor showing the least.
The effect of coagulant on the relative breakdown rates of $\alpha_S^-$ and B-caseins during cheese storage can be seen by comparing the electrophoretic patterns in Figure 4. Each band in the electrophoretogram represents a different protein or protein breakdown product, and the intensity of the band corresponds to the amount of protein present. When specific proteins such as $\alpha_S^-$ or B-caseins are degraded during storage the corresponding electrophoretic bands become fainter. This loss in band intensity was used to quantify the breakdown of $\alpha_S^-$ and B-casein. Cheeses made with *Endothia* showed approximately 70% breakdown of B-casein and 40% breakdown of $\alpha_S^-$ casein between days 3 and 57 of storage. In contrast, only about 28% of B-casein and 50% of $\alpha_S^-$ casein were degraded in cheeses made with *Mucor* during the same time period. Cheeses made with chymosin showed nearly 70% breakdown of $\alpha_S^-$ casein but only slight breakdown of B-casein. The three coagulants also gave different electrophoretic patterns (Figure 4) revealing differences in the pattern of proteolysis during storage. Thus, coagulant affected not only the overall rate of proteolysis but also the relative rates at which the specific caseins ($\alpha_S^-$ and B-) were degraded and the pattern of breakdown. In technical terms, the coagulants differed in both residual activity and specificity.

As is always the case with research data, it is important to recognize that these findings apply to the specific cheese making conditions used in our studies. For example, we used a cook temperature of 41°C. However, had we chosen a higher cook temperature such as 44°C our results could have been quite different because *Endothia parasitica* protease is more sensitive to heat inactivation than *Mucor miehei* or chymosin. Presumably, as cooking temperature is increased, *Endothia* cheeses become less proteolytic relative to those made with *Mucor* or chymosin due to greater thermal inactivation of the *Endothia* protease. In short, we will not have a complete picture of the impact of coagulant on Mozzarella cheese until the various factors that interact with coagulant activity such as cook temperature are systematically studied.

**Functional Development During Storage**

**Textural Properties.** Several rheological properties of unmelted cheese texture were measured during aging using the Instron Universal Testing Machine. Changes in cheese hardness, defined as the force required to compress the sample by 50% in two consecutive “bites” (hardness 1 and hardness 2), are shown in Figure 5. Both hardness 1 and 2 decreased significantly during storage, indicating that all cheeses softened with age. Coagulant did not significantly affect hardness 1 ($P = 0.18$) but did affect hardness 2. *Endothia* cheeses had the lowest average values for both hardness 1 and 2 as the cheeses aged, indicating that *Endothia* cheeses underwent greater softening than those made with *Mucor* or chymosin.
Changes in springiness, defined as the rebound height of the cheese sample after being compressed for the first time by 50%, are compared in Figure 6. All cheeses became less springy during aging. Coagulant did not have a statistically significant effect on springiness \((P = .22)\); nevertheless, cheeses made with *Endothia* had lower average values (less springy) than *Mucor* or chymosin cheeses.

Changes in cohesiveness, gumminess, and chewiness are shown in Figures 7 - 9, respectively. Although these three parameters measure important fundamental aspects of cheese texture, their relationship to cheese functional properties such as shreddability is poorly understood. Perhaps what is most important is that two of the three parameters were significantly affected by coagulant (cohesiveness marginally exceeded the .05 level of probability; \(P = .06\)), with *Endothia* cheeses having lower average values for each of these parameters than *Mucor* or chymosin cheeses. Thus, with respect to the various textural parameters, cheese made with *Endothia* consistently stood apart from those made with the other coagulants. These differences could have important implications for cheese shredding properties; however, definitive statements will require further studies. Again, it is important to note that these findings apply to the specific cheesemaking conditions that were used in these experiments.

**Melting Properties.** Changes in meltability during storage, as assessed by a modified Schrieber test, are compared in Figure 10. This test measures the increase in area of the cheese sample as it melts and spreads during heating. Meltability of all cheeses increased significantly during aging, and a significant interaction occurred between coagulant and aging time. That is, *Endothia* cheeses showed a greater increase in meltability than the other cheeses during storage.

Changes in apparent viscosity (AV), a measure of melted consistency, during aging are shown in Figure 11. The AV of Mozzarella cheese decreases as its melted consistency becomes more flowable and less elastic. The AV of our experimental cheeses decreased significantly during storage, typical of the "mellowing" process that occurs in Mozzarella texture during the first few weeks of aging. The effect of coagulant on AV was not significant \((P = .10)\); however, average AV levels differed consistently among coagulants during storage, with *Endothia* cheeses showing the lowest and *Mucor* cheeses the highest average AV throughout aging (Figure 11).

Figure 12 shows the development of free oil formation, a measure of oiling off, during storage. Free oil increased significantly in all cheeses over time but the effect of coagulant marginally exceeded the .05 level of probability \((P = .06)\). It is worth noting that *Endothia* cheeses developed substantially higher average free oil values during the latter stages of storage (beyond 20 days).
DISCUSSION

The data presented above show unequivocally that, under the cheesemaking conditions used in this research, coagulant type strongly influences the breakdown of protein in Mozzarella cheese during short-term aging. The effect of coagulant on cheese functional properties is less readily discerned. With regards to functionality, it is important to recognize that cheese textural and melting properties are very difficult to measure with precision. Often, large random variations occur in the analyses that make it difficult to distinguish statistically significant treatment effects (e.g. effect of coagulant) unless the experiment is repeated many times. Unfortunately, it is usually not possible to repeat cheesemaking experiments more than a few times. Our results are based on three cheese making trials, which may not have been sufficient to distinguish the effect of coagulant on some parameters such as springiness, free oil and hardness.

Another difficulty occurs when analytical data have a high degree of nonuniform random variation, such as the AV data from our experiments. In this situation it is necessary to transform the data into logarithmic values in order to conduct a valid statistical analysis with maximum statistical power. Analyzed in this way, AV was found to be significantly affected by coagulant.

In summary, given the nature of the analytical measurements, we believe that there is adequate basis to conclude that coagulant type has important implications for the development of both melted and unmelted functional properties of Mozzarella cheese.

The following example illustrates a practical application of these findings. Pizza restaurants generally require that Mozzarella cheese be aged for a week or two before use in order to develop the desired melt and stretch. A recent survey of cheeses collected from various pizza restaurants showed that apparent viscosity of most cheeses fell in the range of 10 - 40% yield, with some restaurants consistently falling at the low end of that range and others at the high end depending on the restaurant’s particular expectations for melted consistency. What effect does coagulant have on the aging requirement for Mozzarella cheese that is to be used as an ingredient for pizza? For example, will it take longer for a cheese made with Endothia parasitica to attain a melted consistency with an apparent viscosity value of, say 20% yield, than for the same cheese made with Mucor miehei? An approximate answer can be derived from the data in Figure 13, which shows that Mucor cheeses, on the average, took about two weeks longer to reach AV = 20% yield than Endothia cheeses. Of course, these specific values apply only to our experimental conditions, and different results might be obtained if we change those conditions.
conditions, for example, by using a higher cook temperature as discussed earlier. Nevertheless, it is evident that coagulant has the potential to influence cheese shelf life, storage and distribution requirements. Our results suggest that highly proteolytic coagulants that remain active in Mozzarella cheese can result in accelerated functional changes during storage and aging.

REFERENCES


ACKNOWLEDGMENTS

This research was funded by the Northeast Dairy Foods Research Center.

TABLE 1. Average composition of Mozzarella cheeses made with three different coagulants.

<table>
<thead>
<tr>
<th></th>
<th>Endothia parasitica</th>
<th>Mucor miehei</th>
<th>Fermentation produced chymosin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>44.41</td>
<td>44.11</td>
<td>44.53</td>
</tr>
<tr>
<td>Fat</td>
<td>22.1</td>
<td>22.0</td>
<td>21.9</td>
</tr>
<tr>
<td>FDB</td>
<td>39.7</td>
<td>39.4</td>
<td>39.5</td>
</tr>
<tr>
<td>Salt</td>
<td>1.55</td>
<td>1.46</td>
<td>1.51</td>
</tr>
<tr>
<td>Total Protein</td>
<td>27.83</td>
<td>28.16</td>
<td>27.97</td>
</tr>
<tr>
<td>Calcium</td>
<td>.81</td>
<td>.79</td>
<td>.80</td>
</tr>
<tr>
<td>pH (day 3)</td>
<td>5.15</td>
<td>5.16</td>
<td>5.17</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

1. Changes in TCA soluble nitrogen during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

2. Changes in pH 4.6 soluble nitrogen during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

3. Changes in water soluble nitrogen during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

4. Electrophoretic patterns during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

5. Changes in hardness 1 (closed symbols) and hardness 2 (open symbols) during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

6. Changes in springiness during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

7. Changes in cohesiveness during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

8. Changes in gumminess during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

9. Changes in chewiness during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.

10. Changes in meltability during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = *Endothia parasitica*; MM = *Mucor miehei*.
11. Changes in apparent viscosity during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = Endothia parasitica; MM = Mucor miehei.

12. Changes in free oil formation during storage (4°C) of Mozzarella cheeses made with three different coagulants: CHY = fermentation produced chymosin; EP = Endothia parasitica; MM = Mucor miehei.

13. Effect of coagulant on aging time required to attain apparent viscosity = 20% yield.

Figure 1

- CHY
- EP
- MM

STORAGE (DAYS)
Figure 2

The graph illustrates the pH of 4.6 Sol. N / TN (%) over different storage days for three different treatments: CHY, EP, and MM. The y-axis represents the pH values, ranging from 0 to 15, while the x-axis represents the storage days, ranging from 0 to 50. Each treatment has a distinct line indicating its pH trend over time.
Figure 3

Storage (DAYS)

- CHY
- EP
- MM
Figure 4

CHY

EP

MM

\( \beta \)

\( \alpha_s \)

3 21 57

DAYS

1991-8
Figure 5

![Graph showing the effect of storage on CHY 1, CHY 2, EP 1, EP 2, MM 1, and MM 2 over a 60-day period. The graph plots storage (in days) on the x-axis and some measured value on the y-axis, with different markers and line styles for each category.](image-url)
Figure 7

- CHY
- EP
- MM

STORAGE (DAYS)
Figure 8

GUMMINESS

STORAGE (DAYS)
**Figure 9**

![Graph showing storage (days) vs. value for different categories: CHY, EP, MM. The graph has a y-axis range from 500 to 200 and an x-axis range from 0 to 60 days. The graph includes various markers and lines representing each category.]
Figure 10

MELTABILITY (mm)

STORAGE (DAYS)

- CHY
- EP
- MM
Figure 11

Graph showing the relationship between storage (days) and the degradation of a substance. The graph compares three different conditions: CHY, EP, and MM. The x-axis represents storage days, ranging from 0 to 60, and the y-axis represents the degradation levels from 0 to 150. The lines show a decrease in degradation over time for each condition.
Figure 12

This graph illustrates the change in free oil percentage over storage time for different samples labeled CHY, EP, and MM. The x-axis represents storage time in days, ranging from 0 to 50, and the y-axis represents free oil percentage, ranging from 0 to 8. The lines show an increasing trend in free oil with storage time.
Figure 13

- CHY
- EP
- MM
MANUFACTURE OF CHEESE MADE WITH SIMPLESSE

RENÉ SNOOK

The dairy industry and especially Mozzarella manufacturers are at the precipice of a great opportunity.

Every major food category, especially frozen foods has demonstrated an upsurge in the portion of their business converting to "healthy" and nutritionally controlled products.

Pizza is about to undergo the same growth phenomenon where Tombstone Lite and other manufacturers' brands will soon be introducing their own versions of healthier pizzas. This trend will carry into Food Service quickly. In fact, when Pizza Hut tested their Lite Pizza, it ended up being almost 30% of their volume!

There is obviously an opportunity for manufacturers of performance directed products, such as Mozzarella to spearhead this market growth. The challenge is to deliver the taste and functionality promise that consumers (and the industry) demand.

Simplesse® all natural fat substitute is a protein based dairy ingredient that can be used by the manufacturer to replace fat without loss of taste or functionality.

Just to show you how mainstream fat reduction and fat substitutes have become, I would like to share with you one of my favorite cartoons.

According to the latest Calorie Control Council Survey 1991 over 75% of consumers use lowfat/low calorie products at least once every two weeks although they are not entirely satisfied with the taste.

However, taste is very important to the consumer, where 75% of their food selections are based on taste alone or taste and health. Only 25% of consumers are willing to trade off taste.

64% of the consumers surveyed understand the need for fat replacers. 67% (124 million) consumers are using lowfat products and want more products in more food categories as a means to maintain overall better health. (Calorie Control Council 1991). This is not a niche market! But for the product opportunity to be a success the new products must taste good.

There is a real opportunity for manufacturers to begin now to provide good tasting
lowfat products and build their brand awareness. Since as consumer awareness of fat in cheese will increase with implementation of the new Nutrition and Education in Labeling Act, the cheese industry will ultimately be hurt. The wholesome “halo” we have enjoyed for so long may disappear. As you know, most consumers are not aware of the fact that cheese is higher in fat than ice cream.

To substantiate these trends, we have seen increasing concern by consumers over fat. In 1990, consumer concerns regarding fat had just overtaken cholesterol at 46% and 44% respectively. According to the 1991 FMI Trends survey, the nutritional concern regarding fat has increased to 62% and 58% for cholesterol. Sodium and calorie concerns remain essentially unchanged. Consumers are looking for fast but healthy foods to maintain overall better health.

We believe that these trends will continue since the leading opinion makers, health professionals also agree that fat is the number one health concern since fat has been linked to heart disease, various forms of cancer, diabetes and hypertension. We have been saying this for about a year and now this year consumer concerns parallel those of health professionals last year!

The dairy industry has been quick to respond to the consumer desire for healthier products. Cheese manufacturers in particularly have responded by introducing “Lite” cheese. Two years ago there were only ten brands of “Lite” cheese and today there are over 120 brands available. The biggest challenge for a successful product is taste and performance which we know is key to trial and repeat business. Unfortunately, the cheese industry is still struggling to deliver taste and performance.

Based on acceptance panels run on commercially available products, as fat is reduced, overall liking in all products tend to decrease. You will note that this drop is much less severe in products which rely upon thickeners for mouthfeel such as salad dressings and sour cream where technology has advanced to improve the acceptance of these products. In natural cheese however, acceptance drops dramatically as fat is reduced to about 23%.

This can be explained if we look at the role of fat in its contribution to mouthfeel properties.

In natural cheese, fat contributes to the mouthfeel of the product when chewed into a fluid, the thickness and creaminess of the mass, the lacked of sensed particulate, cohesiveness and rate of clearing. As well, fat contributes to opacity and flavor.

Traditional strategies for replacing fat have included: The use of the addition of whey to the cheese by ultrafiltration, or high heat of pasteurization to entrap whey proteins and bind water, and the occasional addition
of whey protein to the starter tank for the same purpose. Homogenization has also been used in the cultured products area to enhance creaminess. The problem with these methods have been inconsistency in incorporation of the whey proteins, overall quality of the whey protein (including flavor) and subsequent quality of the cheese.

Some cheese manufacturers have also tried to use the ingredients that are available to the formulated foods industry such as thickeners and gums to bind water. The disadvantage has been inhibition of starter and rennet activity, and the tendency toward pastiness, especially with ripening. Side fermentations of the carbohydrates are also common. Non microparticulated proteins have also been used, but may cause toughening of the cheese matrix with the added protein and losses of the protein to the whey due to poor rehydration and solubility.

Simplesse can replace the attributes that fat contributes to cheese resulting in products that taste just as good as their full fat counterparts.

As you may already know, the Simplesse principle is achieved by the technology that controls particle size to yield particles within a 0.1 - 3.0 micron size range that are perceived as creamy and not as individual particles.

Currently two forms of Simplesse are commercially available. Simplesse 300, which received early publicity, is composed of egg white and milk protein. Simplesse 100, which is composed of whey protein, has been used in the development of cheese applications over the past two years.

As you may have heard, we recently reached agreement with the FDA that Simplesse 100 meets the standards of identity and GRAS status of whey protein concentrate.

This means, Simplesse 100 can be used in any food category which can use whey protein. In the product ingredient legend, Simplesse 100 would be labeled as whey protein concentrate.

As I mentioned, Simplesse 100 is 100% whey protein concentrate. It is currently sold as a liquid having the pH stability properties of whey protein, i.e. stable between pH of 3.5-7.0. The product is viscous, shear-thinning and pumpable and thickens with heat above 68C to become more creamy. This is extremely helpful in the processing of UHT sauces for example.

Simplesse is shipped refrigerated in 5 gal. and 55 gal. Scholle bag in box. The current shelf life is 30 days from the date of manufacture. We are continuing to improve the shelf life.
In cheese, Simplesse enhances the quality of natural cheese by enhancing cheese flavor and increasing opacity due to light scattering by the Simplesse particles. In Mozzarella cheese, Simplesse also enhances the whiteness and opacity of the melt, even after cooling.

With the incorporation of Simplesse, lactose/galactose levels are consistent with those in full fat and part skim Mozzarella.

The protein particles also increase water binding and improve manufacturers' ability to control moisture uptake without syneresis.

The addition of Simplesse to cheese milk will not result in the interaction of Simplesse particles with casein or impact functionality of the cheese.

Firmness and high elasticity defects are so common with lowfat natural cheese. With Simplesse, we can actually increase softness and decrease elasticity.

The chewed mass (bolus) is also closer to full fat product with a creamier mouthfeel rather than chew to a mealy/coarse and noncohesive mass.

Since the microparticulated protein is entrapped in the curd, yield is enhanced over cheese produced at comparable fat and moisture levels.

By choosing different levels of Simplesse, different textural attributes may be obtained in the cheese.

As Simplesse levels are increased from 1 to 3%, sensory springiness (elasticity) decreases for cheese at the same fat level. For Swiss cheese, low level of Simplesse may be used to provide for gas entrapment for eye formation. A higher level of Simplesse may be used for semi soft varieties such as Port Salut.

Firmness can also be modified by selection of Simplesse level at similar fat and moisture contents.

For a Mozzarella, the cheese should be soft enough to provide the required "bite", but not so soft as to be pasty and still low enough to allow the appropriate stretch with melting.

I mentioned earlier that Simplesse does not interfere with stretch and melt due to entrapment of the Simplesse particles without interaction with casein. Let me demonstrate this by transmission electron micrographs.
Simplesse microparticles are entrapped in the casein network of natural cheese and appeal unaltered throughout ripening.

In working with Simplesse, it can be added to your cheese milk prior to pasteurization, before or after standardization. Studies have shown that Simplesse will not separate out with the fat. Simplesse microparticles are also stable to standard pasteurization temperatures. In fact, some manufacturers have been working with Simplesse in UHT and Aseptically processed products.

The Simplesse usage will be dependent on the variety of cheese and its fat level, as well as manufacturing considerations.

For Mozzarella, usage levels range from 1-2%. Minor manufacturing modifications are necessary such as reduction of cook temperatures and lower pH at molding. All other manufacturing modifications are similar to those used to manipulate functionality in traditional product manufacture.

Based on these studies, you can see that lowfat Mozzarella made with Simplesse can have the same functionality as that expected from the traditional higher fat products.

We believe that the usage of Simplesse will bring some very positive benefits to the dairy industry, particularly to cheese makers. First by expansion of a natural dairy protein into traditionally oil-based categories such as spreads, salad dressing and mayonnaise, usage of whey protein should increase significantly.

In cheese, Simplesse will expand consumption as consumers discover healthier choices and re-enter the category or increase frequency of eatings.

By increasing the demand for whey protein, we should be able to bring increased returns to the milk industry and cheese producers. Quality and consistency will improve as whey protein is elevated from a waste product to an added value ingredient for the industry.

In conclusion, Simplesse will enable the cheese manufacturer to produce lowfat cheese products that deliver taste and functionality, while giving consumers foods they can use as part of a healthier lifestyle.

The Simplesse logo will communicate to the consumer that the product that bears it will deliver all of the taste and lowfat.
Proceedings Order Form

To receive additional copies of the 1991 Italian Cheese Seminar Proceedings, copy this form and send it with a check or money order to:

Poule-Poulenc
Forschall Products-1991 Proceedings
D. Box 592
Madison, WI 53701

Name ____________________________

Company __________________________

Address ____________________________

City, State, Zip ______________________

Phone ____________________________

Quantity __________________________

Price ($13.50 each) __________________

Postage & Handling ($4.00 per book) ______________

Total ____________________________

Italian Cheese Seminar Proceedings Policy

Each exhibiting company, guest speaker, trade publication and Italian and Specialty Cheese Company in attendance will receive one free copy of the proceedings.

Proceedings from previous years are available upon request.