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## Proceedings from the 24th Annual Marschall Italian Cheese Seminar

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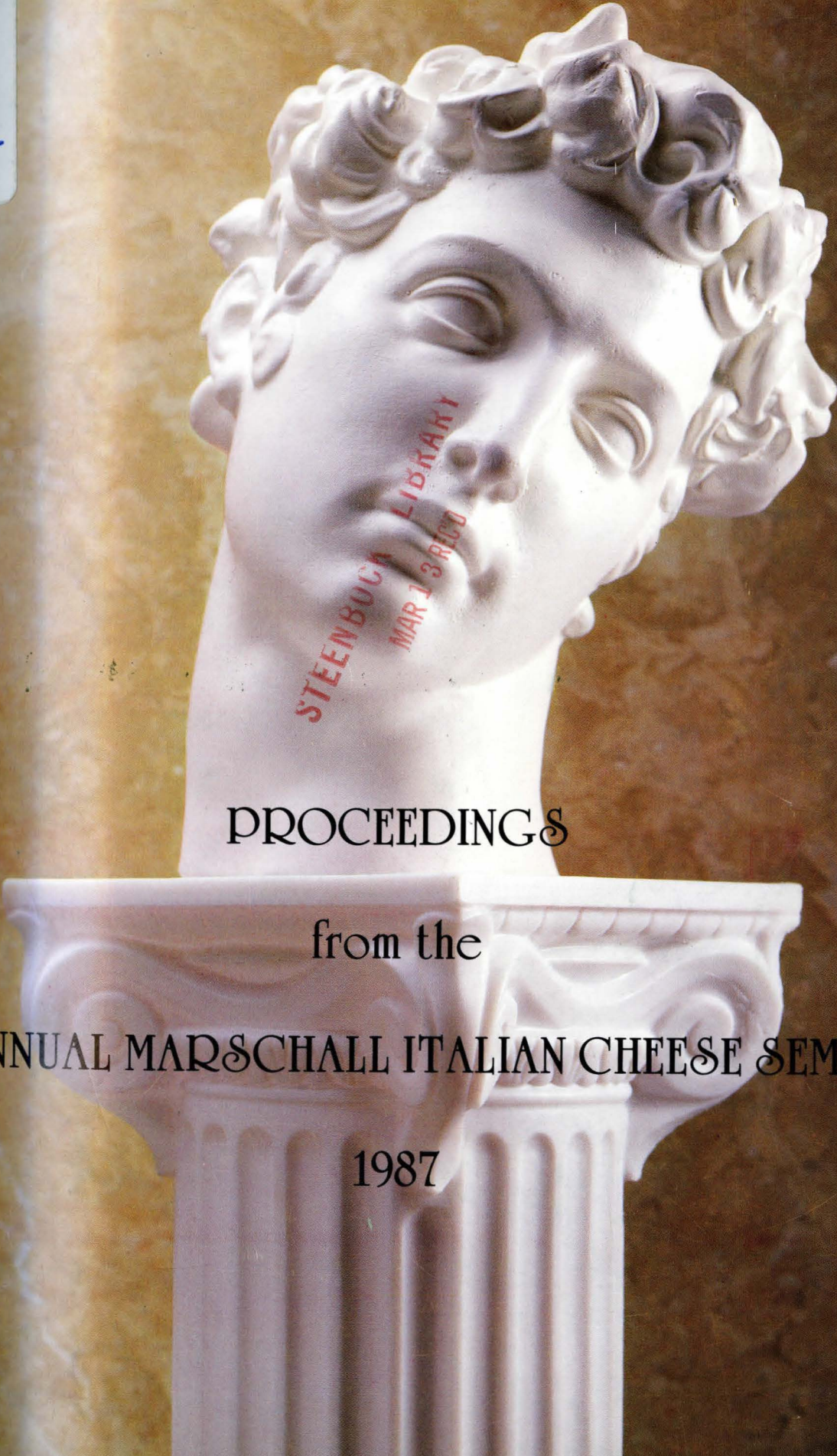
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PROCEEDINGS

from the

24th ANNUAL MARSCHELL ITALIAN CHEESE SEMINAR

1987

PROCEEDINGS  
from the  
24TH ANNUAL MARSCHALL  
ITALIAN CHEESE SEMINAR

September 16 & 17, 1987

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The following paper was presented by R. H. Deibel, President, Deibel Laboratories, 845 E. Johnson Street, Madison, Wisconsin 53703, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

## CHEESE RECALLS ASSOCIATED WITH PATHOGENIC BACTERIA

By R. H. Deibel

### Introduction

A decade ago the incidence of regulatory activity with cheese products due to microbiological problems was rare. These sporadic incidents usually involved Staphylococcus aureus and its production of a food-poisoning toxin that was produced during the fermentation period. With the passage of time, recalls due to cheese contamination with pathogenic bacteria has increased to the extent that hardly a month goes by without at least one recall to Listeria, Salmonella or Staphylococcus aureus.

What has happened in the intervening years to produce this dramatic change? Probably the most profound cause was the finding of Listeria in cheese and outbreaks of listeriosis that in some instances caused death and/or serious infections. Some other factors that have contributed to increased regulatory activity can be associated with:

- a) an increased sensitivity and simplification of methodology (Staphylococcus toxin detection)
- b) the discovery of "new" pathogens (Listeria, Yersinia)
- c) significantly increased scrutiny of all cheeses by the FDA and
- d) an increased public awareness.

Undoubtedly, cheese can be associated with the dissemination of pathogenic bacteria or food-poisoning toxins. The problems appear to be well defined and the challenge to exclude those pathogens now confronts not only the cheese industry but the entire food industry as well.

The organisms most commonly associated with cheese recalls are Listeria monocytogenes, Salmonella and Staphylococcus aureus—approximately in that order. Thus, the focus of this presentation will be on these three pathogens although other bacteria may occasionally be involved in recalled cheeses.

### Recalls Associated With Listeria

In the normal healthy adult, infections with Listeria produce a disease that is similar to the "flu." After a day or two the symptoms subside and the infected person returns to normal. However, the disease in infants, the elderly and immuno-compromised people (those undergoing anti-cancer or corticosteroid therapy) are more susceptible to infections that can be quite serious. Pregnant women are also more susceptible and infection may result in abortion. This is not uncommon and it occurs in both humans and animals.

Listeria grow free in nature and occur on or grow in soil, dust, plants, improperly prepared silage, feces, insects, animal feeds, raw and prepared meats, raw milk, fresh vegetables and in many food processing environments especially if they are wet. The finding of Listeria in raw milk merits extended comment. Depending on the survey, twenty to forty percent of patron samples are contaminated with the organism. Factors such as season and geographical area appear to effect the incidence results. Thus, raw milk must be considered as a likely source of the organism and all efforts to avoid plant contamination from this source should be considered.

Listeria have been found in just about all types of soft, semisoft and hard cheeses. Sour cream and cottage cheeses have not, as yet, been implicated in a recall. Early on it was thought that Listeria contamination was peculiar to soft and surface-ripened cheeses. More recent findings have dispelled this contention.

Listeria outbreaks have also been associated with contaminated grade A milk (postpasteurization contamination) and coleslaw made with contaminated cabbage. The organisms have been found in many different types of sausage (no recalls as yet) and ice cream (many recalls) but definitive outbreaks associated with these products have not been reported. The infectious dose has not been established but data from the Jalisco cheese outbreak in California indicates that about 1,000 to 100,000 is the dose range for "normal" individuals and a dose as low as 100 organisms for "susceptible" individuals was reported. The FDA has stated that in the absence of substantial dose data it has no alternative other than the enforcement of a zero standard.

Generally, the organism is considered to be killed by pasteurization procedures. Although there is some controversy regarding this point, if pasteurization doesn't effect a kill it radically reduces the number of Listeria. In essence, the FDA regards pasteurization as a killing procedure.

The organism is very resistant to salt (it can survive 20% for days) and drying. It can survive for extended periods in lightly salted products with a pH range of 4.8 to 5.5 (cheese and sausage). A very peculiar property of Listeria is its ability to grow at refrigeration temperatures (as low as 34° F). Although it grows slowly at these low temperatures within several weeks to a month (or well within a product's normal shelf life) its growth in a refrigerated display case can exceed thousands of organisms per gram of product. The effect on the infectious dose is readily apparent.

### Recalls Associated With Salmonella

The incidence of recalls associated with Salmonella-contaminated cheese is significantly lower than that of Listeria-contaminated cheese. The subsequent handling of product in the manufacture of a cold-pack cheese necessitates an increased vigilance of the finished product. A Salmonella recall has been associated with this type of product but apparently no infections were reported.

Spray dried cheeses (and cheese products) are also susceptible to Salmonella contamination; and, in one recalled product the count of Salmonella exceeded 100 per gram. The microbiological quality of spices and dried whey that are mixed with cheeses and cheese products are also the responsibility of the cheese manufacturer.

Recently, the second largest outbreak of salmonellosis that occurred in North America (the largest being the Jewel outbreak in Chicago) was associated with natural cheddar cheese manufactured in Canada. Approximately 1,500 people were infected. Another recent outbreak in Canada involved 150 cases (one death), and again the cheese was cheddar.

Salmonella infections usually produce vomiting and/or diarrhea as the prominent symptoms. The "normal" healthy individual has an uneventful recovery within several days. Again the "susceptible" individuals (infants, the elderly and immuno-compromised) may face a life-threatening situation.

The organism occurs in fresh milk but substantial incidence data is lacking. Unlike Listeria; Salmonella does not grow in nature. It can grow in the gut of many different animals (insects, snakes, frogs, birds, chickens, pigs, cows, man) and once implanted in a food-processing area it definitely can survive and grow for years.

It can survive in low acid or high salt environments (although it cannot grow in these environments). The organism is killed by pasteurization and its occurrence in cheese usually reflects post-pasteurization contamination. Dose studies with naturally-contaminated Salmonella foods indicate about 10 to 100 organisms are required. A definitive difference in injectivity has been associated with various strains. Even though the dose data with Salmonella is substantially superior to that with Listeria, the FDA still has essentially a zero tolerance.

#### Recalls Associated With Staphylococcus aureus

The incidence of recalls associated with Staphylococcus aureus is relatively low. Usually, Staphylococcus contamination (with the production of detectable toxin) represents process failure. Slow vats due to:

- a) improper storage of starter cultures
- b) improper scale up or handling of starters or
- c) bacteriophage

may afford the growth of this organism.

The organism produces an enterotoxin as it grows. With a slow vat, there may be sufficient Staphylococcus growth to produce toxin. Eventually sufficient acid is produced by the starter to inhibit Staphylococcus growth and the acid may even kill it. However, the toxin is relatively acid resistant and it can survive in cheese for years. More importantly, the toxin is extremely heat resistant. In fact, it has been reported recently that it can survive some thermal processes for canned goods! It can readily survive pasteurization.

Some operators believe that substandard cheese from slow vats can be used in the manufacture of process cheese. Be careful! An analysis for toxins is indicated as the toxin may survive the cooking temperatures used in process cheese manufacture.



### How Can Recalls Be Avoided?

First and perhaps foremost consider the milk used for cheesemaking. Recent recalls (*Listeria* and *Staphylococcus*) involving cheese made with raw milk indicate that a 60 day holding period will not kill pathogens. Likewise, the use of heated milk (heated but below pasteurization times and temperatures) will not kill pathogens. The use of small quantities of raw milk for flavor production in a tank of pasteurized milk is just another method of inoculating pathogens into the cheese.

Lastly, check your pasteurization equipment frequently to insure its functionality.

In regard to starter cultures:

- a) avoid whey starters
- b) be careful and avoid temperature abuse of frozen cultures--the use of a low temperature thermometer in the freezing cabinet is recommended
- c) initiate a multiple starter program and
- d) avoid the employment of quaternary ammonium compounds (quats) in any vessel or pipe where the milk will eventually come in contact with the starter.

Finally, consider initiating a quality control program. Some cheese manufacturers have a pathogen profile run on each manufacturing lot. Others, attempting to decrease the analytical expenses, will have a profile run once a week or every other week. This is essentially an audit-type program.

In the last analysis, the cheesemaker cannot be assured absolutely that his product will not be recalled or associated with an illness outbreak. However, a consideration of the points enumerated above will help to decrease the potential for these undesirable occurrences.

The following paper was presented by Warren S. Clark, Jr., Executive Director, American Dairy Products Institute, 130 North Franklin Street, Chicago, Illinois 60606, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin on September 16 and 17, 1987.

## A LOOK AT THE WHEY PROCESSING INDUSTRY

By Warren S. Clark, Jr., Ph.D.

### ABSTRACT

Since 1971, when the Whey Products Institute was organized to serve the needs of whey processors by coordinating programs that would enable whey processing to attain its full potential as an important segment of the overall dairy industry, advances in processing technology and market development have been identified as basic goals for unified and coordinated industry support. Significant advances have been made in both areas. This paper reviews the development of the U.S. whey processing industry to where it is today, and projects what lies ahead for whey processors on the basis of technological developments, supply-demand forecasts and other considerations.

The status of whey and whey products in the U.S. today directly relates to formal development efforts on behalf of the industry by its national trade association—The Whey Products Institute—and its new successor organization, The American Dairy Products Institute.

The Whey Products Institute was organized in 1971 as the national trade association of the whey products industry, for the primary purposes of encouraging technological developments in support of whey processing, to develop viable markets for whey products, and to promote the use of these wholesome, nutritious and economical products, as ingredients in a wide range of human food and animal feed products. For over 15 years, these original goals were pursued on behalf of whey processors while, at the same time, accepting the challenge of progress through change that benefits not only whey processors, but, also, the entire dairy industry. On April 17, 1986, members of the Whey Products Institute voted to merge WPI with the American Dry Milk Institute to form the American Dairy Products Institute. A year later, on April 9 of this year, the Institute expanded the scope of its activities when the Evaporated Milk Association became a part of ADPI. Accordingly, the American Dairy Products Institute now is responsible for meeting the existing needs and future challenges for an increasing segment of the processed dairy products industry.

With the assistance of a series of slides presenting both whey and whey product production and utilization for over a decade, I will review first the development of the U.S. whey processing industry to where it is today; and, secondly, project what I believe lies ahead for whey processors on the basis of technological developments, supply-demand forecasts and other considerations. Much of the information presented in my slides this morning is included in Tables I and II of this paper, scheduled to be published as part of the 1987 Marschall Italian Cheese Seminar Proceedings. Should additional information about whey products be desired, I invite you to contact the American Dairy Products Institute.

### Production of whey and whey products

My initial slides will serve to define the magnitude of the U.S. whey processing industry. In 1973, the first year complete industry statistics were compiled, fluid whey production in the U.S. totaled 28.7 billion pounds, of which 24.1 billion pounds were of sweet-type whey and 4.6 billion pounds were of acid-type whey. This volume of fluid whey was equivalent to 1.9 billion pounds of whey solids. By 1986, 52.8 billion pounds of fluid whey were produced, 47.0 billion pounds being of the sweet-type and 5.8 billion pounds of acid-type. Whey solids represented by this production were 3.4 billion pounds—an increase from 1973 of 79 percent.

Dry whey is the primary end product of the whey processing industry; in 1986 it comprised 65% of the production of finished whey and whey products. In view thereof, it may be of interest to consider how the production of this product has changed during the past several years. In 1973, 770 million pounds of dry whey were produced, one-half of which was processed for human food use, the remainder for animal feed utilization. In 1986, 985 million pounds of dry whey were produced, nearly 87 percent of which was intended for human food use and approximately 13 percent intended for animal feed. Two notes are in order regarding the current year's figures: first, dry whey manufacture for human food utilization has risen significantly, while that intended for animal feed has decreased; second, during the same time, significant amounts of value-added (modified) whey products have begun to be produced and these have been reported separately from dry whey since 1975.

The production of modified whey products, which include reduced-lactose whey, reduced-minerals whey and whey protein concentrate, was some 172 million pounds in 1975. Production rose to slightly over 230 million pounds in 1982, and in 1986 approximated 180 million pounds. Lactose, the carbohydrate derived from milk and also commonly known as milk sugar, is a common co-product of the manufacture of modified wheys. Yearly lactose production generally ranges from 130 to 160 million pounds; in 1986 it was 133 million pounds, of which 118 million were manufactured for human food use—primarily in infant formulas—and 15 million pounds were processed for animal feed utilization.

The data regarding production of whey and whey products were generated either directly by the U.S. Department of Agriculture or calculated using USDA production data. Information regarding the utilization of whey products is collected annually by the American Dairy Products Institute in a confidential survey of whey processors and resellers. End-use statistics were first tabulated for 1975; thus, 1986 marks the twelfth consecutive year of such a compilation. In the first year of this endeavor, 70 percent of the USDA-reported whey solids processed were included in the survey. This year, approximately 85 percent of such whey solids were included in the survey. In addition to the higher percentage of whey solids included in the current report, industry record keeping has improved significantly and the data reported are more reliable.

### Utilization of whey and whey products

Sweet-type concentrated whey utilization in human foods was 73.2 million pounds in 1986. In 1975, 82.1 million pounds were reported to be used for human foods. The primary categories of use were dairy, bakery and prepared dry mixes. Sweet-type dry whey use rose from 233.1 million pounds in 1975 to 471.1 million pounds in 1986. Primary use categories were dairy and bakery, with the prepared dry mix and confectionery industries also using significant quantities.

Whey solids use for animal feeds also has risen significantly since 1975—from 328 million to 562 million pounds. Dairy/calf/cattle feeds head the specific use, with swine feeds and pet foods also being significant markets.

An area of whey utilization that is still very much a challenge is that of acid-type whey. Acid-type whey—in the U. S. typically derived from cottage cheese manufacture—has a titratable acidity above 0.16% prior to any pH adjustment for processing purposes. In 1975, 8.8 million pounds of acid-type whey were used in human foods and another 6.5 million pounds used in animal feeds. In 1986, 5.6 million pounds were reported as being used in human foods and 19.7 million pounds used in animal feeds. While the volume increase reported for use in 1986 was significantly above that in 1975 (25.3 million versus 15.3 million pounds), only 6 percent of the acid-type whey supply reportedly was processed. Additional quantities of such whey found a use in fermentation—but, significant quantities also were disposed of in waste treatment plants.

I hope these visuals have been helpful in positioning the U.S. whey processing industry today in relation to where it was some 10-15 years ago. Next we need to project "where whey is going." Many years ago Little Miss Muffet contentedly—until the untimely arrival of an Arachnid—enjoyed curds and whey. But, what about now? Poor Little Miss Muffet most certainly would be confused for today she probably would find it difficult to recognize her whey—and, in fact—she might even lose her "whey."

#### Future outlook for whey processing

Whey processing industry development began with fluid whey, which then was concentrated and dried. New developments added membrane technology to the existing traditional processes resulting in modified whey products such as reduced-lactose and reduced-minerals whey, whey protein concentrate, lactalbumin and lactose. Based upon her knowledge and experience, Little Miss Muffet quite likely wouldn't recognize these as whey products.

We've quickly transgressed from yesterday through today as far as whey processing is concerned—but, what about the future? First, I believe that whey solids will continue to be used effectively and profitably in both human foods and animal feeds. Current emphasis is toward human food utilization, because of economic returns, and I believe the trend will continue in this direction.

Second, notwithstanding a multitude of short-term uncertainties, I believe that total U.S. milk production will decrease in the future. And, given that the first priority of milk use is as Class I utilization, this will mean less milk for manufacturing—to be divided among processors of cheese-whey and butter-nonfat dry milk. With lower volumes of milk and whey available, the supply-demand situation will improve markedly and product prices increase—to a point where it will be comfortably-profitable (rather than un- or marginally- so) to regularly process whey solids. In fact, I believe it's safe to say that situation exists today.

Third, the trend already in existence in whey processing today will spread to other areas of the dairy industry. My reference here is to fractionation, with the various separated components of milk selected and formulated to give the specific product desired for ingredient purposes. Accordingly, "blends" or blended products will become the "name of the game." These blends will not be limited to those containing only dairy ingredients, but will reflect a broad range of combinations with grain proteins and vegetable fats.

Current consumer preferences regarding food intake are for more protein and fiber, no significant change in carbohydrates, but with continued decreases in fat. Certainly, whey and the various whey components fit this preference pattern quite well.

At one time, the future for whey appeared to depend upon teaching cows to drink it. They do so, and with very positive end results, both in terms of body growth and milk production. Accordingly, this is one alternative for future whey utilization and the extent of practice will depend heavily on supply volume and location, as well as on the viable alternatives for processing.

In considering whey handling and utilization in the future, one needs to consider where whey volumes will be originating. This will depend heavily on where milk is produced. Using a map of the U.S., areas can be designated that represent future possible high concentration milk production areas—based upon established production, production efficiencies, and/or consumption demand. These cover: 1.) Washington-Oregon-Idaho; 2.) Southern California-east; 3.) Wisconsin-Minnesota-Iowa; 4.) Northeast; and 5.) Southeast. Primary supplies of whey from these areas certainly will present industry challenges—and, with them, opportunities.

Current institute efforts are focused heavily on whey permeates, lactose, and increasing the utilization for all whey products. Increased utilization, hopefully, will not be in substitution for other dairy solids now in use. Among food products holding greatest potential for utilizing whey solids are beverage drinks, snack foods and a multitude of natural and health foods. Increased utilization, however, can only be accomplished through extensive research in the area of product development—a direct responsibility of processors.

I believe that the strongest demand in the future will be for value-added whey products (modified wheys), as well as for components derived from milk or whey that will be used in preparing any number of bioengineered foods.

### Conclusion

In summary, the future for whey (processing and utilization) will be limited only by lack of a belief in the future of the industry and lack of foresight to properly prepare for it. Whey processors will not be "ultra-filtered" out of existence. An active whey processing segment will add strength and stability to the entire U.S., and world dairy industry.

Table I. ESTIMATED U.S. FLUID WHEY AND WHEY SOLIDS PRODUCTION (BY TYPE) AND RESULTING QUANTITY OF WHEY SOLIDS FURTHER PROCESSED

	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985 <sup>1</sup>	1986 <sup>2</sup>
	million lbs														
<b>Sweet-type Whey Production</b>															
Cheese Production <sup>3</sup>	2,605	2,685	2,937	2,811	3,320	3,359	3,520	3,715	3,984	4,278	4,540	4,819	4,674	5,025	5,226
Calculated Fluid Whey <sup>4</sup>	23,445	24,165	26,433	25,299	29,880	30,231	31,680	33,435	35,856	38,502	40,860	43,371	42,066	45,255	47,024
Calculated Whey Solids <sup>4</sup>	1,524	1,571	1,718	1,645	1,942	1,965	2,059	2,173	2,331	2,502	2,656	2,819	2,734	2,940	3,057
<b>Acid-type Whey Production</b>															
Cottage Cheese Production <sup>3</sup>	784	763	690	701	711	684	688	664	667	648	629	618	603	960	959
Calculated Fluid Whey <sup>4</sup>	4,704	4,578	4,140	4,206	4,266	4,104	4,128	3,984	4,002	3,888	3,774	3,708	3,618	5,760	5,754
Calculated Whey Solids <sup>4</sup>	306	297	269	273	277	267	268	259	260	253	245	241	235	374	374
<b>Total Whey Production (fluid basis):</b>	<b>28,149</b>	<b>28,743</b>	<b>30,573</b>	<b>29,505</b>	<b>34,146</b>	<b>34,335</b>	<b>35,808</b>	<b>37,419</b>	<b>39,858</b>	<b>42,390</b>	<b>44,634</b>	<b>47,079</b>	<b>45,684</b>	<b>51,015</b>	<b>52,788</b>
<b>Total Whey Production (solids basis):</b>	<b>1,830</b>	<b>1,868</b>	<b>1,987</b>	<b>1,918</b>	<b>2,219</b>	<b>2,232</b>	<b>2,327</b>	<b>2,432</b>	<b>2,591</b>	<b>2,754</b>	<b>2,901</b>	<b>3,060</b>	<b>2,969</b>	<b>3,316</b>	<b>3,431</b>
<b>Whey Solids Further Processed:</b>															
<b>A-Concentrated Whey Solids<sup>4</sup></b>	70	70	61	88	146	145	144	100	86	113	142	136	130	51	42
<b>B-Dry Whey</b>															
- Human Food	377	384	453	439	480	473	515	529	534	594	611	680	725	812	857
- Animal Feed	385	389	399	157	182	155	196	203	156	184	179	212	173	175	132
<b>C-Modified Dry Whey Products</b>															
- Reduced Lactose Whey & Reduced Minerals Whey	- <sup>7</sup>	-	-	142	128	144	166	137	158	140	132	86	60	96 <sup>8</sup>	92 <sup>8</sup>
- Whey Protein Concentrate <sup>9</sup>	-	-	-	8	8	7	9	10	4	4	71	86	96	105	86
<b>D-Whey Solids in Wet Blends</b>	-	-	-	81	74	68	96	103	144	163	144	124	136	136	116
<b>E-Whey Solids Utilized for Lactose<sup>10</sup></b>	141	184	210	222	170	174	183	175	224	259	230	208	198	197	213
<b>Total Whey Solids Further Processed (A+B+C+D+E):</b>	<b>973</b>	<b>1,027</b>	<b>1,123</b>	<b>1,159</b>	<b>1,214</b>	<b>1,194</b>	<b>1,339</b>	<b>1,287</b>	<b>1,337</b>	<b>1,486</b>	<b>1,540</b>	<b>1,562</b>	<b>1,546</b>	<b>1,572</b>	<b>1,538</b>
<b>Total Whey Solids Further Processed as % of Total Whey Production (solids basis):</b>	<b>53.2%</b>	<b>55.0%</b>	<b>56.5%</b>	<b>60.4%</b>	<b>54.7%</b>	<b>53.5%</b>	<b>57.5%</b>	<b>52.9%</b>	<b>51.6%</b>	<b>54.0%</b>	<b>53.1%</b>	<b>51.0%</b>	<b>52.1%</b>	<b>47.4%</b>	<b>44.8%</b>

<sup>1</sup>Revised<sup>2</sup>Preliminary; pending revision.<sup>3</sup>Agricultural Statistics Board, NASS, USDA - Da 2-1.<sup>4</sup>Whey Production: approximately 9 lb/l lb cheese produced (except Cottage) approximately 6 lb/l lb Cottage cheese produced<sup>5</sup>Average total solids content of whey: 6.5%.<sup>6</sup>Average total solids content of concentrated whey: 40%.<sup>7</sup>Data not available.<sup>8</sup>Reduced Lactose and Reduced Minerals Whey combined to avoid disclosure of individual plant operations.<sup>9</sup>Reported as Partially Delactosed/Demineralized Whey through 1981.<sup>10</sup>Approximately 1.6 lb whey solids utilized/1 lb lactose produced.

TABLE II. DRY WHEY PRODUCTION - INTENDED UTILIZATION<sup>1</sup>

<u>Year</u>	<u>Total Dry Whey Production</u>	<u>Human Food Use</u>	<u>Animal Feed Use</u>
1960	277	- <sup>2</sup>	-
1965	404	-	-
1970	621	-	-
1975 <sup>3</sup>	596	439 (73.6%)	157 (26.4%)
1980 <sup>3</sup>	690	535 (77.5%)	155 (22.5%)
1985 <sup>3</sup>	987	812 (82.3%)	175 (17.7%)
1986 <sup>3</sup>	985	853 (86.6%)	132 (13.4%)

<sup>1</sup>Millions of pounds<sup>2</sup>Data unavailable<sup>3</sup>Excludes production of modified dry whey products

The following paper was presented by Kathlyn M. Guidi, President, Specialty Product Marketing, 76 McGillivray Avenue, Toronto, Ontario M5M 2Y2, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

## SPECIALTY CHEESE: FINDING YOUR NICHE

by Kathlyn M. Guidi

### ABSTRACT

For the past 6 to 8 years, specialty cheese production has been popular in the U.S. However, the customer has changed. How they spend for food, has changed. This change will affect the concept and growth opportunities for specialty cheese in the future. Focus has to be on providing "lifestyle" products that answer the need for convenience, through value-added innovation, in addition to the quality, variety and intrigue, customers already expect from a specialty cheese. It means we've got to find new avenues for incorporating cheese into people's diets. Several examples are cited. The recommendation is also made that cheese-marketers think like a consumer and talk to retail customers, in order to stay on top of products and services that continue to meet the needs of the customer.

Six to eight years ago, getting into the specialty cheese business generally meant copying the Europeans, with either a cheese type, a marketing approach (i.e. labels, shapes, packaging), or developing a new positioning statement that stressed quality, variety and general intrigue of the product.

It was a logical, healthy approach. One which has increased domestic cheese sales in the deli; and has prompted introduction of American specialty cheeses (i.e. string, cheese fudge and marbled) which have become everyday institutions in the cheese case.

Today, I'd like to suggest that the age of 'copying the Europeans' has seen its day. Replaced by the age of 'Americans as Innovators', in expanding specialty cheese usage, thus giving specialty cheese an even more convenient 'lifestyle fit' with the consumer. It's time that domestic specialty cheeses achieve the same level of distinction held internationally by U.S. wines or 'Americana' gourmet cooking (i.e. cajun or tex-mex) for being proprietary and innovative, with a varietal quality all its own.

Now don't get me wrong, there still is room for growth in domestic production of European-style cheeses. In fact, I'll cite some later. However, I'm not going to discuss the rationale for pursuing domestic Havarti, Jarlsberg, goat or soft-ripened cheese types. This information is fairly easy to get from the USDA, the WMMB, WCMA, etc. Plus, if that's the only approach we look at, we'll be back into the competitive 'rat-race' of several years ago, in no time.

Rather, the key points I'd like to make about finding specialty cheese niche opportunities, are:

- 1) Customer spending for food has changed.



- 2) This change will affect the concept and growth opportunities for specialty cheese in the future.
- 3) Ways for you to stay on top of products and services that continue to meet the needs of the customer.

First of all, recognize that the customer has changed. Psychographic data on the lifestyles of "DINKS" (dual income no kids), "YUPPIES," "OOMPIES," (older, on top, middle-aged professionals, aged 40-49), singles and single parent families, is everywhere. But the impact of their changing food buying habits in the supermarket, can best be seen by looking at deli sales, and the breakdown of sales within deli product categories.

In 1986, deli sales increased by 13.5 percent. This is the 5th straight year of double digit growth with no end in sight. No other department in the supermarket has shown this kind of consistent growth rate.<sup>1</sup>

#### HOW DELI CATEGORY SALES HAVE CHANGED

1986 vs. 1978  
(percent of department sales)

Category	1986	1978	Category	1986	1978
Sliced Meats (including sausages)	38%	46%	Hot Foods	14%	12%
Cheese	21%	20%	Cold Entrees	2%	N/R
Imported	8%	7%	Sandwiches	3%	N/R
Domestic	13%	13%	Other	7%	3%
Salads	13%	10%	Packed Goods	5%	7%
Puddings and Gelatins	2%	2%			

N/R = Not Reported

"Cheese has increased slightly, its percentage of total deli sales, while the cold meats category has experienced a drop of 12 percentage points in its position as the department's biggest sales producer. Inroads in the category have been made by the growing popularity of salads, hot foods, entrees, sandwiches and related items in the 'other' category."<sup>2</sup>

Retailers are still expecting growth in the deli cheese center, but it is no longer the top growth area. Take-out and prepared foods are now in the spotlight.

Does this news strike fear of 'flat cheese sales' in your heart especially, if you just got your specialty cheese act 'rolling'? It shouldn't, because cheese consumption, and in particular specialty cheese consumption, is still on the rise. In fact, I remember the seemingly over optimistic projection made by a Kroger buyer at that first specialty cheese marketing seminar in

1982. He said consumption of cheese would be 30 lbs. per person by 1990. Today we know that this is not a far fetched number. (23.2 lbs. of cheese was consumed per person in 1986, a 7.0 percent increase over 1985).<sup>3</sup>

This brings me to point number two. Consumer change will affect the concept and growth opportunities for specialty cheese in the future.

I suggest that in order to maintain growth, the concept of 'specialty cheese' has to become broader. The focus has to be on providing 'lifestyle' products that answer the need for convenience, through 'value-added innovation', in addition to the quality, variety and intrigue, customers already expect from specialty cheese. It means we've got to find new avenues for incorporating the popular food cheese, into people's diets.

Here are some ways cheese-marketers can adapt their product to the needs of the consumer.

### 1. PACKAGING INNOVATION

It's finally happened, the zip lock bag Sargento is using on shredded cheese, and the resealable label used by Kraft, are the answer to many years of consumer prayers. The application by these companies may not be on specialty cheeses, but who says it has to stop with basic varieties? Many companies pre-cut or grate specialty cheeses. But what about the 1 lb. packages of string cheese sold in the deli? Isn't there a better way to offer freshness and convenience to these dedicated string cheese customers? How about individually wrapping string cheese and putting the pieces in a nylon mesh bag (the kind used for grapes and berries)? The extra cost would be worth it to the customer.

### 2. NEW SELLING VISTAS WITHIN THE STORE

A few years ago consumers voted cheese, the 4th most popular sandwich ingredient.<sup>4</sup> For years Gallo has been marketing a cheese and salami pack. How about taking that idea one step further by offering the consumer a variety of sliced specialty cheeses (i.e. Havarti, Gouda or Provolone) for sandwiches, right in the peg rack sliced meat section? Redundant within the store? No more so than dairy and deli pre-cut cheeses, or having bologna in the deli and in the packaged meat case. A common cheese and sausage "country of origin" theme, can tie this idea together.

### 3. GET YOUR SHARE OF DELI "TAKE-OUT FOOD" GROWTH

Pizza is still the most popular take-out food in America. But today's customer likes to try new things. Help retailers continue to build their pizza business by suggesting pizzas that use new cheeses, for their in-store fresh pizza programs. Canada really excels here. You see Hawaiian pizza (pineapple and ham with Mozzarella), Swiss cheese and ham pizza, gourmet goat cheese-topped pizza with pesto instead of tomato sauce, and Mexican pizza that uses cheddar, seasoned refried beans and ground beef on a crust.

Some delis are including fried cheese and sauce on their hot take-out menus. But consider these ways of selling more cheese through the carry-out, too.

- Breaded Mini Camembert - Dorman-Roth sells it in a kit for home preparation. The product is from Germany. Retailers could offer this item hot, or ready to microwave, by breading at store level. Or, you could sell it to them ready to warm.
- Hot Cheese Curds - Sound crazy? If you can do it with pretzels, why not with curds? Many kids I know love curds microwaved until the cheese is stringy and soft. Are you aware that 60 percent of U.S. households have microwaves? That's more than dishwashers! Promote curds as "microwavable."
- Fully Prepared, Cheese Fondue - The only commercial one I know of is from Switzerland, yet fresh cheese fondue could really offer convenience while being upscale and sophisticated. In fact, I can envision a whole line of cheese fondues using different herbs, cheeses, etc.
- What's the logical extension of cheese fondue? To my way of thinking its cheese soups; (because the processing is probably similar). They should be fresh (i.e. refrigerated), prepackaged and microwavable of course. Include cheddar and/or Swiss cheese based, but think exotic too, like Neufchatel fruit soup, eaten cold for brunch.
- Salads are a whopping 13 percent of U.S. deli sales. The WMMB has some great recipes for cheese salads. Why not take a formula to a salad manufacturer or to a retail commissary. Growth in the salad category is on variety styles.
- One of my favorite new foods from Canada is a popular fast food from Quebec called "Poutine" (pronounced Poo-tin). It is French fries topped with plenty of cheese curds and a gravy, that's more like a white sauce. It's served hot in a little paper tub with a fork. No respectable canteen truck is without it.

Poutine is luscious . . . and fattening, but so are the biscuits and gravy so many restaurants are offering.

- In New York last summer I ran across a father-daughter team selling 'Cheesh,' a fantastic product that is nothing more than a high cheese content, quiche. After eating 'Cheesh' I know why 'Real Men Don't Eat Quiche.' There's not enough cheese in it.

The point of these examples is that we've only scratched the surface of selling more cheese to consumers. However, we've got to be creative as to the ways we offer it, and help the retailer meet consumer demand for convenient, upscale take-out foods.

#### 4. UNDERDEVELOPED SPECIALTY CHEESE NICHES

- Italian Marscapone - There is a delicious, quite authentic version made in Canada. Marscapone is the ultimate triple creme cheese used as a spread, or as a convenient alternate to whipped cream.
- English Devon Cream similar to Creme Fraiche, but with a longer shelf life. It's wonderfully convenient and rich as a topping for fruit or as a sauce base.

- Raclette Cheese - The machines are quite vogue in cookware stores. And, Raclette restaurants are popping up all over. The only cheeses promoted for Raclette are from Europe. What a shame.
- Mozzarella - Now most of you would hardly call it a specialty cheese, but specialty stores and companies like Polly-O from New York, are creating a whole new and profitable niche for this old standby.

Polly-O sells balls of fresh Mozzarella packed in water under the Fior Di Latte™ name. The cheese curd is eaten as is, dipped in tomato sauce, or marinated in an herb-oil dressing to be eaten with crisp French bread. This fresh cheese is also terrific sliced in a tomato salad with Basil dressing.

Many specialty stores roll fresh Mozzarella thin, top it with Prosciutto, ham or salami, then roll it back up, pinwheel fashion. The final product is sold in thick slices. Anco cheese is selling the finished product in supermarkets in New York.

These new Mozzarella concepts are truly innovative here in America. Yet, they offer strong sales potential for the deli, because they build on the upscale variety foods that people expect from deli cheese centers, while offering something new in a cheese, consumers already enjoy.

As you can see, many of the 'innovations' that can add to your specialty cheese sales growth, while offering value and lifestyle convenience for consumers in purchasing and consuming cheese, are right on your doorstep. These specialty cheese niche ideas use an expanded concept of 'specialty' cheese beyond our past definition that was based on variety, quality and intrigue alone.

Finally, how can you stay on top of products and services that continue to meet the needs of the customer?

I encourage you to do two things that are not innovative, but rather are the golden, most tried and true rules of marketing.

- 1) Think like a customer. Don't think of your cheese product as 'finished' just because it has left your plant. Stay in touch with how and when consumers are using your product, how it is sold, how much are they buying per purchase, where they eat it, etc. Always be thinking of ways you can make the purchase of your product more attractive (i.e. convenient) for consumers.
- 2) Talk to your retail customers. Not just through your brokers and distributors, but face to face, across the desk, at trade shows, or by at least reading the same trade publications they do, so that you keep abreast of the key issues and trends they face.

Ask buyers what they see as the next step for their business? Ask how you can serve them in that development process? It's amazing the good ideas retailers have for using your product. But they are seldom heard by the people who can make the ideas happen, simply because these people (cheese-marketers), don't think to ask.

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The following paper was presented by Jeffrey T. Barach, Ph.D., Director of Dairy and Agricultural Fermentation Products, Biotechnology, Miles Laboratories, Inc., P.O. Box 932, Elkhart, Indiana 46515, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 and 17, 1987.

### Starter Strain Characterization For An Italian DSS Culture Program

by Jeffrey T. Barach, Ph.D., Larry L. Talbott, M.S.  
and Paula J. Leonhard, B. S.

#### ABSTRACT

A successful Defined-Strain Starter (DSS) culture program depends on having a collection of well characterized strains on hand to accommodate use in a diversity of Italian cheese types. If conditions in the field indicate cheese manufacturing difficulties, the program must support the rapid identification and substitution of the problem strain with an acceptable replacement. This paper reviews the properties and characteristics of single strain isolates made from traditional mixed-strain Italian cheese cultures.

Starter organisms used in Italian cheese manufacturing predominately are strains of Streptococcus thermophilus and Lactobacillus bulgaricus or helveticus. These cultures have been traditionally grown together in a symbiotic relationship in whey starter or buffered starter media. Cheese milk is inoculated with the ripened starter at about 1 percent or more depending on the initial acidity of the milk and activity of the starter. These thermophilic organisms differ significantly in their ability to produce acid. The coccus will produce little more than 1 percent lactic acid in milk where the rod typically can produce 2 percent or more. In some cheese plants, the rods and coccus are carried separately and inoculated together into the bulk starter. In other plants, cultures are always grown together and the balance is maintained by manipulating parameters such as time, temperature, nutrients, pH and the degree of ripening. A 1:1 ratio of cocci to rod is fairly standard for acid ripe buffered starters. Using pH control such as the Biolac Thermolac starter system, we find a 6:4 to 8:2 coccus to rod ratio is often preferred. This is because the cells are generally more active than when produced in a conventional medium. The special needs of the Italian cheesemaker, regarding the use of the pH controlled bulk starter, were addressed at this meeting last year by Carl Brothersen. Newly developed control equipment can pasteurize, cool down, prompt the inoculation, control pH and auto-cool. Advanced instrumentation can even change pH set points during starter growth and precisely control the ratio and activity of the mixed culture population.

Last year at this seminar, Dr. Randy Thunell gave us the background and general concepts involved in a Defined-Strain Starter (DSS) approach (2). Figure 1 shows the essential elements of this type of a program. These include (a) whey monitoring for bacteriophage and (b) strain replacement. As you can imagine the key to successful strain substitution involves having a bank of well characterized strains. Technical Service can then choose from these strains and recommend back-up cultures for replacement with little or no disruption to the cheese make procedure or to the properties of the finished cheese.

The focus of this presentation is to review the properties and characteristics of single rod and coccus isolates taken from traditional mixed-strain Italian cheese cultures. In understanding the properties of these strains, a logical selection procedure can be used to predict which cultures will work best in different cheese varieties and rapid substitution of strains can be performed if cheese manufacturing difficulties occur.

Before discussing characteristics of individual strains, let's review properties of the thermophilic microorganisms most commonly used in Italian cheese making. These properties, shown in Table 1, are often used by taxonomists as a basis to classify and identify different organisms by genus and species. In looking closely at the properties of these organisms, as they relate to cheesemaking, we see in Table 2, that the characteristics can be divided into two general categories: starter related properties and cheese related properties. Each will have an impact on different aspects of cheesemaking especially when considering the different types of Italian cheese made.

For any starter program,, such as conventional bulk starter with culture rotation or state-of-the-art DSS program with pH control, the primary function of the starter is acid production. Figure 2 depicts the relationships between temperature, growth rate and acid production. This type of data on all cultures allows selection of strains with desirable acid production properties. It also allows one to predict relative growth rates of strains when grown together. Of course, these organisms produce certain metabolic by-products which stimulate each other and the actual dynamics of symbiotic growth must be tested to demonstrate if good or poor compatibility exists between coccus and rod strains.

Bacteriophage susceptibility is probably the next most important characteristic to consider for strain selection. We know phage exist at some level in every cheese plant. The success of a good operation depends on sanitation, handling procedures and the durability of the starter program. To select strains for our new program we looked for phage in the field as well as using in-house phage banks to select-out phage susceptible strains. Table 3 shows the results of the field sampling program. Sixty-nine percent of samples obtained contained phage and some of these whey samples were further shown to contain phage for both cocci and rods. Table 4 shows an example of using multiple whey samples to screen and select against phage susceptible strains. Occasionally, we had to drop phage "free" strains because they didn't pass other qualifying tests.

In a group of phage "free" strains, which have similar characteristics, one may ask how many of these are actually the same strain? Determining plasmid profiles, such as shown in Figure 3, gives a fingerprint of the strain and allows them to be compared on the basis of their DNA content. Sometimes this is the most rapid way to differentiate between similar strains.

It is an advantage to have a program which can accommodate a variety of different bulk-starter media preferences. In Table 5, we see that not all cultures perform the same in all media. The ranking here is based on speed of acid development in milk of the ripened bulk starter. Also, considered here is rod to coccus ratios. It was interesting for us to discover that most all cocci performed in a similar manner and that the rod contributed the greatest variability. Consequently, ranking was based on rod selection.

The rod cultures are responsible for controlling many of the cheese related properties described earlier in this paper. This is particularly true of aged cheeses where enzymatic changes occur during ripening. The two most common rods (*L. bulgaricus* and *L. helveticus*) used in Italian cheese making can easily be differentiated by their ability to ferment maltose

(Table 6). Our program has several of each species to provide for different needs in cheesemaking.

Galactose utilization by the starter is of particular interest to mozzarella makers where the cheese is destined for pizza. The correct level of galactose in the cheese can determine the amount of browning which occurs during cooking. Table 7 shows classification of galactose utilization and you can easily see the possibilities of mixing and matching strains based on this trait.

The ability of a rod culture to produce a "pink ring" defect in aged Italian cheese can be predicted using a laboratory assay (Table 8). This property has been associated with the culture's ability to alter the oxidation/reduction potential in the cheese as it ages.

Cultures can also be ranked on their ability and the way they digest milk protein. Table 9 shows the results of a laboratory screening study where extensive protein breakdown was allowed to occur. Cultures are characterized on their tendency to liberate bitter peptides during casein proteolysis.

In an attempt to quantify proteolytic characteristics and compare them with organolytic profiles shown in Table 9, we looked at the ability of individual strains to digest casein and to hydrolyze a synthetic substrate. The data in Table 10 suggests a correlation between flavor profile and proteolytic function of the rod strains, however, no such correlation was seen for the cocci strains.

The final characteristic evaluated was salt tolerance of the *S. thermophilus* strains. Data in Table 11 show most cocci have no significant growth at 1.5 percent NaCl in a 6 hour time period. When the cultures are allowed to grow for 24 hours, however, growth can occur in the presence of less than 2.5 percent NaCl. The rod cultures are currently being studied for their growth in media with salt.

In summary, by thoroughly classifying and characterizing strains in our new Italian DSS, we have the ability to select and substitute strains based on functional characteristics as a starter and properties related to cheese flavor and texture development.

## **References**

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- Thunell, R. K. 1986. DSS Cultures and Thermolac Media: Applications in Italian Cheese Manufacture. 23rd Annual Marschall Invitational Italian Cheese Seminar pp. 53-59.



Table 1  
Classical Properties  
of Rod and Coccus  
Cultures

<u>Property</u>	<u>S. thermophilus</u>	<u>L. bulgaricus</u>	<u>L. helveticus</u>
Morphology	Spherical (Coccus) in pairs or chains	Rods, singles or pairs	Rods, singles or pairs
Growth Temperature	Optimum 40-44°C Max. 50°C Min. 20°C	Optimum 40-45°C Max. 53°C Min. 15°C	Optimum 40-45°C Max. 53°C Min. 15°C
Salt Tolerance	2% NaCl - No growth	2% NaCl - No growth	2% NaCl - No growth
Final % lactic acid in milk	= 1%	> 2%	> 2%
Lactic Acid Configuration/ Optical Rotation	L(+)	D(-)	DL

Table 2

Characterization Traits for Italian Strains

Starter Related

Acid Production Rate  
Phage Susceptibility  
Strain Compatibility  
Temperature Profile  
Plasmid Profile  
Starter Media Preferences

Cheese Related

Galactose Utilization  
Proteolysis  
Salt tolerance  
Flavor/Off Flavor  
Oxidation/Reduction Profile

Table 3  
Field Whey Sampling Program

<u>PLANT</u>	<u>LOCATION</u>	<u># SAMPLES ROD.</u>	<u># SAMPLES POSITIVE</u>	<u>PHAGE FOR: COCCI/ROD</u>
A	Northern, IL	7	4	Yes Yes
B	Northern, WI	5	4	Yes Yes
C	Central, WI	9	7	Yes No
D	Eastern, WI	3	0	No No
E	Western, WI	7	6	Yes Yes
F	Western, WI	8	3	Yes No
G	Eastern, WI	9	9	Yes Yes
		48	33 (69%)	

Table 4

Example of Screening Single Strain Cocci  
for Phage Susceptibility

Whey Containing Phage\*

<u>Strain Isolate</u>	<u>Whey 1/φ</u>	<u>Whey 2/φ</u>	<u>Whey 3/φ</u>	<u>Whey 4/φ</u>
A (C180)	-	-	-	-
B - Deleted	-	+++	-	+++
C (C130)	-	-	-	-
D (C140)	-	-	-	-
E (C170)	-	-	-	-
F - Deleted	++	-	-	-
G - Deleted	-	-	+++	-
H - Deleted	-	-	+++	-
I (C200)	-	-	-	-
J - Deleted	-	-	-	-
K - Deleted	-	-	-	-

\* pluses indicate plaque size from spot assay

Table 5

Performance of Cultures in Selected Bulk-Starter Media  
Based on Rapid Acid Production and Rod:Cocci Ratios

CR (Acid Ripe)

R110	Best Performance
R150	↑
R160	↕
R120	↓
R130	
R140	
R170	Not Best Performance

Thermolac (pH Controlled)

R110	Best Performance
R150	↑
R160	↕
R170	↓
R120	
R130	
R140	Not Best Performance

TMR (Acid Ripe)

R110	Best Performance
R160	↑
R150	↕
R120	↓
R140	
R130	
R170	Not Best Performance

Table 6

Differentiation Between *Bulgaricus* and *Helveticus*

L. bulgaricus - Maltose Negative

L. helveticus - Maltose Positive

StrainMaltose Fermentation Reaction

R110	-
R120	+
R130	+
R140	-
R150	+
R160	-
R170	-

Table 7

Galactose Fermentation Profiles  
of Italian DSS Cultures

<u>Rod Cultures</u>	<u>Utilization of Galactose</u>
R110	Weak
R120	Weak/moderate
R130	Strong
R140	Strong
R150	Strong
R160	Strong
R170	Weak
<u>Cocci Cultures</u>	
C110	Negative
C120	Delayed/strong
C130	Negative
C140	Negative
C150	Negative
C160	Negative
C170	Delayed/moderate
C180	Negative
C190	Delayed/moderate
C200	Negative

Table 8

Laboratory Assay to Detect  
Tendency to produce "Pink Ring"  
in Aged Italian Cheese

<u>Rod Cultures</u>	<u>Reaction</u>
R120	Strong
R140	Strong
R130	Moderate
R150	Moderate
R160	Moderate
R110	Negative
R170	Negative
<u>Cocci Cultures</u>	
All	Negative

\* Procedure of Shannon and Olson (1969 - JDS 52: 1678)

Table 9  
 Flavor of Proteolized Milk After  
 Extensive Protein Breakdown

<u>Rod Cultures</u>	<u>Taste (Bitterness Level)*</u>
R170	Intense
R110	Definite
R140	Definite
R150	Absent
R160	Absent
R120	Absent
R130	Absent
<u>Cocci Cultures</u>	
C150	Extreme
C200	Intense
C120	Intense
C110	Definite
C160	Possible
C140	Possible
C180	Possible
C130	Possible
C170	Possible
C190	Possible

\* Scale: absent, possible, definite, intense and extreme

Table 10  
 Proteolytic Characteristics of Italian DSS Cultures

<u>Rod Cultures</u>	<u>Casein Hydrolysis PAU/10g DWB</u>	<u>Aminoamidase PEP/10 g DWB</u>	<u>Ratio PAU/PEP</u>
R170	3.8	4.3	1
R110	9.9	11.7	1
R140	-	-	-
R130 *	0.7	13.8	20
R160	3.4	41.0	12
R120 *	2.5	62.0	25
R150 *	1.2	69.6	58
<u>Cocci Cultures</u>			
C150	0.5	12.5	25
C120	1.5	9.8	7
C200	2.5	8.3	3
C110	0.3	4.5	15
C160	0.1	15.9	159
C130	0.1	3.4	34
C140	0.1	7.9	79
C180	0.1	0.2	2
C170	0.4	6.9	17
C190	1.8	0.3	<1

\* L. helveticus

Table 11

Salt Tolerance of 19 *S. thermophilus* strains  
used in Italian Cheese Manufacturing

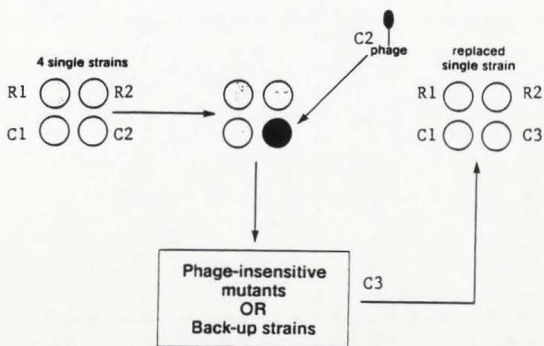
<u>Strain</u>	Inhibitory Salt Level (%) For:	
	<u>Rapid Growth</u>	<u>Extended Growth</u>
C150	2.0	2.5
C140	1.5	2.5
C160	1.5	2.5
C180	1.5	2.5
C190	1.5	2.5
C200	1.5	2.5
C220	1.5	2.5
C230	1.5	2.5
C240	1.5	2.5
C260	1.5	2.5
C290	1.5	2.5
C110	1.5	2.0
C270	1.5	2.0
C120	1.5	1.5
C170	1.5	1.5
C210	1.0	2.5
C250	1.0	2.0
C280	1.0	2.0
C130	1.0	1.5

## DSS PROGRAM COMPONENTS

1987-4

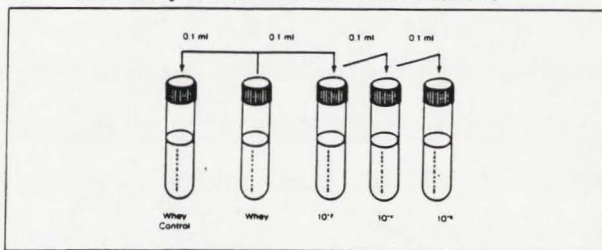
Figure 1

## Strain Replacement



## Phage Monitoring

Add Whey to BCP Milk and Make Dilutions



Incubate Tubes and Interpret Results

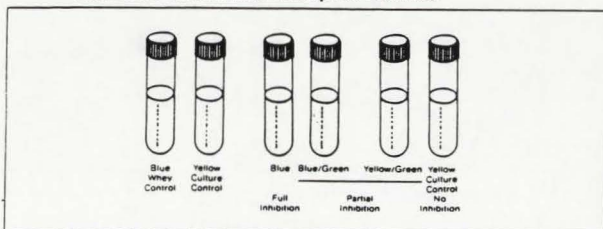




Figure 2. Growth And Temperature Profile For Selected Rod And Cocci Isolates

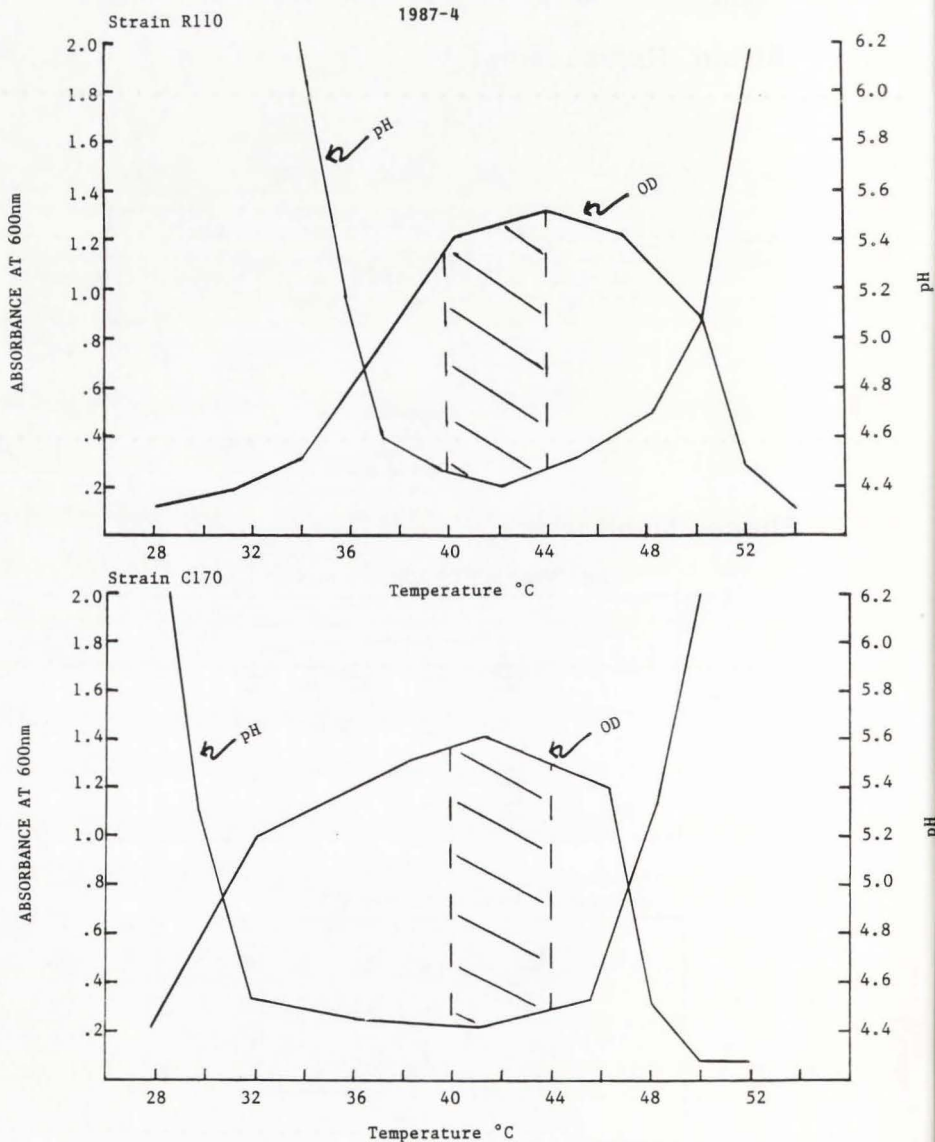
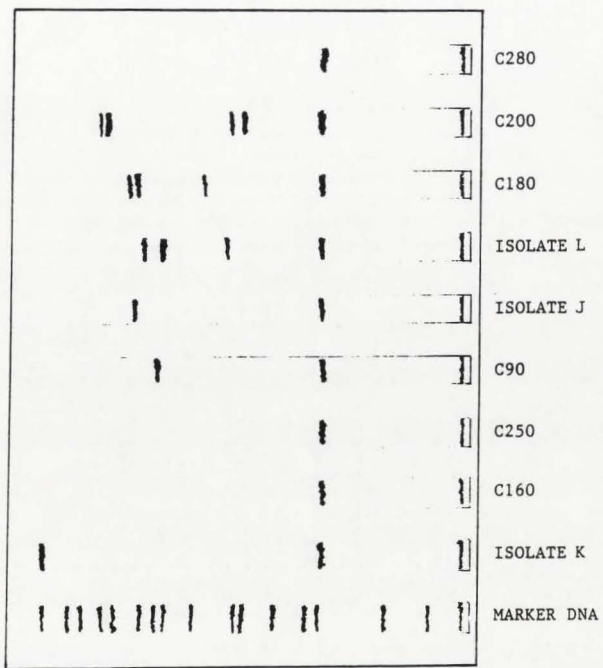


Figure 3

PLASMID DNA PROFILES OF SELECTED S. THERMOPHILUS STRAINS

The following paper was presented by D.M. Irvine, Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada, at the 24th Annual Marschall Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

### BACTERIOLOGICAL STANDARDS AND QUALITY OF CANADIAN ITALIAN CHEESE

By D. M. Irvine

Milk is an excellent growth medium for different kinds of organisms. It is therefore essential that milk be cooled as quickly as possible to keep bacterial numbers to a minimum. Milk is cooled quickly in bulk tanks to 5°C where it is held for a maximum of 2 days. These temperatures inhibit bacterial growth and it is normal to obtain milk with less than 5,000 bacteria per cm<sup>3</sup>. Psychrophiles can grow in cold milk and their numbers increase rapidly after 96 hours. These psychrophiles are reputed to cause flavour problems and protein digestion which results in loss of yield.

Aside from the organisms that cause flavour and other defects, there are a number of pathogens that are of vital concern to the cheesemaker. These include pathogenic organisms of the following type: Mycobacterium tuberculosis, Staphylococcus aureus, Streptococcus pyogenes, Coxiella burnetti, Clostridium botulinum, Bacillus anthrax, Salmonella spp., Shigella, E. coli, Campylobacter spp., Yersinia enterocolitica (ICMSF, 1978; IDF, 1980), B. cereus and Cl. perfringens. All of the above except the sporeformers and the enterococci are destroyed by pasteurization (Chapman and Sharpe, 1981).

In cheesemaking, all phases of the operation must be monitored to ensure that pathogenic organisms are destroyed or the making procedures or time of aging effectively eliminate all pathogens.

We have recently had a problem of Salmonella muenster in a raw milk Cheddar cheese. We monitored these cheese and found that some of the Salmonella lasted for 135 days in a well made raw milk Cheddar cheese (Woods *et al.* 1984). Salmonella typhimurium from Cheddar cheese has also been implicated in a Canadian foodborne outbreak (D'Aoust, 1985). Listeria monocytogenes is a potential foodborne pathogen and has been detected in our milk supply. Yersinia enterocolitica has been isolated from milk (Sarrouy, 1972).

There is justifiable cause for concern about these organisms in the cheese industry. Let us define the terms for a better understanding. The term coliforms includes Escherichia coli and several other species. The term faecal coliforms is an attempt to find a rapid method for establishing the presence of E. coli. Numbers of these organisms in cheese suggest a lack of cleanliness and sanitation. The presence of E. coli in foods is not always closely correlated with the occurrence of salmonella or other pathogenic organisms. In general, the presence of numbers of coliforms suggests inadequate processing, post-pasteurization contamination or microbial multiplication which could allow a wide range of pathogenic and toxigenic organisms. The presence of Staphylococcus aureus which usually indicates contamination from the skin, mouth, nose and hands of workers but also poorly cleaned equipment may be a source of contamination. These staphylococci can grow in milk and cheese and Zehren and Zehren (1968) reported a number of instances of foodborne poisoning from cheese made in

the U.S. These organisms grow in milk during the making operation and in low acid milk and may produce enterotoxins A.B.C.D.

The Health Protection Branch of the Department of Health and Welfare Canada established fairly rigid standards for cheese in 1978 (Table 1). These standards were based in part on a study done at the University of Guelph and it was determined that most factories can meet these standards.

I will discuss an overview of the Canadian Cheese Industry. The types of cheese made in Canada are listed in Table 2. The amount of cheese of the major varieties that is produced is listed in Table 3.

Italian cheese is made in three areas of Canada. Ontario has 14 factories, British Columbia and Alberta have 2 factories, and Quebec has 4 factories.

The data that will be presented are from Ontario factories only.\* The cheese are sampled and analyzed by the Ontario Ministry of Health and also by the Canada Department of Agriculture. The cheese may be sampled at the cheese factory or from supermarkets and may be fresh or a few months or years old. The data are biased in that a factory that has bacteriological problems will be sampled more frequently. The cheese are analyzed for coliforms, Escherichia coli, Staph. aureus and routinely. The cheese are also analyzed for the presence of Yersinia enterocolitica, Campylobacter spp., Salmonella spp. and Listeria monocytogenes. The cheese is also routinely analyzed for pasteurization by the phosphatase test and for compositional standards.

In 1985, seventeen hundred cheese of all types were sampled at the plant level by the Ontario Department of Health. Eighty (4.6 percent) exceeded the Federal standard for E. coli and six (0.34%) exceeded the standard for Staphylococcus aureus. Fourteen (0.8 percent) were positive in the phosphatase test.

Italian type cheese were analyzed for coliforms and the data are presented in Table 4. For the sake of brevity, only the major types of cheese are listed in Table 4. The Escherichia coli that were excessive are shown in Table 5 for the various types of cheese. The Staphylococcus aureus counts that were excessive are indicated in Table 6.

The data indicate that there has been no problems with Salmonella spp., Yersinia spp. or Listeria in factory made cheese.

The data in these tables indicate that the Italian factories in Ontario can conform to the standards and indeed most of the plants are doing so. It is important that cheese be monitored for excessive bacterial counts routinely so that any problem can be rectified. The routine analyses by the Ontario Ministry of Health and the Canada Department of Agriculture, and sporadic checks by the Health Protection Branch of the Department of Health and Welfare Canada, ensure that Canadians enjoy fine Italian cheese without risk.

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\* The author wishes to acknowledge the assistance of Dr. D. Styliadis of the Ontario Ministry of Health and H. MacKenzie of the Canada Department of Agriculture.

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TABLE 1. Maximum bacterial standards for Canadian cheese.\*

Organism	Pasteurized milk	Raw milk
Coliforms	500/g	5,000
<u>E.Coli</u>	100/g	500
<u>Staph. aureus</u>	100/g	1,000

\* Food and Drug Act and Regulations, Canada

<u>Yersinia enterocolitica</u>	)	0 count in 25 grams of cheese.
Campylobacter spp.	)	
Salmonella spp.	)	
<u>Listeria monocytogenes</u>	)	

TABLE 2. Types of Italian cheese manufactured in Canada.

Grana	Pasta Filata	Soft
Romano	Cacciocavallo	Ricotta
Parmesan	Mozzarella	Cremona
Bra	Scamorze	Basket
Canestrato	Caciotta	Fresca
Crotonese	Casata	Italian Brick
-	Burrini	Primo sale
	Passita	
Other - Montasio	Trece	
Friulano	Provolone	
Stella Alpina	Twisted	
Fontina	Boccicini	
Arabic	Pizza	
Goat's milk		

**TABLE 3.** Manufacture of Italian type cheese in Canada in tonnes.

Cheese	1985
Buttiri	4
Cacciocavallo	168
Friulano	971
Mozzarella	58,381
Parmesan	2,438
Provolone	645
Romano	501
Scamorze	130
Ricotta	2,997

Dairy Market Review 1986. Agriculture Canada.

**TABLE 4.** Coliform determinations of various types of cheese.

Year	Satisfactory	Excessive
<u>Ricotta cheese</u>		
1986	78	16
1985	126	22
1984	83	19
1983	111	10
1982	126	27
<u>Mozzarella cheese</u>		
1986	108	1
1985	165	5
1984	114	3
1983	179	5
1982	184	8
<u>Friulano cheese</u>		
1986	54	6
1985	95	23
1984	67	3
1983	53	9
1982	70	23
<u>Pasta Filata cheese</u>		
1986	68	7
1985	154	12
1984	100	4
1983	179	14
1982	199	24
<u>Romano cheese</u>		
1986	24	-
1985	29	-
1984	16	-
1983	41	-
1982	45	-



TABLE 5. *Escherichia coli* counts of cheese that were excessive.

Year	Cheese	Excessive
1986	Boccicini	8
	Casata	2
	Mozzarella	16
	Provolone	2
	Scamorze	3
	Friulano	8
	Other	31
	Ricotta	17
1985	Friulano	15
	Ricotta	8
	Mozzarella	4
	Brine	1
	Other	3
1984	Ricotta	8
	Friulano	4
	Mozzarella	2
	Casata	1
	Other	3
1983	Friulano	11
	Scamorze	2
	Cacciocavallo	2
	Ricotta	2
	Other	4
1982	Friulano	40
	Ricotta	14
	Provolone	6
	Canestrato	22
	Basket	4
	Cacciocavallo	14
	Casata	4
	Other	6

**TABLE 6.** Staphylococcus aureus analyses of cheese from 1982-1986.

Year	No. of samples	Excessive	Type of cheese
1986	925	2	Mozzarella
1985	569	1	Ricotta
1984	380	1	Mozarella
1983	563	0	
1982	624	31*	

\* All of these violations were from a single factory which is now out of business.

The following paper was presented by Tom Capparelli, Sales Representative, A.M. Lock, Inc., 12904 DuPont Circle, Tampa, FL 33625, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

### METAL DETECTION - HOW IT IS USED IN THE CHEESE INDUSTRY TO PROTECT YOUR CUSTOMER AND YOU

By Tom Capparelli

#### ABSTRACT

Metal detection is an easy and inexpensive way to check your cheese for all grades of tramp metal including stainless steel! With today's high costs of litigation and product recall, it makes sense to use metal detectors on all your products. You have worked hard to win your customers trust and loyalty. A metal detector will help you keep that customer.

Metal detection is winning widespread acceptance in the cheese industry. A look at the reasons will show why metal detection is so important and that your product should also be checked for metal contamination.

**MACHINERY PROTECTION** - The inspection of large cheese blocks can easily be justified by eliminating the chance of metal damaging or breaking your grinding equipment causing costly repairs and lost production down time. Usually you have more than one supplier and they probably do not use metal detectors yet. You will also prevent the one large piece of metal from fragmenting into many small pieces that will go on to the customer.

**QUALITY ASSURANCE** - Injury to the customer, loss of customer goodwill, adverse publicity and legal retribution can all have a detrimental effect on the cheese processor. A "quality image" built up over many years can be quickly lost.

#### **WHERE DOES THIS METAL COME FROM?**

1. Raw materials and ingredients to the cheese maker with metal.
2. Personnel can lose pens, jewelry, coins, paper clips, buttons.
3. Production and packaging.

Despite most stringent controls possible on production techniques, metal contamination frequently does occur. Every screening, grinding, cutting, mixing or packaging process introduces tramp metal into the cheese.

#### **WHERE METAL DETECTORS ARE USED**

- Inspection of incoming blocks of cheese and other ingredients.
- Inspection of finished products in the final package or for bulk product, the use of a metal detector with a pipeline detector or incline drop through system on your dried grated cheeses and milk powders.

Record keeping of all contamination found is a useful preventative measure. By finding the origin of the contamination, steps may be taken to eliminate the problem by demanding improved quality from raw material suppliers, changing maintenance procedures, etc.

The occurrence of metal in a product is often an unpredictable and random event and its frequency depends on the product under inspection.

How can producers avoid the danger? Firstly, by improving their methods of working and secondly, by using electronic metal detectors.

The following notes offer guidance on the good manufacturing practice necessary to minimize the risk of metal contamination.

## **I. Prevention.**

### **A. Raw Material Control.**

1. Specifications for raw material should state that they be free from foreign-body contamination and should indicate specific precautions according to the type of ingredients involved, for example:
  - Powdered and granular products to be sieved.
  - Pallets should be in good condition and free from loose nails, broken wood, etc.
2. Bulk deliveries should be received through pipelines fitted with permanent magnets.
3. De-bagging and de-boxing should be carried out in a separate dispense area where further inspection can be carried out before transfer to covered bins. Containers should be provided for waste packaging materials.
4. Canned products should be opened using a punch-type can opener.
5. Prior to mixing, all dry ingredients should be sieved and passed through or over a permanent magnet or preferably through a free fall metal detector. Liquid products, if practicable, should be passed through an in-line filter.

### **B. Production.**

1. All ingredients, prepared mixes and finished products should be covered wherever possible.
2. Production equipment should be examined daily for signs of wear, paying particular attention to mincer blades cutting systems, mixers, etc.
3. Re-usable baking or molding tins must be kept inverted until immediately prior to filling. Where possible, the containers and lids should be air rinsed and/or vacuumed.

4. Cans and other rigid packaging containers should be inverted, air rinsed and/or vacuumed (not while on magnetic conveyors), as near as possible to the filling point. The production line should then be covered until final capping, lidding, etc.

### C. Personnel

1. All personnel should be trained in basic food hygiene, including the prevention of foreign body contamination.
2. There should be a system for reporting signs of foreign body contamination or defective machinery, etc. by production staff to management, and for taking prompt action on these reports.
3. Protective clothing should only be fitted with inside pockets. Before issue, new, laundered and repaired, protective clothing should be checked for loose buttons, pins, etc.
4. Staff should not wear decorative jewelry, watches or hair grips in production areas and should be discouraged from taking personal belongings into these areas.
5. Staples, drawing pens and paper clips should not be used on documents taken into or displayed in production areas.
6. Personnel under notice of termination of employment should not work in any production area.

### D. Maintenance

1. All maintenance personnel should be trained in the requirements of the food industry.
2. Maintenance should be planned so that wear and tear can be remedied before defects occur. Particular attention should be given to slicing and mincing blades, woven wire conveyors and sieves.
3. Routine maintenance should take place outside of production hours or on equipment which is completely screened off from adjacent food plant.
4. Emergency repairs should be carried out by experienced staff, equipped with clean protective clothing, an enclosed box for tools, a magnetized blanket for spreading around or under the workpoint and a small vacuum, brush and magnet for cleaning down afterwards.

Welding, riveting, drilling or soldering should not take place in plant being used for production or packing.

5. Outside contractors should be instructed on the standards to which they must conform and be supervised.

6. The installation of new plant and major alterations should be screened off from production areas using floor to ceiling polythene and/or hardboard partitions.
7. Step bridges over production lines to have fully enclosed floors and sides and must be regularly checked by quality control or hygiene teams.
8. The following practices are not acceptable:
  - The use of tape, wire and other temporary repair methods for the long term repair of plant.
  - Missing and loose screws and other fittings.
  - Swarf and wire debris on or around equipment.
9. All equipment repaired in workshops should be cleaned down and vacuumed, not compressed air blown, before being returned to production areas.
10. On completion of repairs, maintenance and installations, the plant and surrounding area should be inspected by a member of the quality control or hygiene teams before production commences.
11. Housekeeping should be organized for workshops so that floors are swept and vacuumed at least daily. A magnetized grid or mat should be fitted to the exit of workshops with a notice instructing personnel to scrape their feet.

A review of the sources of metal contamination shows that it is necessary to detect both ferrous and non-ferrous metal. Cheese producers use a great deal of stainless steel in their factories and thus it is vital that metal detectors have specific capability for these metal alloys.

Most metal detectors consist of a metal box with an open aperture through the center. The product under test is transported through the aperture, usually on a non-metallic conveyor band but sometimes down an inclined chute or pipeline.

Around the aperture are wound three coils of wire. The center coil is oscillated at high frequency and can be regarded as the "transmitter." This induces an identical voltage in each of the two outer coils which can be regarded as the "receivers." These two coils are connected in such a way as to cancel each other out to give a resulting zero volts. When a piece of metal passes through the aperture, this disturbs the electro-magnetic field and an out of balance voltage results. This voltage is processed and amplified.

In normal operation the detector should be in a state of perfect balance with a zero output. Maintaining this state of perfect balance is essential for long term reliable operation. Changes in temperature, humidity, the aging of detector head materials, however, all tend to create a constant out of balance voltage known as "drift." This drift may be offset by manual balance controls which require periodic attention if the performance of the detector is to remain constant. More advanced metal detectors incorporate automatic balance control (A.B.C.) which totally eliminates any operator attention. This ensures that the detector permanently operates at peak performance and has the added advantage that the equipment may be installed in relatively inaccessible positions.

**SENSITIVITY:**

The most common question asked when considering metal detection equipment is "what size metal can we expect to detect." The answer is not easy and to be meaningful, needs to be qualified by additional data. An equally, if not more important question, but one that is seldom considered, is that of repeatability and stability.

Highly sensitive equipment is of no value if that sensitivity is not permanently maintained, or if multiple metal pieces "fool the detector" or if the detector goes "blind" after a large metal piece passes or if periodic adjustment is needed by an operator to maintain sensitivity without frequent false rejections. All metal detectors are not equal, despite having rather similar specifications.

Whether you want to admit it or not, metal contamination is a large problem and you can not afford to operate without a metal detector for protection.

The following paper was presented by M. E. Johnson, Assistant Scientist, Walter V. Price Cheese Research Institute, University of Wisconsin, Madison, 1605 Linden Drive, Madison, Wisconsin, 53706, U.S.A., especially for the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum of the Dane County Exposition Center, Madison, Wisconsin, on September 16 and 17, 1987.

### DEVELOPMENT OF A SPECIALTY CHEESE

By James Beyer, Mark E. Johnson, Brian Riesterer  
and Tom Blattner

#### ABSTRACT

In the past few years there has been a heightened enthusiasm by U.S. cheesemakers to manufacture European varieties of cheese. This is in response to an awareness that perhaps U.S. cheesemakers have underestimated the desire of consumers for new or different tastes in cheese. This enthusiasm has been tempered by reluctance to manufacture European or specialty cheeses due to uncertainty of marketing and acceptability by consumers, as well as a lack of manufacturing know-how. This paper will describe the manufacture of a creamy Havarti style cheese. This cheese will be compared by sensory labs and consumer acceptability taste panels to Danish Havarti.

As with any cheese there are a variety of manufacturing methods to produce a similar cheese. It is almost certain that the following manufacturing procedure will deviate from manufacturing schedules of Havarti cheeses produced in European countries and domestic Havarti cheese manufacturers. The conditions of manufacture will almost surely vary with a given cheese plant. But such is the nature of the beast! The cheese described in this paper has been successfully made in a 5000 lb. capacity vat at the UW dairy processing plant.

#### Manufacture of Creamy Havarti style cheese

- Milk:** Milk is standardized to 5.05% fat or an approximate casein to fat ratio of .44. The cheese will have a fat on the dry basis of at least .60. Milk is pasteurized after standardization at 163°F/16 sec.
- Culture:** Commercially available cultures containing Streptococcus lactis, Streptococcus cremoris, Streptococcus lactis var. diacetylactis and Leuconostoc cremoris are grown in 12.5 percent reconstituted non-fat dry milk at 72°F for 16 hr. Commercial cultures containing only Streptococcus cremoris and Leuconostoc cremoris have also been used. One percent culture is added to the milk at 90° F.
- Color:** Level and type of color used depends on shade and intensity desired. Ice cream color is used at a rate of .5 oz. per 1000 lb. milk. Color is added 10 minutes prior to rennet addition.
- Rennet Addition:** Milk is ripened at 90°F for 40 minutes prior to the addition of rennet. Single strength calf rennet is used at the rate of 2.3 oz. per 1000 lb. of milk.



- Cutting:** Thirty minutes after rennet addition the curd is cut with 3/8 inch knives. The curd is soft at cutting. Cutting the curd soft insures adequate whey expulsion. High fat milks naturally have softer clots and allowing more time to firm the curd may be fruitless.
- Healing:** The cut curds are allowed to sit for 10-15 minutes before agitation. The soft curds will shatter if not allowed to heal. Excessive fat losses will also result if the curds are agitated too abruptly.
- Whey Removal:** Thirty minutes after cutting 20 percent of the original milk volume is removed as whey. The whey may have an approximate fat level of .5 to .6 percent. Without whey removal the cheese may have an acid flavor. Care must be taken to prevent shattering of the soft curd. As an alternate procedure whey could be removed once the curd has firmed sufficiently during cooking.
- Cooking:** Hot water (equal to the amount of whey removed) is slowly added back to the remaining curd/whey mixture until the cooking temperature of 105°F is reached. Steam may have to be added to the vat jacket to heat the whey to 105°F. The temperature is increased from 90°F to 105°F over 30 minutes to prevent case hardening of the rather large curd particles. The curd is cooked at 105°F for 1 hour. The curd will become much firmer and is easily pumped into hoops without shattering. Lower cook temperatures will increase moisture content of the cheese resulting in pasty cheese. Decreased cook times may result in too soft a curd that may shatter during pumping.
- Hooping:** Whey is drained to about curd level and then the curd/whey mixture is pumped into standard brick hoops (6 x 5 x 10 inches). The whey will have .2 to .3 percent fat. Cheese will have more and larger openings if more whey is drained before hooping.
- Turning:** Once filled, the hoops are turned to prevent uneven openness in the cheese. Turning continues every 20 minutes for the first hour and then once every hour until the curd pH is 5.7 to 5.8 (about 3 to 4 hours after hooping).
- Brining:** Cheeses are placed in 23% brine at 40°F for 1 day.
- Drying:** Cheeses are allowed to dry (40°F) for 2 to 4 days.
- Curing:** Cheeses are cured at 55°F for 1-2 weeks and then stored at 45°F until ready for consumption at 8-12 weeks.
- Cheese Composition:** 37 to 38 percent fat, 37 to 38 percent H<sub>2</sub>O, 1.5 to 1.7 percent salt, pH at 4 days 5.1 to 5.2, and fat in the dry matter of .60 to .61. Yield is approximately 12 lb. per 100 lb. of milk. Although the cheese may appear firm initially, by 8 weeks the body will have softened. Higher moisture cheeses will become excessively soft and pasty and not easily sliced. Higher moisture cheeses tend to also have a

they taint flavor. This defect has been traced to inadequate whey expulsion and whey drainage.

Sensory  
Analysis:

Descriptive and consumer preference panels were run on the cheese and directly compared to Danish Havarti. The results are shown in Table 1 and Table 2. The descriptive taste panel (32 people) is a direct comparison of several attributes and is useful in detecting differences between the cheeses. The flavor attributes are similar, however the body of our cheese is firmer than the Danish Havarti. This would also account for the differences in stickiness and creaminess. The Danish Havarti might be older than our 8 week old cheese. The overall preference of the cheeses showed no significant difference.

The consumer preference panel (208 people) showed that both cheeses were well liked. The Danish Havarti had more responses in the like very much category; however, the mean scores were not significantly different.

Conclusion: The manufacturing schedule for creamy Havarti style cheese presented in this paper produced a cheese closely resembling Danish Havarti and equally as acceptable by consumers. The method of manufacture did not involve exotic cultures and could easily be manufactured in most existing cheese plants.

The cheese described here is not Danish Havarti and cheese connoisseurs would likely readily separate the two cheeses. Consumer preference panels seem to indicate that a cheesemaker does not have to make exact copies of an existing European variety but can produce their own unique specialty cheese acceptable to consumers.

This work was supported by funds from the Wisconsin Milk Marketing Board.

Table 1. Summary of mean scores for the descriptive sensory analysis of Havarti Cheese.

Sample Attributes							
Samples	Flavor Intensity <sup>1</sup>	Sharpness of Flavor <sup>2</sup>	Aftertaste <sup>3</sup>	Overall Firmness <sup>4</sup>	Stickiness <sup>5</sup>	Creaminess of Texture <sup>6</sup>	Overall Preference <sup>7</sup>
Experimental #4145	4.52a	3.96a	3.98a	3.72b	3.59b	4.70b	4.77a
Danish Havarti	4.71a	4.22a	3.96a	3.07a	4.27a	5.40a	4.61a
Value (LSD 5%)	NS	NS	NS	S(.50)	S(.54)	S(.40)	NS

- 1Scale: 1 = very weak to 7 = very sharp or biting  
 2Scale: 1 = not sharp or biting to 7 = very sharp or biting  
 3Scale: 1 = no aftertaste to 7 - pronounced aftertaste  
 4Scale: 1 = soft to 7 = firm  
 5Scale: 1 = not sticky to 7 - very sticky  
 6Scale: 1 = not smooth, not creamy to 7 = very smooth, very creamy  
 7Scale: 1 = dislike extremely to 7 = like extremely

a,b Mean scores in the same column with the same superscript are not significantly different at the 5% level.

S = significant at the 5% level; NS = not significant.

Date of evaluation: July 7, 1987.

**Table 2.** Response frequencies and mean scores for the consumer preference panel evaluation of Havarti Cheese.

Preference rating	Assigned numerical score	Samples	
		Commercial	Experimental
(---Frequency of responses--)			
Like very much	7	96	79
Like moderately	6	75	85
Like Slightly	5	24	29
Neither like nor dislike	4	4	6
Dislike slightly	3	8	4
Dislike moderately	2	1	3
Dislike very much	1	0	2
Total responses: n=208			
MEAN SCORES:		6.17 <sup>a</sup>	6.02 <sup>a</sup>
STATISTICAL ANALYSIS:			
F value		NS	
LSD (at 5% level)		.20	

S = significant at 5% level; NS = not significant

a, b Mean scores with same superscripts are not significantly different at 5% level.

Date of Evaluation: July 16, 1987

The following paper was presented by C. A. Ernstrom, Professor, Department of Nutrition and Food Sciences, Utah State University, Logan, Utah 84322, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

## RESIDUAL MILK CLOTTING ENZYMES IN CURD

By C. A. Ernstrom, Ph.D.

### ABSTRACT

The amount of residual milk clotting enzyme left in cheese curd (including calf rennet) can be a significant factor in causing bitter flavors in medium and aged cheese. Retention of calf rennet in curd is favored by acidity at setting and draining, reduced cooking temperatures and use of too much rennet. Porcine (pig) pepsin does not survive Cheddar cheese manufacture and does not contribute to body break down. Neither does it cause bitterness in cured Cheddar cheese.

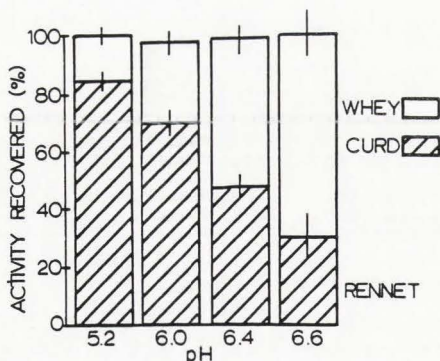
Calf rennet has long been considered the coagulant of choice for cheese manufacturers. However, other approved coagulants are now on the market and in commercial use. These include clotting enzymes derived from Mucor miehei, from Mucor pusillus var. Lindt, from Endothia parasitica, extracts from adult cattle stomachs and porcine (pig) pepsin.

The major role of these enzymes is milk clotting. However, they also play a part in the breakdown of proteins during cheese curing. Many researchers have considered this role to be essential and of considerable significance while others think the part played by milk clotting enzymes in cheese curing is secondary to that of microorganisms and in some cases detrimental to cheese quality.

There are hundreds of proteolytic enzymes in the world and most of them will clot milk. However, only a very few have proved successful as coagulants for the cheese industry. Most of them are too proteolytic and make the cheese bitter and sometimes pasty.

We must recognize that if milk clotting enzymes contribute to protein breakdown during cheese curing, some of the enzyme must remain active in the curd. An evaluation of the amount of various milk clotting enzymes that remain in cheese curd and their influence on protein degradation has been the object of studies in our laboratories over the past several years, and an active program in this area is still continuing.

A simple diffusion test was reported in 1977 and improved in 1985 that enables us to measure the amount of residual milk clotting activity in cheese curd and whey. Using this test we have shown that when 3 oz. calf rennet are used per 1000 lbs. of milk about 7% of the chymosin added to the milk remains active in Cheddar cheese after pressing. This is equivalent to about 10 milk clotting units of activity per kilogram of cheese. There are cheese making practices that can increase or decrease the amount of enzyme left in the curd. The affinity of calf rennet (chymosin) for curd is markedly affected by pH of the milk. Figure 1 shows how calf rennet (chymosin) distributes itself between curd and whey in freshly coagulated milk set at various pH values.



**Figure 1.** Effect of pH on the distribution of rennet between curd and whey in freshly coagulated milk. (J. Dairy Sci. 60:862. 1977)

Note that the more acid the milk, the more rennet stays with the curd. The same is true for porcine (pig) pepsin, but it is not true for the microbial rennets. When Cheddar cheese is made with *Mucor miehei* or *Mucor pusillus* rennets, only about 2% of the enzyme stays in the curd, and pH has absolutely no effect on the affinity of these enzymes for curd. There is plenty of evidence in the literature that the microbial rennets are more proteolytic than calf rennet (chymosin), and it is probable that the only reason they can be used at all for cheese making is that such a small amount remains in the cheese.

A second factor that affects the amount of residual coagulant in the curd is the amount used to coagulate the milk. Tables 1 and 2 illustrate the amount of calf rennet (chymosin) in curd when set with normal and 3 times normal levels of enzyme in milk at pH 6.6 and 1/3 normal levels in milk set at pH 6.2.

**Table 1.** Residual chymosin in cheese at various times of ripening.

Treatment	Percent of original chymosin activity			
	1 day	3 mo	6 mo	9 mo
Chymosin, pH 6.2 (2245 CU/454 kg milk)	12.7 ± .1	10.8 ± .1	10.3 ± .6	10.1 ± .6
Chymosin, pH 6.6 (6745 CU/454 kg milk)	6.3 ± .5	5.1 ± .6	4.9 ± .6	4.8 ± .5
Chymosin, pH 6.6 (20235 CU/454 kg milk)	13.1 ± .9	11.0 ± 1	10.4 ± 1.5	9.7 ± .9

**Table 2.** Activity of residual chymosin per unit weight of cheese.

Treatment	Mean clotting units of chymosin/kg cheese			
	1 day	3 mo	6 mo	9 mo
Chymosin, pH 6.2 (2245 CU/454 kg milk)	6.5	5.5	5.3	5.2
Chymosin, pH 6.6 (6745 CU/454 kg milk)	9.6	7.8	7.4	7.2
Chymosin, pH 6.6 (20235 CU/454 kg milk)	59.9	50.5	45.9	44.4

Tripling the use of rennet increased the percentage retention of enzyme in the curd as well as the total units of activity per kilogram of cheese. Note also that when one third normal amount of rennet was used in milk at pH 6.2, percent retention increased due to the pH affinity effect, but the total amount of residual enzyme in the curd was lower than normal. There was only a slight reduction in enzyme activity during 9 months curing of the cheese.

A third factor that affects residual enzyme in curd is the stability of the enzyme. For example, the microbial milk clotting enzyme derived from *Endothia parasitica* cannot be used for Cheddar cheese because it causes bitter flavors and a mealy body. However, it is used successfully for Swiss cheese and some Italian varieties that employ cooking temperatures in excess of 115°F. This enzyme is stable enough to survive Cheddar cheese cooking but not stable enough to survive high cooking temperatures used for Swiss cheese. This enzyme does not contribute to the curing of Swiss or Parmesan cheese because it has been inactivated, whereas in Cheddar cheese where it does survive, its effect on cheese quality is detrimental.

During normal Cheddar cheese manufacture, about 35 percent of calf rennet activity is destroyed during cooking at 102°F. If cooking temperatures are reduced, one could expect less enzyme inactivation and more residual enzyme activity in the curd.

The original *Mucor* microbial rennets are very stable and no activity is destroyed during Cheddar cheese making. Most of the activity ends up in the whey. New modified versions of these enzymes are now being sold which are less stable to heat. We have no data on their exact stability, but it is claimed by the manufacturers that they have about the same stability as calf rennet.

Porcine (pig) pepsin is probably the most unstable of all the common milk clotting enzymes. This enzyme normally functions in the environment of hog stomachs that are very acid (pH = 2.0) where it has very strong protein digesting activity. However, at pH 6.6-6.7 as in milk, its activity and stability are both substantially diminished. Therefore, if porcine pepsin is added to cheese milk it undergoes a certain amount of inactivation even while it is clotting the milk. For this reason, studies on the use of porcine pepsin as a coagulant for cheese making have often reported soft sets and excessive fat losses.

This problem is easily overcome by simply using more pepsin. This is not generally a concern since porcine pepsin is very inexpensive. The more important question is whether pepsin survives the cheese making process and remains active in the curd during curing. In numerous trials that we have run, the answer is clearly no. Porcine pepsin does not survive Cheddar cheese manufacture if the pH of the milk is higher than 6.4 at setting.

Tables 3 and 4 illustrate the amount of residual pepsin left in Cheddar cheese when milk was set at pH 6.2 and 6.6 compared to calf rennet in milk set at pH 6.6.

**Table 3.** Residual porcine pepsin and chymosin in cheese at various ripening times.

Treatment	Percent of original enzyme activity			
	1 day	3 mo	6 mo	9 mo
Porcine pepsin, pH 6.2 (990 CU/454 kg milk)	5.0 ± .2	4.2 ± .3	4.0 ± .3	3.8 ± .2
Porcine pepsin, pH 6.6 (6745 CU/454 kg milk)	0.0	0.0	0.0	0.0
Chymosin, pH 6.6 (6745 CU/454 kg milk)	7.0 ± .5	5.5 ± .1	5.2 ± .1	5.0 ± 1

**Table 4** Activity of enzyme recovered per unit weight of cheese.

Treatment	Mean clotting units of enzyme/kg cheese			
	1 day	3 mo	6 mo	9 mo
Porcine pepsin, pH 6.2 (990 CU/454 kg milk))	1.1	.9	.9	.8
Porcine pepsin, pH 6.6 (6745 CU/454 kg milk)	0	0	0	0
Chymosin, pH 6.6 (6745 CU/454 kg milk)	10.7	8.3	7.9	7.6

In all cases the amount of enzyme added to the milk was adjusted to give a normal setting time of 30 minutes. When set at pH 6.2 a small amount of residual pepsin remained in the curd, but when set at pH 6.6 there was none. This is just another illustration of the very great effect of pH on pepsin stability.

It is well documented that residual milk clotting enzymes in Cheddar cheese curd enhance protein breakdown. The question of importance is whether such breakdown is helpful or detrimental to cheese quality.

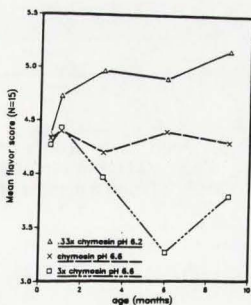


The rate of protein breakdown expressed as water soluble nitrogen as a percent of total nitrogen for Cheddar cheese set with 3 oz. calf rennet per 1000 lbs. of milk at pH 6.6, and a comparable amount of clotting activity from pig pepsin in milk set at pH 6.6 and 6.2, is given in figure 2.

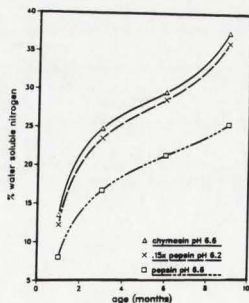
Note that only 15 percent of the pepsin used at pH 6.6 was needed at pH 6.2 to give the same clotting activity. Calf rennet gave the most rapid protein breakdown and pepsin set at pH 6.6 the least. Since none of the pepsin survived cheese making (pH 6.6), protein breakdown in this cheese was entirely due to activity of microorganisms or to proteolytic enzymes in the milk. The small amount of residual pepsin in cheese from milk set at pH 6.2 caused soluble nitrogen changes similar to chymosin.

Cheese used for this study was cured at 50°F and graded at 2 weeks, and 1,3,6, and 9 months of age. Five trained judges graded the cheese on a scale of 1 to 6 in which 1 was unsaleable, 3 satisfactory and 6 superior. Flavor scores for cheese made with 3 levels of calf rennet are in figure 3.

When only 1/3 the normal level of calf rennet was used, flavor scores increased steadily throughout curing. Normal levels resulted in flavor scores that were relatively constant throughout curing while 3 times normal usage caused general deterioration in flavor quality. The most common flavor criticism in the high and normal calf rennet cheese was "bitter". No bitterness was ever detected in any of the cheese set with only 1/3 the normal level of calf rennet. There is little doubt that calf rennet (chymosin) can cause bitter flavors in cured Cheddar cheese if too much enzyme is left in the curd. Since some bitterness was detected in 6- and 9-month Cheddar cheese made with normal levels of calf rennet (3 oz/1000 lb milk), it appears that normal levels are borderline in terms of ability to produce bitterness. Cheese making factors such as reduced cooking temperatures and acid milk at setting will favor retention of this traditional enzyme in the curd.



**Figure 2.** Soluble nitrogen in Cheddar cheese (made with chymosin and two levels of porcine pepsin) at 1, 3, 6 and 9 months of age.



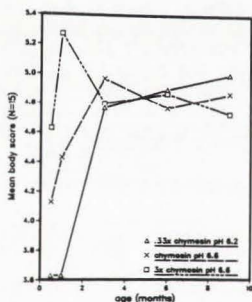
**Figure 3.** Mean flavor scores of Cheddar cheese (made with three levels of chymosin at .5, 1, 3, 6 and 9 months of age.

The body scores of the same cheese are shown in figure 4.

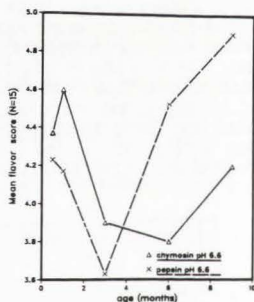
Cheese containing the highest level of rennet had the best body up to 3 months of age. Next was cheese made with normal levels of rennet, and poorest was cheese containing the least residual enzyme. However, after 3 months of aging there were no detectable differences.

It is quite clear that residual calf rennet has a favorable effect on the body of young or mild Cheddar cheese, but this effect diminishes as the cheese ages.

Cheddar cheese made from the same milk and starter but set with normal levels of calf rennet and pig pepsin exhibited flavor scores shown in figure 5. During the first three months of curing, the flavor of the calf rennet cheese was judged superior to the pepsin cheese. The major criticism of the pepsin cheese was bland, and lacks flavor. However, at 6 and 9 months of age, the pepsin cheese was judged substantially better than the calf rennet cheese. The major criticism of the calf rennet cheese at this time was bitterness.



**Figure 4.** Mean body scores of Cheddar cheese (made with chymosin at .5, 1, 3, 6 and 9 months of age).



**Figure 5.** Mean flavor scores Cheddar cheese (made with chymosin and porcine pepsin) at .5, 1, 3 and 6 months of age.

Body scores of the same cheese are depicted in figure 6.

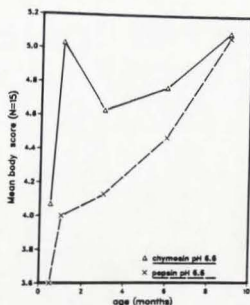


Figure 6. Mean body scores of Cheddar cheese (made with chymosin and porcine pepsin) at .5, 1, 3, 6 and 9 months of age.

Residual chymosin activity in the cheese produced rapid body breakdown during the first 3 months of curing. The pepsin cheese containing no residual enzyme activity remained curdy longer, but after 6-9 months was judged practically equivalent to the chymosin cheese.

Reports have come to my attention of an apparent increase in bitterness in Cheddar cheese during recent years. Some people have even suggested that a little bitterness is part of good aged Cheddar cheese flavor. Our studies have shown rather conclusively that too much residual milk clotting enzyme (even calf rennet) left in the cheese curd can cause bitterness. We are currently repeating this work with the microbial enzymes and with bovine pepsin, but these studies are not yet complete.

Practices that could be responsible for increased bitterness might be too low cooking temperatures (especially in stirred-curd cheese in an effort to maintain high moisture), lower pH at setting, and excessive use of coagulant.

Porcine pepsin when used as a coagulant does not survive Cheddar cheese manufacture and therefore has no effect on body breakdown and does not produce bitterness in cured cheese.

Pig pepsin is not recommended as a coagulant for mild cheese under 3 months of age since body breakdown is too slow. On the other hand, for medium and aged cheese, it could be an excellent coagulant particularly if used as part of a blend that would permit a substantial reduction in the amount of calf rennet needed for coagulation.

Certain starter strains also are capable of producing bitterness in Cheddar cheese, but the role of residual rennet as a causative factor in producing this defect has been largely overlooked.

The following paper was presented by Khem M. Shahani, Professor, Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, Nebraska 68503-0919, at the 24th Annual Marshall Invitational Italian Cheese Seminar, held in the FORUM Building of the Dane County Exposition Center, Madison, Wisconsin on September 16 and 17, 1987.

## ROLE OF LIPASES AND OTHER ENZYMES IN FLAVOR DEVELOPMENT

by Custy F. Fernandes, M. S. (Technol.)  
and Khem M. Shahani, Ph.D.

### ABSTRACT

The fresh curd when stored at low temperature develops a typical cheese flavor following ripening. The curd is ripened by the microbial enzymes and enzymes that are deliberately added to the curd. Lipases and proteases have been used to accelerate cheese ripening as individual and as a "cock-tail." The enzymes may be added to the curds as such or they may be encapsulated and then added to the curd. The role of enzymes in flavor development is discussed.

The economic advantage to rapid development of appropriate cheese aroma and taste in a shorter time would result in considerable savings in refrigeration and labor costs, and reduce capital investment. Accelerated ripening of cheese has been studied using such methods as incorporation of enzymes responsible for ripening, back-slopping and encapsulation of cell free extracts for enhanced flavor development. All the aforementioned methodologies basically depend upon the involvement of enzymes but differ in method of applying the enzymes. If cheese ripening is to be accelerated with the aid of enzymes, it is essential that the role of various enzymes in flavor biogenesis be elucidated.

### Flavor Biogenesis

The understanding of cheese flavor biogenesis is important so that cheeses can be produced repeatedly without variation in aroma, taste and texture. During cheese ripening a series of enzymatic reactions proceed very gradually modifying the freshly mechanically worked curd to the desired final ripe cheese texture and flavor. Cheese ripening enzymes result from contaminating bacteria present in raw milk, inherent milk enzymes, intentionally added enzymes and enzymes of starter cultures and secondary flora. The enzymes, lipase(s), protease(s) and lactase, hydrolyze lipids, proteins and lactose, respectively, giving rise to flavor moieties and/or flavor precursors.

Protease(s) and lipase(s) are the most important enzymes for the flavor development of cheese. During ripening there is a steady increase in concentration of liberated fatty acids and total soluble nitrogen. Lipases release the fatty acids from triglycerides while the proteinases and peptidases together liberate amino acids from the casein. These reactions have been shown to trigger the development of cheese flavor.

### (a) Role of Lipases in Flavor Biogenesis

Milk fat contains fatty acids and small quantities of oxy fatty acids. The fatty acids and oxy fatty acids which are released by lipases from the aforementioned sources contribute to the flavor of cheeses. Lipases of psychrotrophic bacteria are not denatured completely during heat treatment of milk and are lipolytic during ripening, but the inherent lipase of milk, on the other hand are inactivated completely during pasteurization. However, in the cheeses made from unpasteurized milk, inherent milk lipase effects considerable lipolytic action. The starter cultures and secondary flora, are lipolytic and produce lipase which are responsible for lipolysis, such as the *Penicillium roqueforti* and *Penicillium camemberti* in Blue-vein and Camembert cheeses, respectively. In addition, lipases are usually added to Italian cheese, *viz* Parmesan, Provolone, and Romano, to intensify their flavor.

Methylketones are another major group of compounds which contribute to cheese flavor and are derived from the fatty acid liberated by lipolysis. For instance, in Brie, Camembert and Blue-vein cheeses, 2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone have been identified. Some of the liberated fatty acids are metabolized further by the secondary flora present in cheese. Fatty acids have been shown to be enzymatically oxidized by the secondary flora into methylketones through the  $\beta$  oxidation pathway. The  $\beta$ -keto acids are subsequently decarboxylated into 2-methylketones. In addition, the oxy fatty acids found in milk fat may also be the source of methylketones, following enzymatic decarboxylation.

The large fluctuations in concentration of individual methylketones during Blue-vein cheese ripening, suggests that they may be converted plausibly, into secondary alcohols. It has been shown that methylketones are reduced by spores and mycelium of *P. roqueforti* into corresponding secondary alcohols. Further, the  $\delta$  hydroxy and  $\delta$  keto fatty acids liberated during lipolysis undergo spontaneous ring closure producing lactones.

### (b) Role of Proteases in Flavor Biogenesis

Initially, proteolysis of milk casein is generally brought about by chymosin, which continues to be proteolytic during the entire ripening period producing a large number of peptides. However, chymosin does not liberate amino acids. The amino acids are liberated by exopeptidase and endopeptidases present in starter cultures and secondary flora.

Proteinase activity results in the formation of bitter peptides and if these peptides are not broken down further into smaller non-bitter peptides by peptidases, then the cheese flavor is adversely affected. Additionally, the peptides also contribute to other flavor, such as the brothy flavor of Swiss cheese.

Some of the other compounds which contribute to cheese flavor include carboxylic acid, amines, thiols, esters, substituted amines, etc. Some of these compounds are formed through enzymatic reactions while others are products of non-enzymatic reactions. The compounds are produced enzymatically by starter cultures and secondary flora through deamination, decarboxylation, and transamination reactions. These compounds have been suggested to intensify cheese flavor.

### Accelerated Ripening with Enzymes

The biogenesis of cheese flavor has been the focus of study for many years and the knowledge procured thus far has been applied to expedite cheese ripening. Individual and mixture of enzymes have been added to milk or curds to accelerate cheese ripening have shown the following results:

#### (a) Individual enzymes

Lipase(s), protease(s) and lactase have been added individually to milk and/or curd during ripening.

(i) **Lipases(s):** gastric lipase has been used to accelerate ripening and flavor development of many cheese types, including Cheddar, Provolone and Ras cheeses. Lipase addition, enhances the rate of fatty acids liberation which accelerates flavor development relative to the control. These studies indicated that liberated fatty acid profiles of the accelerated process were identical to the control and the total quantities of short chain liberated fatty acids (C4 to C6) were important for development of typical Cheddar cheese flavor during ripening. The addition of calf lipase and increasing the ripening temperature (from 7°C to 13°C) result in a significant increase in liberation of fatty acids. Although neutrase, a neutral protease has little effect on liberation of free acids, it has a synergistic effect in the presence of lipase.

Generally, the addition of lipase accelerates the flavor development in cheeses, and reduces the ripening time. However, there may be one shortcoming. The lipase continue to be active following ripening and can cause the development of strong rancid flavors.

(ii) **Protease(s):** microbial proteases have also been added to milk and unripened curds to enhance flavor development. Addition of protease increases the level of soluble nitrogen and free amino acids. As the concentration of the protease increases there is an increase in the amount of amino acids that are liberated. Unacceptable bitter flavor can often be a problem if there is gross proteolysis with liberation of bitter peptides. This usually occurs at higher enzyme concentrations. In addition, proteases are also known to modify the texture of cheese.

Enzymatic reactions are temperature dependent and the enzyme activities increase with rise in temperature until the optimum temperature is reached. Ripening at higher temperature (13°C rather than 7°C) following addition of protease increased the free amino acids concentration and the reduced ripening time.

(iii) **Lactase:** although this enzyme has been attempted to accelerate the ripening of cheese, the results have been mixed. It has been suggested that lactose hydrolysis would expediate the ripening and reduce ripening time because of rapid acid development. However, this aspect has still to be elucidated.

#### (b) Cock-tail of Enzymes

During the ripening process, several enzymes are active at the same time. Therefore, addition of a single principle enzyme such as lipase and/or protease may result in complete characteristic flavor development. Hence, combination of enzymes have been utilized to

reduce the ripening time of cheese. Further starter enzymes have also been added along with lipases and proteases to fully develop the cheese flavor.

When a mixture of fungal protease and fungal lipase were used, Cheddar cheese developed a higher soluble protein and free fatty acid levels and displayed better flavor than did control cheeses following 3 months of ripening. Similar observations were also reported when the fungal protease P 53 was used in combination with the Meito lipase.

The level of enzyme added to accelerate cheese ripening is very important. High levels of enzyme during ripening may result in excessive enzymatic reactions which may culminate into undesired characteristics. Ras cheese treated with fungal protease and lipase, and pregastric lipase Capalase K, at low concentration improved the quality of cheese without any noticeable flavor defect. But at higher concentration of the same enzyme mixture resulted in defective cheese during a 45 day storage period.

Addition of crude cell free extract of Lactobacillus casei isolated from Cheddar cheese, resulted in bitter flavor development. The bitter flavor development has been attributed to the complexity of L. casei protease and to disturbance in the sequence of enzyme intervention during ripening. The bitter flavor was still present when the crude extract of a Lactobacillus species of simpler peptidase system was added to the cheese curd.

Crude extracts of Kluveromyces lactis and Saccharomyces cerevisiae increase the proteolysis and accelerated flavor development in Cheddar cheese. P. roqueforti extract resulted in pronounced bitter flavor in Saint-Paulin cheese during ripening. Whereas a combination of Penicillium proteases with either S. lactis or L. casei autolysates resulted in a cheese of better quality.

The proteolytic and lipolytic activities of crude enzyme preparations obtained from P. roqueforti, Geotrichum candidum and Streptococcus faecalis var. liquefaciens have been used to accelerate ripening of Crescenza cheese. The proteolytic extract from S. faecalis var. liquefaciens damaged Crescenza cheese structure. However, a combination of the fungal extracts improved texture and the cheese developed stronger flavor.

In other attempts to evaluate the combination of a gross proteolytic agent and an exopeptidase, capable of releasing potentially flavorful low molecular weight peptides, along with a mixture of neutrase and cell free extract of S. lactis was added to Cheddar cheese curd. Results demonstrated the possibility of reducing the amount of neutrase to a level which does not weaken the body of cheese. Similarly, when a blend of Rulactine and Piccantase were added to Ras cheese at low levels, the flavor development was accelerated, but at higher concentration the same enzyme preparation resulted in flavor and texture defects.

Ripening of Gouda cheese was accelerated when either heat-shocked or freeze-dried lactobacilli were added to milk. Overall lactobacilli resulted in reduced bitterness in the experimental cheese when compared to the control; however, Lactobacillus helveticus consistently produced an acetaldehyde-like flavor and one lactobacilli strain generated a peppery taste sensation. A commercial enzyme preparation Nutrage, is known to produce a typical Cheddar cheese flavor in approximately half the usual ripening time.

Addition of a single enzyme would favor the breakdown of one of the cheese components faster than the others, which very often leads to a disturbance in flavor balance of the cheese through over production of some of the flavor components. Further research needs to be

pursued using combination of enzymes to evaluate their role in accelerating cheese ripening. Other enzymes should also be investigated (i.e. enzymes involved in  $\beta$ -oxidation, reductases, decarboxylases, deaminases, transferases, thiolases, enzymes involved in formation of diacetyl, etc.) and more attention should be given to extracts obtained from microorganisms normally present in the cheese since those extracts will be expected to contain the appropriate enzyme mixtures in the desired concentrations. Thus it seems plausible to accelerate the development of cheese flavor by either using whole cell free extracts or a combination of cell free extracts.

### (c) Microencapsulation and liposome entrapment of enzymes

During ripening the various enzymes which are active have different optimum pH, and act at different times during ripening. For example, when bacterial cells are intact, the bacterial endocellular enzyme cannot act on cheese constituents. However, following cell lysis these endocellular enzymes are released and they contribute to flavor through lipolysis and proteolysis and other enzymatic reactions. Therefore, a system has been developed in which the cell-free extracts of selected bacteria with appropriate substances were encapsulated in milk fat and the resulting microcapsules were added to milk before clotting. Products were formed within capsules during cheese ripening. *S. lactis* subsp. *diacetylactis* was evaluated for diacetyl and acetoin production, while *Pseudomonas putida* and *Brevibacterium linens* for production of methanethiol and a mixture of *Gluconabacter oxydans* for the production of acetic acid and *S. lactis* var. *maltigenes* for production of aldehydes and alcohols. The last two coencapsulated extracts also demonstrated recycling of Nicotineamide dinucleotide. Cheeses made with intact capsules added to milk contained substantially more enzymatic end products than cheeses to which enzyme extracts were added. It was also reported that capsule stability was improved by encapsulation in a high melting fraction of fat.

Liposomes are used in the medical field to optimize the action of drugs by transporting them to target areas thus circumventing drug waste, inactivation and anatomical barriers. The application of liposomes technology to cheese ripening has also been considered.

Adaptation of liposome technology for accelerated cheese ripening has been utilized for the following reasons. Liposomes can be prepared in sizes similar to bacterial cells and are expected to be distributed in the curd in a similar way. Liposomes could therefore be considered to be bacterial cells containing selected mixtures of enzymes which are released much faster in the cheese. For instance, phospholipids vesicles will also protect potential substrates in milk until after the cheese curd has been formed and pressed; therefore reducing bitterness and losses in yield resulting from direct addition of an exogenous proteinase to cheese milk. Liposomes technology also offers the possibility of preparing a wide range of vesicles varying in size, net charge, sensitivity to pH and/or temperature. These different preparations can be applied to different types of cheese.

### (d) Back-slopping

Additionally, cheese scrappings obtained from ripened cheese can be incorporated into the fresh curd. This technique is called as back slopping. By using this technique, the starter cultures and secondary flora can be added to fresh curd and reduce ripening time as their enzymes would be in the active state and in balanced proportions.



### Conclusion

In order to exploit the knowledge gained in the biogenesis of cheese flavor, it is essential to know the level of the enzymes required to be added and this could be done with enzyme kinetics. Encapsulation of enzymes to accelerate flavor development certainly holds a lot of promise, but some obstacles have to be overcome. During ripening some of the enzymes are active simultaneously while other enzymes are active at a different time. Thus enzymes may have to be encapsulated in materials that are able to withstand processing temperature and release the enzymes at a particular hydrogen ion concentration, as the pH of ripening curds permits the enzyme to function and develop the flavor. During cheese ripening there is an increase in bacterial count hence the level of enzyme is also increased and these must be taken into consideration to accelerate the ripening of cheeses as they would have a tremendous impact to the industry from an economic angle.

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### PENICILLIN DISTRIBUTION FOLLOWING DIRECT ACIDIFICATION MOZZARELLA CHEESE MANUFACTURE

by Jeffrey J. Ryan, Ph. D.

#### ABSTRACT

Reduction in milk pH required for the manufacture of several fermented dairy foods, including Mozzarella cheese, can be accomplished by direct acidification of the milk. Should antibiotic residues be present in this milk, the potential exists for these residues to be retained in the finished products. The objective of this study was to determine if penicillin G was retained in low-moisture part-skim Mozzarella cheese manufactured from intentionally contaminated milk by a direct acidification procedure. S. lutea cylinder plate assay and B. stearothermophilus disc assay analyses indicated that penicillin G residues were retained in the finished cheese. Considering the potential public health risks, all materials used for Mozzarella cheese manufacture should be analyzed for antibiotic residues.

#### INTRODUCTION

The 1986 production figures for Italian type cheeses indicate that Mozzarella cheese is continuing its steady upward growth trend. Since its inception, the Italian cheese industry has undergone many modifications that have helped processors produce a uniform, high quality product and at the same time reduce production costs. Traditional manufacturing procedures include the use of a thermophilic starter culture for acid production and a suitable milk clotting enzyme for coagulum formation.

As an alternative to the traditional culture set procedures, direct acid set procedures for Mozzarella cheese manufacture have been developed. Following acidification with a food grade acid to pH 5.6, the milk is warmed and a milk clotting enzyme is added to produce a coagulum. The coagulum forms within minutes and is ready for stretching immediately after cooking.

Since the direct acid set procedure does not rely on the biological activity of a starter culture to attain the proper pH for curd stretching, the potential exists for milk contaminated with antibiotics to be inadvertently converted into Mozzarella cheese. Thus, the objective of this study was to determine the distribution of penicillin G in cheese and whey following low-moisture part-skim Mozzarella cheese manufacture by a direct acidification procedure.

## **METHODS**

### **Cheese Manufacture**

Cheese milk for this study was pasteurized at 165°F for 15 seconds, standardized to 2% fat, then stored overnight at refrigeration temperature. The following day, the cold cheese milk was acidified to pH 5.6 with food grade acetic acid then warmed to 90°F. Milk weights of 10.2 pounds were transferred to six laboratory scale cheese vats where the cheese milk was maintained at 90°F. Known amounts of penicillin G concentrate were then added to the cheese vats to achieve cheese milk penicillin concentrations of 0, 5.0, 10.0, 20.1, 40.2 and 80.5 ng/ml. Following agitation and sampling of the cheese milk, single strength calf rennet was added. Within 3-4 minutes, the vats were cut with 1/4-inch cheese knives and the curd was cooked at 105°F for one hour. Following cooking and draining, the curd was stretched by hand, molded, and brine salted for 3 hours at 40°F. Weights of cheese and whey from each vat were recorded and samples were collected for chemical composition and antibiotic analyses. Total solids content of cheese was measured by vacuum drying a two gram sample of cheese at 212°F for five hours. Fat and salt content were determined by the FAO/WHO and Volhard methods, respectively.

### **Penicillin G Determination**

The presence of penicillin G residues in cheese milk, cheese and whey was determined using two different microbiological methods. Method 1 was the *Sarcina lutea* cylinder plate assay as described by the National Center for Antibiotic and Insulin Analysis. This procedure involves the measurement of the size of zones of inhibition surrounding stainless steel cylinders that contain the samples in question. Using a standard curve that relates zone of inhibition to penicillin G concentration, a quantitative estimate of the penicillin G concentration in a milk, cheese, or whey sample can be made.

Preliminary experiments indicated that the *S. lutea* cylinder plate assay was not sensitive enough to detect low level penicillin contamination in the cheese samples. Therefore, the second detection method used was a modification of the *Bacillus stearothermophilus* disc assay procedure. The modification incorporated the use of standard curves that related disc assay zone sizes to penicillin G concentrations. As both procedures require a fluid sample, all cheese samples were diluted 1:4 with citrate buffer prior to the assay. Milk and whey samples were assayed without dilution.

## **RESULTS AND DISCUSSION**

The chemical composition of the six low-moisture part-skim Mozzarella cheeses manufactured in this study is shown in Table 1. Average FDB, moisture, and salt contents were 37.7, 47.5 and 1.47%, respectively. Compositional analyses indicate that all cheeses met the CFR standards of identity and were comparable to that which may be found in the retail market.

**Table 1.** Chemical composition of low-moisture part-skim Mozzarella cheese manufactured from milk intentionally contaminated with penicillin G.

Desired PEN Concentration in Milk (1)	FDB	% Moisture	Salt
0	37.6	46.9	1.37
5.0	36.9	48.4	1.54
10.0	37.7	47.6	1.42
20.1	38.1	47.2	1.45
40.2	38.4	47.1	1.45
80.5	37.6	48.0	1.60

1. in nanograms/milliliter.

Distribution of penicillin G as a percentage of the total ng of penicillin G assayed in the cheese milk is shown in Table 2. Mean *B. stearothermophilus* disc assay zone sizes ranged from 16.3 to 25.0 mm, thus all intentionally contaminated cheese milk samples were considered positive for antibiotics with respect to NCIMS and FDA actionable levels. When low-moisture part-skim Mozzarella cheese was manufactured from milk containing various amounts of added penicillin G, recovery of the antibiotic in the cheese ranged from 12.7 to 16.7 percent with a mean of 14.5 percent. As the cheesemilk penicillin G content increased, percent penicillin G recovered in the cheese decreased. Recovery of penicillin G in the whey fraction ranged from 76.3 to 93.0 percent with a mean of 85.2 percent. Combined recoveries from cheese and whey samples ranged from 94.2 to 108.9 percent.

Data on percent recovery of penicillin G in cheese and whey indicate that when low-moisture part-skim Mozzarella cheese was manufactured from intentionally contaminated cheese milk by a direct acidification procedure, the majority of the antibiotic was removed in the whey. This observation is consistent with the findings of Cayle et al. (J. Food Prot. 49:796, 1986) who found that Cheddar cheese retained approximately 12 percent of the cheese milk penicillin while 86 percent was recovered in the whey.

The concentrations of penicillin G in the cheese milk, cheese and whey are shown in Table 3. As the actual penicillin G concentration in the cheese milk increased, penicillin G concentration of the respective cheese and whey samples also increased.

**Table 2.** Percent of penicillin G recovered in cheese and whey as determined by the modified *Bacillus stearothermophilus* disc assay.

Desired PEN Conc. in milk (1)	Actual PEN Conc. in milk (1)	Mean BSDA zone size of milk	%Pen recovered in cheese	%Pen recovered in whey	Total% PEN recovered
0	0	12.7	--	--	--
5.0	5.8	16.3	DZ(2)	76.3	
10.0	13.9	18.6	16.7	89.3	106.0
20.1	17.8	20.5	15.9	93.0	108.9
40.2	37.4	23.0	12.8	81.4	94.2
80.5	71.5	25.0	12.7	85.5	98.2

1. in nanograms/milliliter.
2. detectable zone.

**Table 3.** Concentration (1) of penicillin G in milk, cheese, and whey as determined by the modified *B. stearothermophilus* disc assay.

Desired PEN Conc. in Milk (1)	Actual PEN Conc. in Milk (1)	Penicillin Conc. in Cheese	Penicillin Conc. in Whey	Percent Increase in Cheese
0	0	0	0	--
5.0	5.8	DZ(2)	4.8	--
10.0	13.9	26.2	13.3	88.5
20.1	17.8	32.7	17.8	83.7
40.2	37.4	53.9	32.6	44.1
80.5	71.5	104.0	65.5	45.4

1. in nanograms/milliliter or gram.
2. detectable zone.

At the 5.8 ng/ml level, the cheese sample produced a detectable zone on the disc assay, but the zone size was outside the limits of the standard curve. For this reason, penicillin concentration of this sample could not be determined without extrapolation of the standard curve. At penicillin concentrations above 5.8 ng/ml level, antibiotic concentrations in the cheese samples were always greater than concentrations in the starting cheese milks. The percent increase in cheese penicillin G concentration over that of the starting cheese milk ranged from 44.1 to 88.5.

Although a majority of the penicillin G contained in the cheese milk was recovered in the whey fraction, data from this study indicate that when low-moisture part-skim Mozzarella cheese is manufactured and analyzed as previously described, antibiotic residues are concentrated in the finished product. Considering the potential public health risks associated with the marketing of finished products containing antibiotic residues, all dairy materials used for Mozzarella cheese manufacture should be screened for antibiotic residues.

The following paper was presented by Allan N. Bringe, Professor, Department of Dairy Science, University of Wisconsin, Madison, Wisconsin 53706, at the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 & 17, 1987.

### CHEESE PLANT PROFITABILITY IS AFFECTED BY SOMATIC CELL COUNT

by Allan N. Bringe, Ph.D.

#### ABSTRACT

Research the last five years has clearly demonstrated the significant reduction in cheese plant yield and in product quality when manufactured from milk with high somatic cell count. Milk price incentives and education of dairy producers assist in adoption of research proven herd management techniques and improved dairy cow environment. This paper discusses factors that the cheese industry needs to consider that affects progress affecting milk quality, lowering somatic cell count and improving profitability of the plant and the dairy farm.

Changes in our industry currently involve a number of new techniques and new technologies. Because of a wide range of responses from the producer there will likely be ever increasing disparities between producers. The future characteristics of dairy production will be greater opportunities for more improvement by some producers and greater hardships for others. The effects on your plant will depend on which producers are your patrons and your ability to get them actively involved in planning for improvement.

There will be significant benefits to the cheese plants that plan for the future by having an understanding of factors affecting profitable production of quality milk. The knowledge, experience and ability of the cheese plant field representatives to provide research proven assistance to the producer will be essential. This involves a willingness to evaluate what procedures are used at milking time. A critique following milking should establish a priority order for the recommendations made.

The industry can improve communications to the producer concerning the testing of milk. The milk hauler is a very significant part of the team in taking accurate samples and communicating back field concerns. Reporting results of tests frequently and near the time of analysis provides the producer a monitoring tool that is valuable for herd management awareness and decisions. The ability of the laboratory to provide repeatable accurate analysis is important not only because of payment but also herd management. Veterinarians and other consultants are depending on plant reports along with Dairy Herd Improvement (DHI) analysis for evaluation of health and nutrition performance.

#### Promote DHI records

Dairy producers need accurate measures of production and correct cow identity to make management decisions and improve performance. Herds on DHI will be shipping more pounds of milk per cow to the cheese plant. Encourage producers to test for Somatic Cell Count (SCC) on individual cows. The knowledge gained from testing can result in uninfected

cows milked first and cows over 200,000 SCC last. This one decision will greatly decrease the rate of new infections and keep the herd milking with a lower SCC.

### What does SCC measure?

The SCC measures the cellular defense mechanism and is the best indicator of the presence of inflammation of the bovine udder. The SCC will provide accurate information on individual cows for the evaluation of success or failure of mastitis prevention and treatment programs.

Significant milk yield losses have shown a linear relationship associated with an increase in SCC. University of Wisconsin research finds a loss of 400 pounds of milk per lactation for each doubling of SCC over 50,000 cells per ml. For example a cow with a lactation average SCC of 800,000 is estimated to have a decrease milk production of 1600 pounds. Table 1 shows how SCC affects daily milk yield. A 100,000 cell increase lowers production more at low cell counts than at high cell counts. Severe reductions in milk yield have already occurred once SCC reaches 400,000 cells.

**Table 1.** Reduction in milk yield per day at various levels of SCC

Daily SCC (cells per ml)	Reduction in Daily Milk Yield (lbs)
50,000	0
100,000	1.5
200,000	3.0
400,000	4.5
800,000	6.0
1,600,000	7.5

\*These losses apply to cows in second and later lactations. Corresponding yield losses in first-lactation cows are one-half these amounts.

### The effective use of SCC

The ability to document economic impact is an essential motivating factor in achieving adoption of herd health management procedures. When SCC increases in the dairy herd the likely cause of microorganism invasion of the mammary glands needs to be found. Accurate cheese plant bulk tank SCC from the last 24 months should be evaluated. The month or months when SCC increased were periods when previous uninfected cows likely became newly infected cows due to management or environment contamination. Eliminate the likely cause and improve conditions. If no changes are made then the herd will likely experience new infections and increased SCC again for the same reasons.

Bulk tank SCC reports do nothing to identify infected cows. Herds with bulk tank data can use the California Mastitis Test (CMT) paddle to identify cows and quarters that contribute to the count. When this system is used it is desirable to record the data of cows quarters reacting to



the CMT reagent. These are likely infected. Be aware that quarters that may be infected will be missed because of the cycle of the battle between bacteria and SCC. The paddle will not read as low counts as reported by the cheese plant laboratory or DHI.

### Benefits of DHI SCC

Herds that have monthly individual cow SCC information have a monitor concerning the effectiveness of the mastitis control procedures used on the herd. It allows measurement of the reduction in subclinical mastitis as management is improved. The rate of new infection is the best indicator of desirable herd management. It is necessary to focus the producers attention on this information as a meaningful monitor of control. A realistic goal is for 85 percent of the cows to have a SCC below 200,000.

### Complexity of the causes

The complexity of mastitis makes solutions to herd mastitis problems challenging. Accurate analysis and effective recommendations require that all factors causing the high SCC be considered. Failure to accumulate sufficient information can misdirect the producer and result in ineffective control of SCC.

Since considerable knowledge of milking equipment, milking management, stray voltage, microbiology, mastitis treatment products and treatment regimes as well as general dairy farm management are required, a team effort may be necessary. The solution of a herd health program may require the cooperative efforts of the owner/herd manager, veterinarian, milking equipment dealer, milk plant fieldman, and county extension agent.

### Recommended control procedures

The research data at this time indicated that SCC will decrease in herds improving mastitis control procedures as follows: Milking equipment installed to 1987 standards and maintained in proper operating condition; use correct milking procedures, including milking time cow preparation, sequencing, and dipping all teats immediately after each milking with a product proven effective; review management of freshening cows, cows to cull, milking infected cows last, source of herd replacements, condition of lots and stall bedding; evaluate dry cow treatment and dry period management. Compare individual cow SCC before dry off and a month after calving to have an indication of effectiveness of dry cow treatment, environment and culling.

Improvements in the mastitis prevention program will require time to see effects. The level of SCC in the herd is due to the rate of infection and the duration of infection. Making decisions on both will likely result in progress.

### Decisions with high SCC cows

The mistake in management has been made! The preventative measures were not adequate. Unfortunately, after infected cows are identified the management options are limited.

Milk cows that are over 200,000 cells last to decrease the spread of infection to the uninfected cows during the milking process. In some herds this may not be practical. The use of automatic backflush in a parlor can be used to accomplish similar results. An alternative method is to identify cows over 200,000 cells by a leg band and milk with a separate milking unit.

Culling is often the most practical means for elimination of chronically infected cows. There is little justification for keeping cows that have consistently high SCC, sporadic flare-ups, and infections that persist in spite of dry cow treatment. These cows are reservoirs of infection which spread to other cows during the milking process.

It is generally recommended that all quarters of all cows be treated with a dry cow antibiotic preparation at drying off. This approach has the advantage of reaching all infected quarters and is effective in prevention of new infections during the dry period. Approved commercially prepared products in single dose containers should be used.

If a dairyman decides to use selective dry cow therapy, SCC results can be used as a guide. Do not expect too much from dry cow treatment. Culling is important. Cows with two or more infected quarters should be evaluated for culling.

#### Prevention vs lactation treatment

Many producers expect to use SCC as a basis for treatment of individual cows. Research has shown that when cows with SCC exceeding 400,000 were treated there was very little effect on milk production during the remainder of that lactation. Producers in danger of loss of their milk market need to evaluate the situation with the veterinarian. Early dry off and dry treatment should be used for cows in late lactation.

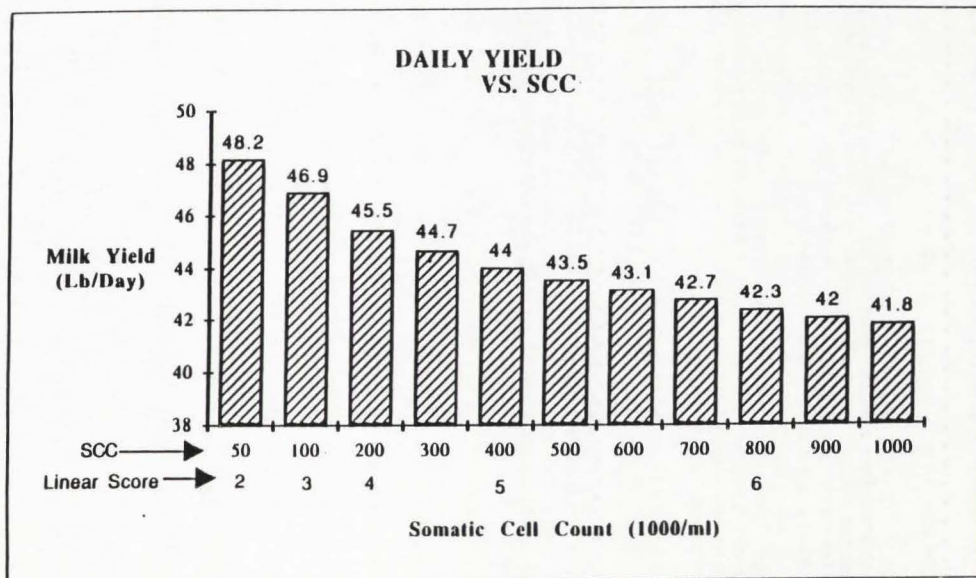
#### Motivation of the producer

The success or failure of lowering bulk milk SCC often depends upon an understanding of human nature as much as an understanding of the scientific basis of disease prevention. To solve the herd mastitis problem on a scientific basis through correcting deficiencies in teat dipping, udder environment, dry cow therapy, milking routine, etc., please remember the people that perform these tasks. They may not yet be convinced that the procedure is worthwhile.

Programs that most often appeal to dairy farmers are relatively simple, inexpensive, have visible results, and demonstrate economic return. Economic incentive is the most powerful motivator; however, it is not the only motivation. The enjoyment of working with dairy cattle and the pride of doing a good job is important. Sensitivity toward producer values will ease communication, help the setting of realistic goals, and enhance adoption of recommended procedures.

The adoption of new procedures will depend on many interrelated personal, social and situational needs. The five stages of the adoption process are as follows:

- 1) Awareness: The producer has learned of a new practice or idea but lacks complete information. This increased awareness may have been the result of attendance at an educational program or by reading an article.
- 2) Information: The producer becomes interested and seeks further knowledge. There is a need to know how and why it works, what its cost will be, its advantages and disadvantages relative to current practices, and if there is economic return. It is important to offer a full explanation of any recommended procedure. Understanding the theory behind a concept is a strong motivating factor.
- 3) Evaluation: The producer will mentally examine the newly acquired information relative to his specific farm. Usually the opinions of others will be requested, particularly other farmers who may have tried the procedure. The veterinarian is often a sounding board for contemplated changes.
- 4) Trial: Many will want to try the concept on a smaller scale before a total commitment is made.
- 5) Adoption: Finally, the full commitment to use the idea or procedure is made. Final adoption of a procedure does not mark the end of the change process. Follow-up encouragement and adjustments to accommodate farm management should be expected. The establishment of an idea and establishment of a practice are not synonymous.



**Figure 1.** Average daily milk yield for various levels of SCC. Adapted from Jones, et al. *Journal of Dairy Science*, 67:1823.

## **WHAT SHOULD YOU DO WITH HIGH SCC COWS?**

### **Lactation Treatment**

1. If in danger of loss of your milk market because of high SCC, it may be necessary to treat cows during the lactation.
2. Early drying off should be used for cows in late lactation.

### **Dry Cow Treatment**

1. All quarters should be treated with a dry cow antibiotic preparation at drying off.
2. If the dairyman decides to use selective dry cow therapy, SCC results should be used as a guide.

### **Milking Order**

1. Milk cows with high SCC last to decrease the spread of infection to uninfected cows during the milking process.
2. An alternative method is to identify cows with high SCC by a leg band and milk these cows with a separate milking unit. The unit should be flushed with clear water, soaked in sanitizer, flushed again with clear water and hung up to drain before using on the next cow.

## **EVALUATION OF HERD MASTITIS CONTROL PROCEDURES:**

- (1) Restore milking equipment to proper operating condition.
- (2) Correct milking procedures, including milking time sanitation (emphasizing dipping of all teats immediately after milking on a routine basis with a product proven effective).
- (3) Review other management practices such as the basis for culling, source of herd replacements, condition of lots and free stall bedding.
- (4) Evaluate and improve mastitis detection and lactation treatment program.
- (5) Evaluate dry cow treatment and management program.  
A comparison of each cow's SCC before drying off and a month after calving will give you an indication of the effectiveness of your dry cow treatment and management program.

The following paper was presented by Dr. David M. Barbano, Associate Professor of Food Science, Cornell University, Ithaca, New York 14853, U.S.A., especially for the 24th Annual Marschall Invitational Italian Cheese Seminar, held in the Forum Building of the Dane County Exposition Center, Madison, Wisconsin, on September 16 and 17, 1987.

### INFLUENCE OF MILK SOMATIC CELL COUNT ON CHEESE MANUFACTURING AND CHEESE YIELD.

By David M. Barbano, Ph.D.  
Robert J. Verdi, Ph.D. Candidate  
Robert Rasmussen, Research Specialist

#### ABSTRACT

Over the past ten years the relationship between milk somatic cell count and milk quality has been investigated. Recent research has found that as milk somatic cell count increases, the level of proteolytic enzyme activity in milk increases. The most important proteolytic enzyme associated with increasing milk somatic cell count is plasmin. Plasmin breaks down casein during milk handling and storage. When milk casein is enzymatically damaged it may be lost in the whey during cheese making and result in lower cheese yields. Other factors besides mastitis may influence native milk protease activity. Many cheese manufacturers have offered milk quality payment premiums so that farmers would have an additional economic incentive to reduce somatic cell counts. Decreasing milk somatic cell count benefits both the cheesemaker and the farmer. The farmer will obtain higher milk production from cows that are free of mastitis and the cheesemaker will obtain higher cheese yields. Current research indicates that there is a sharp drop in cheese yield once milk somatic cell count reaches approximately 100,000 cells per ml and then a more gradual decrease as somatic cell counts increase to 1,000,000 per ml. High milk somatic cell counts may also influence starter culture activity and moisture content of the cheese.

#### INTRODUCTION

Mozzarella cheese yield and quality are two very economically important factors for the Italian cheese industry. All cheese manufacturers want to obtain the best possible cheese yield and quality. Some yield losses and quality defects can result from poor raw milk quality, while other losses and defects relate to poor or incomplete execution of good cheese manufacturing practices.

During the past five years there has been increasing interest in the influence of milk quality on cheese yield. This has been particularly true for milk somatic cell count. Research reports have indicated that increasing milk somatic cell count will cause a decrease in cheese yield (1,2). However, there has been very little published research that has quantitatively characterized the relationship between somatic cell count and cheese yield under practical cheese making conditions. The popularity of somatic cell count in milk premium payment programs has resulted from advances in electronic testing technology that allow rapid and

economical quantitative determination of milk somatic cell count. This gives the cheesemaker an objective and repeatable test on which a milk quality premium can be based, even though the exact quantitative relationship between milk somatic cell count and milk value has not been characterized fully. Characterization of the impact of somatic cell count on milk value is difficult because increasing somatic cell count can influence both cheese yield and cheese quality. Cheese yield improvements are quantitative and recognizable and can be related directly to revenue loss.

Poor milk quality can result in poor cheese quality. Changes in dairy product quality due to poor milk quality are much more difficult to assign a specific economic value for assessment of revenue loss. If high somatic cell count milk causes texture or other quality defects and a customer is dissatisfied with the product, how does the management of a mozzarella cheese plant quantitate and account for the economic loss? The exact magnitude of economic losses due to poor product quality can be easily underestimated and relatively invisible.

The objective of this paper is to describe how mastitis causes cheese yield losses and manufacturing problems in the Italian cheese industry.

### **How Does High Somatic Cell Count Damage Cheese Yield and Quality?**

All milk contains some level of somatic cells. In normal milk, the somatic cells are mostly epithelial cells or other types of somatic cells that do not cause significant milk quality problems. When there is a bacterial infection, tissue damage, or other inflammation of the mammary tissue, the number of somatic cells in the milk increase dramatically. This increase in somatic cell numbers results from a transfer of white blood cells from the blood into milk. In addition, the relative proportions of different types of somatic cells present in milk changes significantly. This change is illustrated in Table 1.

In normal milk, greater than 80 percent of somatic cells are epithelial cells or other somatic cells normally found in milk. As we compare normal milk with <100,000 somatic cells per ml to milk from cows with subclinical mastitis (500,000 cells per ml), the number of lymphocytes increase about 3.9 fold, the neutrophils increase 26 fold and the epithelial cells increase about 2.8 fold. Neutrophils make up nearly 50 percent of somatic cells present in milk from cows with subclinical mastitis. In milk from cows with clinical mastitis (>1,000,000 cells per ml), the neutrophils make up an increasing proportion of the somatic cells present. The exact nature of this change will be highly variable from cow-to-cow, but the general trend of changing ratios of somatic cell types illustrated in Table 1 would be typical of the changes in types of somatic cells that occur during the course of an infection.

Why is the proportion of somatic cell types present in milk different in normal milk than in milk from cows with mastitis? The specific function of a neutrophil, which is a type of white blood cell, is to destroy invading bacterial cells, foreign proteins, and tissue debris in an area of tissue inflammation and infection. Therefore, during mastitis, which is an infection and inflammation of the mammary tissue, it is reasonable to expect an increase in the proportion of white blood cells called neutrophils.

How do neutrophils fight infection? A neutrophil is a white blood cell that moves quickly to areas of tissue damage and infection. A neutrophil has a very potent arsenal of weapons to fight infection. These weapons include extremely active proteases, lipases, phospholipases, and specific chemicals that are inhibitory to bacteria. All of these enzymes and bacterial inhibitors are carried into milk with the neutrophils.



What else happens during mastitis? Because of the mammary tissue damage resulting from mastitis, there is a leakage of blood plasma into the milk. Blood plasma contains many enzymes such as proteases and lipases which can accelerate the breakdown of milk protein and fat.

Currently, it is thought that the most important enzyme (for cheese yield and quality) that enters milk from the blood plasma is a proteolytic enzyme called plasmin. Plasmin will break down milk casein. Most recent research information indicates that plasmin cannot be inactivated by normal milk pasteurization and may even survive some ultra high temperature (UHT) processing conditions.

The primary mastitis induced changes in milk that will influence dairy product quality and cheese yield are the result of changes in the amount and/or activity of proteolytic and lipolytic enzymes in milk. These enzymes digest and breakdown protein and fat causing yield losses, and possibly result in the development of undesirable flavor and texture.

Thus, the most important point to recognize is that the cause of cheese quality and yield problems (related to mastitis) is increased proteolytic and lipolytic enzyme activity in milk. The yield potential and quality of milk will continue to decrease with time. The rate of milk component breakdown will increase with increasing milk temperature. Commingling of high somatic cell count milk with low somatic cell count milk may result in some damage to milk casein and fat in the low somatic cell count milk. The amount will depend on how long and at what temperature the milk is stored.

#### How Well Does A Change In Milk Somatic Cell Count Reflect A Change In Milk Proteolytic Enzyme Activity and Milk Quality?

Our research (3,4) and research conducted in other laboratories (5,6) indicates that when somatic cell count increases the proteolytic enzyme activity in milk increases. Based on this observation we have assumed that when somatic cell count decreases after mastitis has been eliminated, that the level of proteolytic activity in milk returns to pre-infection levels. Since this is a very important question, an experiment was designed to obtain the answer.

The proteolytic activity of milk was studied before, during and after mastitis. An inoculum of Streptococcus agalactiae was infused into one quarter of each of six cows to elicit an experimental infection. Uninfected quarters in each cow were used as controls. Bacteriological cultures and milk somatic cell counts were used to monitor infection status. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to measure milk proteolytic activity (7).

Somatic cell count and total milk proteolytic activity increased significantly in infected quarters. No changes were observed in somatic cell counts or milk proteolytic activity from the uninfected quarters during or after the infections. After elimination of the infections by use of antibiotics, somatic cell counts in milk from the infected quarters decreased to levels not statistically different from the pre-infection period. Total proteolytic activity of milk from the previously infected quarters decreased after the infections were eliminated, but still remained significantly higher than pre-infection levels. Total proteolytic activity of milk from the previously infected quarters decreased after the infections were eliminated, but still remained significantly higher than pre-infection levels. The increased level of milk proteolytic activity in treatment quarters post-infection when compared to pre-infection proteolytic activity may have been due to continued leakage of blood plasma into milk resulting in higher plasmin activity.

Therefore, some detrimental effects of mastitis on milk quality may continue after the infection has been eliminated and milk somatic cell counts have returned to low levels. Severe and repeated infections in the same cow over several lactations may cause an elevation of background milk proteolytic activity for that cow due to cumulative mammary tissue damage. This aspect of milk proteolytic activity and the impact of mastitis needs to be studied in more detail.

Other factors in addition to mastitis may influence native milk proteolytic activity and casein damage. These factors are very poorly documented and further research is needed to identify the best indicators of milk quality for payment programs.

Milk somatic cell count is a very effective tool for the farmer for mastitis control. It appears that a simple testing method that would measure milk proteolytic activity or undamaged milk casein may be a better index of milk quality and potential cheese yield for the cheesemaker than milk somatic cell count.

### How Much Does Somatic Cell Count Influence Cheese Yield?

Cheese yield is dependent on two factors: 1) cheese yield potential of milk, and 2) manufacturing cheese yield efficiency. The cheese yield potential of any milk is determined by the amount of casein and fat in milk. In addition, the physical condition of milk casein and fat (ie. the amount of proteolytic and lipolytic damage) will influence the efficiency of their recovery as cheese. The modified Van Slyke and Price cheese yield formula (shown below) is one method used to predict mozzarella cheese yield from chemical analysis of milk composition (8). As milk casein and fat decrease, cheese yield will decrease.

$$\text{PERCENT YIELD} = \frac{(0.85F + (C - 0.1)) 1.13}{1 - W}$$

- F = Percentage of fat in the milk  
 C = Percentage of casein in the milk  
 W = Percent of moisture in the cheese/100

There is conclusive evidence that an increase in milk somatic cell count is accompanied by an increase in proteolytic activity in milk (7). It has always been said that high somatic cell count milk had low casein. For many years it has been assumed that a cow made less casein and more whey proteins during an infection. However, several investigators have determined that this is not the case in the normal range of somatic cell counts from 100,000 to 1,000,000 and that milk casein is actually being broken down by proteolytic enzymes. When this occurs large casein molecules are broken into smaller casein fragments that are lost into the cheese whey and do not contribute to cheese yield. Therefore, (in the cheese yield formula shown above) enzymatic damage to casein would have the same impact on cheese yield as decreasing casein content of the milk.

It is very difficult to determine exactly how much a change in milk somatic cell count will change cheese yield. Currently, there is no formula or calculation that can be used that will tell a cheese maker how much cheese yield will decrease if the somatic cell count increases in 100,000 cell per ml increments.

A study is being conducted currently at Cornell University to determine a quantitative relationship between increasing milk somatic cell count and Cheddar cheese yield. Results from the work on Cheddar cheese would be applicable to mozzarella. The range of somatic cell counts being studied is from 30,000 to 1,300,000 cells per ml. Each batch of cheese is made from 100 lbs of milk. Batches of milk are collected at the Cornell dairy farm and cheese is made from one half of the batch at approximately 24 hours after milking and another batch of cheese is made from the other half of the batch of milk after 120 hours of 4°C storage. Preliminary results from 44 batches of Cheddar cheese indicate that the major impacts of increasing milk somatic cell count are on yield, moisture, and starter culture activity. Cheese yield decreases with increasing somatic cell count but not in a linear fashion. When the milk somatic cell count reaches approximately 100,000 cells per ml, there is a sudden decrease (about 1 percent) in cheese yield. As milk somatic cell count increases from 100,000 to 1,300,000 the rate of decrease in cheese yield is much slower and may be an additional 1 to 2 percent depending on the milk handling conditions (ie. time and temperature). Milk handling and cheese making conditions used in this study represent best case milk handling conditions observed changes are probably conservative. Average psychrotrophic bacteria counts of one day old raw milk in this study were less than 15,000 per ml and less than 250,000 per ml for five day old raw milk. Bacteria counts were maintained at low levels by good sanitation, rapid milk cooling, and storage at 4°C.

Increasing milk somatic cell count cause cheese moisture to increase and starter culture activity to decrease. Over the range from less than 100,000 to 1,300,000 somatic cells per ml, the moisture content of the cheese may increase by 1.6 percent and the make time (time from addition of starter to a milling pH of 5.30) may be increased by 15 minutes. The changes in moisture and starter culture activity could adversely influence cheese quality.

### Conclusions

High milk somatic cell counts will cause low cheese yields. In addition, high somatic cell counts may cause problems with cheese moisture control and starter culture activity during cheese manufacture. Increasing milk somatic cell count is correlated with increasing proteolytic enzyme activity in milk and the source of the proteolytic enzymes are from the cow. The bovine proteolytic enzyme plasmin is major cause of casein proteolysis related to mastitis. Other factors may increase plasmin activity in milk in addition to mastitis. These factors need to be identified and better testing methods need to be developed so that milk quality can be quantitatively related to true milk value for cheese manufacture.

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PERCENT YIELD = (C - 0.1) / 1.13

Conclusions

High milk somatic cell counts will cause low cheese yield. In addition, high somatic cell counts may cause problems with cheese moisture control and starter culture activity during cheese manufacturing. Increasing milk somatic cell count is correlated with increasing proteolytic activity in milk and the amount of the proteolytic enzymes are from the cow. The proteolytic enzymes are major cause of casein proteolysis related to mastitis. Over factors may increase plasmin activity in milk in addition to mastitis. These factors need to be defined and better testing methods need to be developed so that milk quality can be determined related to milk usage for cheese manufacture. The proteolytic activity is related to somatic cell count and it is a good indicator of mastitis. However, increased proteolytic activity is not always related to mastitis. In addition, proteolytic activity is also related to other factors such as lactose, urea, and other non-protein nitrogen. The proteolytic activity is also related to the type of starter culture used and the type of cheese manufactured. The proteolytic activity is also related to the type of cheese manufactured. The proteolytic activity is also related to the type of cheese manufactured.

Proceedings of the 18th Annual Meeting of the National Mastitis Council, Washington, D.C.

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TABLE 1. CHANGE IN TYPES OF SOMATIC CELLS PRESENT IN MILKS WITH INCREASING SOMATIC CELL COUNTS.

MILK TYPE (cells per ml)		SOMATIC CELL TYPE		
		LYMPHOCYTES	NEUTROPHILS	EPITHELIAL
NORMAL (<100,000)	% OF TOTAL NUMBER	6.1 6,061	9.1 9,091	84.8 84,848
	INCREASE	3.9x	26x	2.8x
SUBCLINICAL MASTITIS (500,000)	% OF TOTAL NUMBER	4.8 23,809	47.6 238,095	47.6 238,095
	INCREASE	3.9x	26x	2.8x
CLINICAL MASTITIS (1,000,000)	% OF TOTAL NUMBER	2.6 25,848	71.6 716,000	25.8 258,182
	INCREASE	4.3x	79x	3.0x