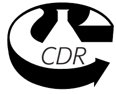


# Wisconsin Center for Dairy Research

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## CDR Annual Report

Published March 1, 1998, by the Wisconsin Center for Dairy Research.

Our annual report is a technical overview of CDR funded research and other Center activities during fiscal year 1997. We prepared this report for organizations funding CDR and for fellow dairy researchers. This document describes projects in progress and interpretations of data gathered to date. It is not a peer-reviewed publication.

Please seek the author's written consent before reprinting, referencing, or publicizing any reports contained in this document.

For more information call Karen Paulus at (608) 262-8015.
(E-mail: Paulus@cdr.wisc.edu)

Dear Colleagues,

The value of a research center can be measured in many ways. We can total the number of ongoing research projects (51), or the number of industry partners we have worked with the past few years (82). We can look at current applications programs (5), the number of participants in our conferences, workshops, short-courses, etc. (over 2,000), our annual budget ($3.5 million), or the total number of CDR staff (28). We could showcase many accomplishments from the past year—including initiating a whey applications program and seeing industry use technology CDR developed: baking/pizza cheese, milkfat fractions, specialty cheeses, 60% reduced-fat Cheddar, etc. In addition, we formalized a Cheese Industry Team of 15 initial members who receive cheese-related technical services, and increased international involvement through CODEX, IDF, and scientific presentations.

However, I'd like to emphasize that the true value of CDR is two-fold. The first is the partnerships we developed with our industry, Wisconsin Milk Marketing Board, and Dairy Management Inc. Second, and most important, is the strength of the CDR staff and university researchers. I believe these people, as individuals, are some of the most dedicated professionals in the business. As the Center has grown in numbers and reputation individuals have grown and matured to accept the enormous responsibility that comes with prominence. They represent the heart, soul, and strength of CDR, and I am honored to be a part of this team.

Sincerely,

J. Russell Bishop

**Our Mission Statement**

*The Wisconsin Center for Dairy Research will serve as a national leader in strategic research to improve the competitive position of the dairy industry by linking Center/University faculty, staff, students and the dairy/food industries to address key issues resulting in transfer of technology and communication of information.*

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Investigation of baked milkfat flavor development in milkfat ingredients for the bakery and food industries

Technology for improving the flavor and consumer acceptability of fat-free Cheddar cheese

Improvement of functionality, flavor, and stability of butter

Intensified flavors in cheddar cheese and cheese ingredients for enhanced applications in food

Increased functionalities for milkfat fractions, especially as related to baking applications

# CDR was established in 1986 to:

- **Provide technical expertise to strengthen the economy of the dairy industry**
- **Re-establish a focus on dairy research at University of Wisconsin-Madison**
- **Foster multidisciplinary research and transfer information and technology**
- **Integrate milk production, processing and marketing research**

# Committees

### Administrative Committee

The Administrative Committee is responsible for policy formulation and appointment of the CDR Director. Its members (FY 1996-1997) are:
J. Russell Bishop, CDR
Joe von Elbe, Dept. of Food Science
Janet Greger, Graduate School
Neal Jorgensen, College of Agricultural and Life Sciences
Leslie Lamb WMMB
Bill Haines, DMI
Tom Szalkucki, CDR

### Technical Advisory Committee

The Technical Advisory Committee (TAC) plans the CDR research program, and evaluates and approves research projects for scientific merit. Members (FY 1996-1997) include:

Bishop, J. Russell, CDR
Blaska, Gregory, DMI and WMMB
Bremel, Robert, Department of Dairy Science
Bumbalough, John, Land O' Lakes
Dobson, William D., Dept. of Agricultural Economics
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Haines, Bill, DMI
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Krug, David, DMI and WMMB
Lindsay, Robert, Department of Food Science
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Muck, George, Dean Foods
Ney, Denise, Dept. of Nutritional Sciences
Olson, Norman F., Department of Food Science
Rose, David, WMMB
Sellars, Robert, R. L. Sellars & Associates, Inc.
Szalkucki, Thomas, CDR

## Industry Advisory Committee

The Industry Advisory Committee determines the best methods for commercial investment in CDR projects. Committee members bring an industry perspective to research planning, including a commercial view of the interaction between R&D, marketing, and economics. They include (FY 1996-1997):

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Bumbalough, John, Land O'Lakes Inc.
Campbell, Larry, Hershey Foods
Carr, Jim, Systems Bio Industries
Cobian, Francis, Lake O' Lakes
Crawford, Robert, Borden Foods, Inc.
Everson, Tom, Grande Cheese Co.
Geyer, Jim, Foremost Farms
Gregorich, LaVerne, Grassland Dairy Products Inc.
Haines, Bill, DMI
Johnson, Bob, Campbell Soup Co.
Keel, Tom, Foremost Farms
Kozak, Jerry, NMPF
Krug, David, WMMB
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Leitner, Ken, Alto Dairy Coop
Mathison, Matt, Wisconsin Milk Marketing Board
Mehnert, David, Kraft
Moran, Jim, Kraft-General Foods Inc.
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Narasimmon, Raj, Schreiber Foods, Inc.
Pangier, Doug, Gist-Brocades
Racicot, Linda, Dairy Management Inc.
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Sellars, Robert, R. L. Sellars & Associates, Inc.
Schroeder, Craig, Mid-America Dairymen, Inc. Technology
Sommer, Dean,Alto
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Umhoefer, John, Wisconsin Cheese Makers Assoc.
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Wuethrich, Dallas, Grassland Dairy Products, Inc.

## Program Area Coordinators

Cheese Technology— Robert Lindsay, Dept. of Food Science, University of WI-Madison
Milkfat Utilization—Rich Hartel, Dept. of Food Science, University of WI-Madison
Whey Utilization—Mark Etzel, Dept. of Food Science, University of WI-Madison
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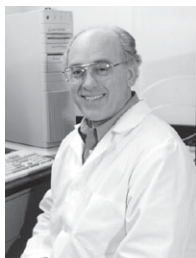

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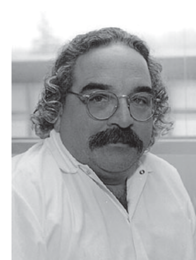

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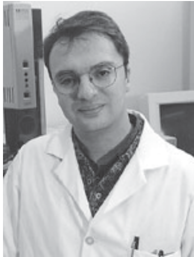
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# Milkfat

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*FINAL REPORT*

# Continued studies of surface melt crystallization techniques for fractionating milkfat

**Personnel**

R.W. Hartel, associate professor, Dept of Food Science; J. Ulrich, professor, University of Bremen; M. Tiedtke, graduate research assistant, University of Bremen.

**Dates**

July 1995 - June 1997

**Funding**

Dairy Management Inc. HRT 96

## Objectives

To evaluate surface layer melt crystallization for multi-step fractionation of milkfat.

To make an economic comparison between surface layer and suspension techniques for fractionating milkfat.

To determine the effects of initial milkfat composition on crystallization kinetics and overall efficiency for fractionating milkfat using surface layer techniques.

## Summary

In the past several years, surface layer crystallization techniques have been used successfully for fractionation of milkfat. Milkfat was cooled as it flowed across a cooling surface in a thin film. By controlling the temperature of the cooling surface, crystallization of milkfat occurred directly on the surface. As a solid layer formed on the cooling surface, a partition between crystalline and liquid portions of milkfat occurred, depending on the temperature gradients and milkfat composition. Temperature of the solid layer was maintained constant during the process by decreasing temperature according to film thickness. To enhance separation of the crystalline layer, a sweating procedure and/or a washing step may be used, which may involve a slight heating of the layer. Finally, the layer was removed from the surface by raising the temperature to melt the solid layer and the hard fraction collected in the liquid form.

In this study, milkfat was crystallized in thin films under a variety of conditions to obtain sufficient data to perform a cost analysis for scale-up to commercial application. Melted milkfat was pumped in a thin film down a stainless steel tube with the temperature inside the tube controlled by a water bath. Temperature was controlled to maintain an approximately constant temperature on the growing surface. Since the resistance to heat transfer increased as the film thickness increased, the temperature of the water bath was continually decreased during crystallization.

During single-step fractionation, the amount of milkfat crystallized on the thin film was governed by the temperature on the cold surface. Figure 1 shows how the melting point of the fractions (both liquid and solid) vary with fractionation temperature, but plotted according to the freezing ratio (ratio of solid to liquid fractions). At low yields of solid fraction (low freezing ratio) the melting point of the solid fraction was quite high - over 48°C. At higher yields (obtained at lower crystallization temperatures), the melting point of the solid fraction decreased, as expected. Fatty acid analysis documented that the content of shorter-chain and unsaturated fatty acids decreased in the higher-melting fractions, also as expected. Clearly, these results show that thin-layer crystallization can be used to fractionate milkfat.

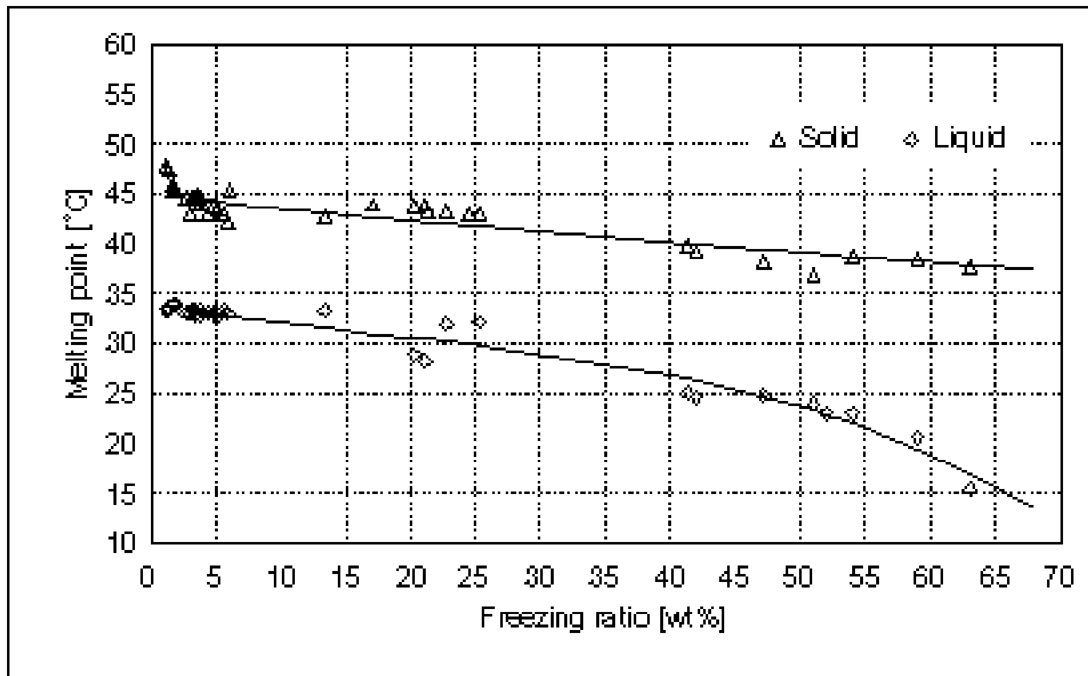


**Figure 1**

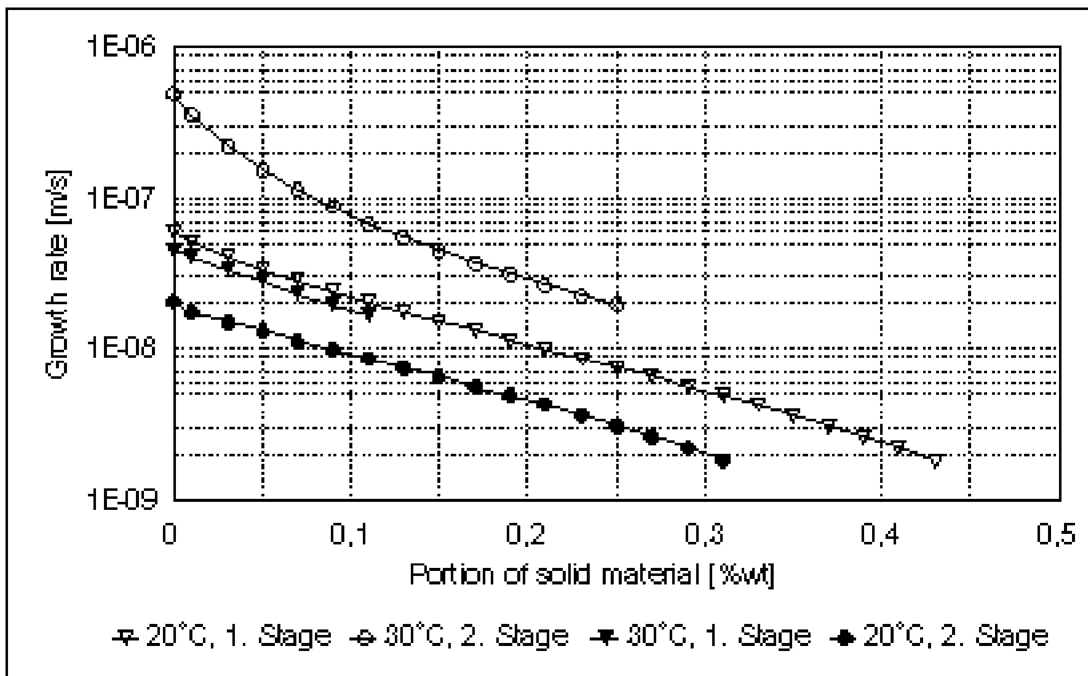


**Figure 2**

In order to determine the efficiency of thin-layer crystallization for fractionation of milkfat, it is necessary to determine growth rates. This allows the total surface area requirements for a given fractionation capacity to be determined. Figure 2 shows how growth rate varies with temperature and stage of fractionation (in a sequential, multi-stage process). First of all, growth rates decreased substantially with time of fractionation (plotted as portion of solid material). That is, the driving force for crystallization decreased with time during crystallization in this process. Also, growth rate decreased significantly at lower temperatures, probably due to the increased viscosity of milkfat at lower temperature. Figure 2 also shows that growth rates are higher if fractionation is first accomplished at 20°C to produce a low-melting fraction and then the solid component is refractionated at 30°C (open symbols). The growth rates are higher than those found during suspension crystallization of milkfat under similar conditions. For sequential fractionation at higher temperature and then lower temperature, growth rates are between 10 and 30% higher than found for suspension crystallization under the same conditions. However, this increase in growth rate is not sufficient to offset the increased surface area needed for fractionation by existing thin-layer technologies, as compared to the commercial suspension fractionation process. For equivalent capacity, thin-layer technology costs approximately twice as much as the corresponding suspension process.

The economic situation can be enhanced by either increasing growth rates or by post-crystallization processing. Continuing studies are underway to further improve the thin-layer technology through optimizing choice of crystallization conditions and post-crystallization separations. Preliminary results show that increases in melting point of the solid fraction of between 1 and 4°C were found when the thin-layer was either heated to "sweat" out entrapped liquid, washed with milkfat or pressed to remove trapped liquid. This process allows faster crystallization to be used (and thus lower surface area) to accomplish the same fractionation.

Techniques that improve separation efficiency between solid and liquid phases to produce more distinct milkfat fractions are needed. Layer-type melt crystallization techniques, popular in the organic chemical industry, have the potential for providing controlled crystallization with minimal separation requirements to produce distinct fractions. In many instances, layer crystallization is more economical than suspension-type crystallization. In this project, we have demonstrated that surface-layer melt crystallization techniques can produce high melting milkfat fractions that are indistinguishable from those produced by existing technologies and at similar yields. Multiple-step fractionations are also feasible with surface-layer crystallization technology, and can produce fractions of similar yield and composition as suspension fractionation. However, due to the slow relative growth rates, the thin-layer fractionation process is approximately twice as expensive as the commercial suspension fractionation technology. Further work is needed to improve the growth rates of milkfat on thin films to optimize separation of solid and liquid components.

## Publications

Tiedtke, M., J. Ulrich and R.W. Hartel, Solid Layer Melt Crystallization - A Fractionation Process for Milkfats, paper presented at Third International Workshop on Crystal Growth of Organic Materials, Washington, DC (1995).

Tiedtke, M., Ulrich, J. and R.W. Hartel, Solid Layer Melt Crystallization - a Fractionation Process for Milkfat, ACS Conference Proceeding Series, Crystal Growth of Organic Materials (Myerson, A.S., Green, D.A., Meenan, P., Eds.) pp. 137-144 (1996).

M. Tiedtke, J. Ulrich and R.W. Hartel, Different Processes for Milkfat Fractionation, proceedings of Bremen International Workshop for Industrial Crystallization (BIWIC), J. Ulrich and L. Wangnick (eds.), University of Bremen Press, pp. 58-61 (1996).

*INTERIM REPORT*

# Effects of milkfat source and composition on crystallization kinetics

**Personnel**

RW Hartel, professor, Dept of Food Science; Colleen Kubitz, professor, Dept of Food Science

**Funding**

Dairy Management Inc.
MF04

**Dates**

June 1997- June 1999

## Objectives

The overall objective is to correlate the variability in anhydrous milkfat with fractionation efficiency. Specific objectives include:

To analyze and identify the key differences in chemical composition and physical properties of anhydrous milkfat produced from different sources (seasonality, regionality, etc.) and materials (cream vs. butter).

To correlate the differences found between AMF samples (Objective 1) with differences in crystallization kinetics and fractionation efficiency.

## Summary

The primary objective of this project is to evaluate the variations in AMF throughout the year, and correlate differences in chemical and physical properties with differences in fractionation efficiency. Fresh AMF will be received on a regular basis, analyzed for chemical and physical properties, and finally, each AMF will be evaluated for crystallization kinetics and fractionation efficiency. In this way, a data base will be generated that correlates chemical composition and physical properties of the AMF with fractionation potential. In addition, through careful correlation of the origin of the AMF (season of production, region of production, processing conditions, etc.), we may be able to define which production parameters play the largest role in causing variability amongst AMF's. These results will ultimately lead to rules to predict optimal fractionation conditions for each milk fat based on a few pertinent measurements.

It is well known that the composition of anhydrous milkfat (AMF) varies based on source and processing conditions, and that these differences can have considerable effect on crystallization kinetics and fractionation efficiency. In fact, prior to fractionation of a new batch of AMF, several experimental tests must be performed to evaluate how well the AMF will fractionate. That is, some AMF's require either higher or lower temperature conditions to obtain satisfactory fractionation. These differences in fractionation behavior between AMF's have been attributed to a variety of sources, including the content of long-chain, saturated fatty acids or the presence of minor non-triglyceride lipid components.

*INTERIM REPORT*

# Kinetics of milkfat crystallization

**Personnel**

R.W. Hartel, professor, Dept of Food Science, D. Illingworth, New Zealand Dairy Research Institute

**Funding**

Wisconsin Milk Marketing Board and New Zealand Dairy Board WMMB 92-8

**Dates**

Feb 1994-January 1998

## Objectives

Determine the effect of crystallization temperature on milkfat crystallization and crystal separation.

Determine the effect of pretreatment and cooling rate on milkfat crystallization.

Determine the effect of milkfat source on milkfat crystallization.

Determine the effect of various mixer and crystallizer conditions and geometries on milkfat crystallization.

Determine the effects of the processing variables (Objectives 1 to 4) on physical properties and yields of milkfat fractions.

Investigate the effects of scale of operation.

## Summary

Recently, experiments have been completed to study several parameters that might influence kinetics of milkfat crystallization and influence the efficiency of fractionation. These include (1) temperature of crystallization, (2) summer vs. winter milkfat, (3) effect of prefiltration, and (4) effect of slow vs. rapid cooling.

As expected, crystallization occurred more rapidly at lower crystallization temperature, as measured by a turbidity technique. Filtration times were longer and rates were slower for slurries formed at lower temperatures, as expected from the increased yield of solid fraction at lower temperatures. The expected trends in fatty acid composition were observed. The content of C4:0 to C10:0 and C18:1 decreased as crystallization temperature increased, whereas content of C16:0 and C18:0 increased with increased crystallization temperature. This matched the corresponding increase in melting point of the solid fraction with increased crystallization temperature.

The winter milkfat, with a higher melting point, crystallized more rapidly than the summer milkfat and produced a solid fraction with higher yield. The slurry from the winter milkfat filtered more slowly than that from summer milkfat, which might be expected from the higher yield. However, no significant differences in crystal size distributions were seen.

Since our milkfat samples were already prefiltered and did not contain visual particulates, there was very little effect of a second filtration. However, the second filtration prior to fractionation resulted in a slightly slower crystallization rate and slightly faster filtration rate. The second filtration also gave slightly higher yield, but this may be related to the level of liquid entrainment. Further analysis of the crystal size distributions and filter cake are underway.

The rate of cooling is known to influence milkfat crystallization. In this study, rapid cooling caused faster crystallization as compared to slower cooling. The

sample that was cooled slowly also filtered more rapidly, perhaps due to the slightly higher yield value measured. However, liquid entrainment and crystal size distribution are being analyzed to better understand this result.

Improvement of fractionation technologies for separating milkfat into valuable food ingredients is necessary to improve process economics and to understand means of producing specific fractions for targeted applications. A better understanding of the kinetics of crystallization of milkfat will allow better control of existing fractionation technologies, and may lead to new and improved techniques. In addition, the relations between mixing conditions in the crystallizer and filtration efficiency will allow more optimal design of fractionation processes.

*INTERIM REPORT*

# Improving thin-layer fractionation technology

**Personnel**

R.W. Hartel, professor, Dept of Food Science, J. Ulrich, professor, University of Bremen

**Dates**

July 1997 - June 1998

**Funding**

Dairy Management Inc.
MF05

## Objective

To improve separation efficiency of surface-layer fractionation of milkfat and update economic analysis.

## Summary

Efforts are underway to improve separation efficiency and crystallization rate to enhance the economic situation of thin-layer fractionation of milkfat. Although we are using the same basic equipment as in past studies, several new approaches are under study.

Sequential crystallization starting with lower temperatures to produce the very-low melting fraction first and then work up to the highest-melting fractions will be studied in greater detail. The first fractionation is carried out at 5°C with a freezing ratio (solid to liquid ratio) of 0.7 to produce a very low-melting fraction (melting point of 10°C). The second fractionation is carried out at 15°C with a freezing ratio of 0.7 in order to produce a low-melting fraction (23°C melting point). The last stage will be operated at 30°C with a freezing ratio of 0.5 to produce middle- and high-melting fractions. In this way, the growth rate of the solid layer will be increased and retention time per stage will be reduced.

Several post-crystallization techniques are also being studied to improve the efficiency of separation. Previous experiments had shown that such post-crystallization techniques could increase melting point of fractions by 1 to 4°C depending on the type of process used. Further studies washing of the thin layers with different materials (milkfat, feed to stage, etc.) are underway. Also, physically squeezing the layer is often used commercially to improve separation in thin-layer separation technology. Squeezing technologies are being evaluated to improve separation efficiency for milkfat fractionation. In this way, crystallization rates can be enhanced by increasing driving force and then attaining the desired separation by physical steps.

Despite the fact that the surface-layer fractionation technique, as we have developed it to date, does not meet our economic goals, the companies that manufacture this equipment are still interested in this project. Since most processes require some washing or pressing step, they agree that the economics of surface-layer technology for milkfat fractionation will be improved by adding these steps. Thus, we are encouraged to proceed with this project for another year to further evaluate purification steps. If successful, the surface-layer technology will provide an alternative approach for milkfat fractionation that may potentially hold advantages in quality and cost over the suspension fractionation process currently used commercially.

*INTERIM REPORT*

# Rheological and structural properties of dairy-based lipid mixtures

**Personnel**

R.W. Hartel, professor, Dept of Food Science, Baomin Liang, associate researcher, Dept of Food Science

**Funding**

Dairy Management Inc. Wisconsin Milk Marketing Board 9703

**Dates**

June 1997 - June 1999

## Objectives

To determine the effects of processing conditions (time, temperature) on crystalline structure of mixed lipids of importance to dairy-based spreads.

To correlate the rheological properties of mixed lipids to their crystalline structure, based on processing conditions, types of fats mixed together and storage conditions.

## Summary

In this project, crystallization of mixed lipids of importance to dairy-based spreads (mixtures of high-melting milkfat fractions with low-melting fractions or canola oil) under different processing conditions, is being investigated in a laboratory system. Crystalline structure is being measured using Confocal Scanning Laser Microscopy (CSLM). The rheological properties of these crystallized fats will then be determined using Dynamic Mechanical Analyzer (DMA) and penetrometer. Processing of the fat mixtures will range from cooling at stagnant conditions to cooling under high shear conditions in a laboratory scale apparatus. At each of these conditions, we will measure production of crystals with time. The production of a certain number, size distribution and shape of fat crystals under different processing conditions will then be related to the rheological properties of the final material.

Often, the solid fat content (SFC) of a fat mixture is used to predict hardness of a product made with those fats. However, processing conditions also influence the rheological characteristics of the final product.

Preliminary experiments clearly show the effect of cooling rate and level of impurities on crystalline structure and the mechanical properties of these products. Slow cooling of a mixture of high-melting with low-melting fractions produced spherulites of about 20 μm when the fat was observed using CSLM. Rapid cooling of the same mixture produced no spherulites, only a random arrangement of strands of lipid crystals. Similar differences were observed when AMF was refined by passing through a chromatography column to remove nontriglyceride components. The AMF crystallized in the form of spherulites, whereas the purified triglycerides crystallized as a random arrangement of strands of lipid crystals. These structural differences are being related to differences in mechanical properties of the fats.

Processing conditions, as well as the characteristics of the fat, influence the rheological characteristics of the final product made from lipid blends. For example, agitation (or working) of butter causes a decrease in hardness without changing SFC. This is related to the number and structure of fat crystals present, and their interactions. However, our understanding of the interactions between the physical chemistry of the lipid mixture and the processing conditions that produce a certain crystalline structure is severely limited since no techniques are available for determining structure in lipid products. This project is a first step in

understanding how crystal structure influences rheological properties of lipid-based products, such as butter and dairy spreads, and will ultimately lead to a better understanding and better control of production of butter and spread products based on milkfat fractions.

*INTERIM REPORT*

# Mechanisms of milkfat nucleation

**Personnel**

R.W. Hartel, professor, Dept of Food Science; Y. Shi, research associate, Dept of Food Science

**Dates**

June 1995 - June 1998

**Funding**

Wisconsin Milk Marketing Board UW 9503

## Objectives

To determine the main parameters influencing nucleation of milkfat and relate these parameters to type (polymorph) and shape of crystal formed.

Determine the effects of milkfat composition, agitation rate and temperature on kinetics of milkfat nucleation in melt crystallization.

## Summary

Recently, we have been defining the relationships between crystallization conditions (nucleation temperature, growth temperature) and physical properties of the fractions (melting point and yield) produced at these conditions. Experimentally, different milkfats were crystallized under the following conditions. The milkfat was cooled to a nucleation temperature and agitated rapidly for a period of 30 seconds. The temperature is then raised to allow growth of the nuclei formed with growth under stagnant conditions. Variable parameters include temperature of nucleation ($T_n$), Reynolds number during nucleation ($N_{re}$), and growth temperature ($T_c$). Solid and liquid fractions were separated by vacuum filtration and weighed. Crystal concentrations in the course of crystallization time were measured by using a photographic technique with image analysis. Maximum melting temperatures (MMT) values of solid fraction samples were analyzed on DSC.

The crystal concentration did not change in the course of entire crystallization with induced nucleation followed by static crystal growth. This implies that all the embryonic nuclei were formed within the inducing time of 30 sec. Thus, nucleation rate (#/ml-min) was found by dividing the crystal concentration with nucleation-inducing time (30 sec). A quantitative relationship existed between crystal concentration and solid fraction yield (wt%), which could be expressed as a power or parabolic equation. Therefore, if the yield of solid fraction was given, the nucleation rate could be calculated by this equation. However, different equations are needed for different AMFs.

At a certain nucleation temperature $T_n$, nucleation rate or solid fraction yield increased as the agitation intensity increased. However, at different $T_n$, the sensitivity of nucleation rate to turbulence ($N_{re}$) was different. At a constant agitation intensity ($N_{re}$), nucleation rate decreased as nucleation temperature ($T_n$) increased between 26.5 and 29°C, and then remained approximately constant at higher $T_n$. The number of nuclei that survived also depended on growth temperature ($T_c$), with more nuclei surviving at lower $T_c$. In previous studies, melting point of the milkfat fraction has been correlated reasonably well with yield of the solid fraction. In these results, however, the relationship between melting point and yield of the solid fraction depended on the conditions of crystallization. In particular, when milkfat was nucleated at higher temperatures (30 - 34°C), the melting points were slightly lower than when milkfat was nucleated at lower temperatures (26.5 - 27.0°C), for a given yield value. This difference is related to compositional differences between these fractions.

In order to control milkfat fractionation technologies, it is critical to understand exactly how milkfat crystals form, and the parameters that influence their formation processes. Through an improved understanding of formation of milkfat crystals, we have developed a more efficient fractionation process.

*FINAL REPORT*

# Phase transition and mechanical properties of dairy products

**Personnel**

R.W. Hartel, professor, Dept of Food Science; Baomin Liang, associate researcher, Dept of Food Science

**Dates**

June 1996 - December 1997

**Funding Agency**

Wisconsin Milk Marketing Board UW9601

## Objectives

To develop the expertise to study the complex phase transitions of milkfat and milkfat fractions using combined modulated DSC and mechanical (DMTA) analysis.

## Summary

This project was essentially an equipment grant intended to provide funds to purchase a dynamic DSC for thermal analysis of milkfat, milkfat fractions and products made with these ingredients. The funds were leveraged with funds from the College of Agricultural and Life Sciences to also purchase a Dynamic Mechanical Analyzer (DMA) to measure mechanical properties of these materials. It was intended that this equipment support ongoing and new research projects in CDR.

A Perkin-Elmer DSC-7 with Pyris 1 software and dynamic capabilities was purchased and delivered in early January, 1997. A Perkin-Elmer DMA was also obtained at the same time using matching funds from CALS. These instruments finally became operational in May, 1997. Since they have been used to investigate thermal behavior and mechanical properties of milkfat and its fractions.

Preliminary studies using the dynamic DSC (DDSC) on a range of fats ranging from simple triglycerides to complex food products was recently completed. Our goal was to develop a base of information to understand the complex transitions obtained using DDSC. Since there is little to no available information on what these transitions indicate, we have been forced to build our knowledge from the ground floor. However, the only lesson learned from this initial study of lipid materials is that great care must be taken to ensure that the observed transitions are really indicative of physical changes in the material. In this preliminary study, we learned that the DSC baseline is critical to obtaining usable information from DDSC. Thus, no conclusions can be drawn at this time. The next step is to go back and repeat each DDSC scan under more controlled conditions. This will be undertaken in early 1998.

Use of the DMA has been much more productive in recent months. After some initial difficulties in obtaining reliable results, we have finally begun to accumulate valuable research information. We are now utilizing the DMA in conjunction with a separately-funded project on "Rheological and Structural Properties of Dairy-Based Lipid Mixtures." In this project, we have evaluated the effects of different cooling rates on mechanical properties of mixtures of high- and low-melting milkfat fractions (50:50 mixture). The fat that was cooled slowly had higher elastic modulus than the one cooled more rapidly. However, since this is contrary to what we expected, we are studying this further. Use of confocal laser scanning microscopy (CSLM) to study the microstructure of these fats will allow us to understand the relationship between microstructure and mechanical properties, according to the objectives of this project. The effects of different ratios of HMF to LMF has also been studied. In this case, our results were expected, where elastic modulus

increased with increasing addition level of HMF. Thus, the fat with higher solid fat content produced harder (more solid) mixtures. The effects of agitation rate and storage time on the mechanical properties of these mixtures are also being studied.

In summary, much more work on the DDSC will be required to assure that we fully understand the capabilities of this unit. However, the DMA is currently being operated in conjunction with other dairy projects to evaluate mechanical properties of products of interest.

Milkfat and milkfat fractions exhibit extremely complex phase behavior due to the wide range of component triglycerides. This phase behavior, and the subsequent effects on mechanical/textural properties, has never been studied in any detail, even though phase transitions are critical to controlling quality and shelf-life of many products. A basic understanding of the reversible and irreversible phase transitions of importance in milkfat and milkfat fractions will ultimately lead to improved control of quality and shelf life of products containing dairy ingredients. This includes butter, chocolate and spreads based on milkfat or milkfat fractions, as well as ice cream, baked goods, and other confections.

One of the primary benefits of this project is maintaining UW-Madison researchers at the forefront of dairy research. Acquisition of this equipment will provide the UW-Madison and CDR with equipment and skills to greatly improve our understanding of the complex interactions between phase behavior and textural properties of foods. Most importantly, we will be better able to serve the users of dairy ingredients (particularly milkfat and milkfat fractions) as they become increasingly important in a wide variety of food products.

*FINAL REPORT*

# Characterization of an esterase from *Lactobacillus helveticus* CNRZ32


### Personnel

Kirk L. Parkin, professor, James L. Steele, associate professor, Jad Arsan, graduate research assistant, Kurt M. Fenster, graduate research assistant, Qing-long Chang, research associate, Department of Food Science


### Dates

July 1996 - December 1997


### Funding

Dairy Management Inc.
PRS97


## Objectives

Purify or partially purify an esterase from *Lactobacillus helveticus*

Characterize the esterase in terms of substrate selectivity for ester hydrolyzing and synthesizing activities.

## Summary


The gene (558 base-pair open reading frame) encoding for the *Lb. helveticus* CNRZ32 esterase was modified to yield a construct which produces a fusion protein containing the esterase and a polyhistidine terminus. This construct was cloned into *Escherichia coli*, the transformed *E. coli* was cultured, and then used to prepare cell-free extracts. The extract was passed through a nickel-nitriloacetic acid-derivatized agarose resin to retain the fusion protein. This matrix interacts with the polyhistidine domain in a manner that can be characterized as an affinity separation process. The fusion protein (esterase) was eluted by a gradient of histidine, and was confirmed to be purified on the basis of electrophoretic homogeneity. The esterase appears to be a monomer of 22,500-25,000 Da, as determined by gel filtration chromatography and SDS-PAGE techniques, and is a basic protein with an isoelectric point of 9.0-9.3. The serine inhibitor, diisopropyl fluoro phosphate inhibited the esterase by 98-100%, whereas phenyl methyl sulfonyl fluoride had little effect on activity. Since the latter is specific for modifying active site histidine residues of Serine protease-type active site constellations, these results suggest the presence of an unusual, or more likely partially protected, Serine protease-type active site. The esterase was also inhibited 75-88% by the thiol reagent, *p*-chloromecuriphenylsulfonic acid, but not iodoacetic acid. The esterase has six serines and a single cysteine residue(s) in its primary structure, and further work on point mutations may be necessary to demonstrate which, if any, of these residues are essential.

Ester hydrolysis activity of the esterase was maximal at 35-40°C, pH 8, in the presence of 3.5-4.0% NaCl; maximum rates using the preferred substrate, phenylacetate, were on the order of 0.6-0.9 mol $min^{-1}$ $mg^{-1}$ protein. Under conditions that simulate those prevailing in a cheese matrix (pH 5.1, 10°C, 4% NaCl), activity was 4% of that observed under optimum conditions. Esterase activity was notably osmo-tolerant, as 29% optimum activity could be observed when NaCl was increased to 25%.

Ester hydrolysis selectivity was determined with various series of substrates to ascertain essential features of enzyme-substrate recognition. Using a *p*-nitrophenyl acyl ester series, selectivity toward the various *n*-acyl derivatives was C4>C2>C3>C5>C6,C8 with corresponsing reaction velocities of 100:88:66:8:3; no activity was detected on C10 and C12 derivatives. Using an acetate ester series, phenyl acetate and phenyl thioacetate were reactive at relative rates of 100:64, with less than 1% activity toward *n*-alkyl or iso-alkyl acetates of 2-6 carbon alcohols. Facile reactivity was also observed with 3-acetoxy pyridine and 4-acetoxy benzal-

dehyde, but not methyl acetylsalicylate. These latter two series of substrates suggest that the esterase can be best classified as an aryl esterase, with greatest selectivity toward *p*-substituted aromatic esters. There was little hydrolysis of tributyrin, indicating a lack of typical lipase-type (interfacial) activity.

Ester synthesizing activity of a celite-adsorbed esterase was found to be near-optimum at a water activity of about 0.21. Maximum initial rates of ester synthesis using the preferred substrate system of hexanoic acid and *n*-propanol were on the order of 6-7 mol $min^{-1}$ $mg^{-1}$ protein, or about 10-fold greater than the rates of hydrolysis on preferred substrates. This ratio is within the range of the relative hydrolytic/synthetic activities of lipases that have been observed, especially those that are considered useful in enzyme syntheses. However, given the variability of ester synthesis activity of different preparations of the esterase (see below), the relationship between hydrolytic and synthetic activities remains to be unequivocally established.

Ester synthesis selectivities were determined using multi-competitive assay mixtures containing either multiple acyl donors (of 4-10 *n*-acyl carbons) or alcohol acceptor substrates at 38°C in hexane as the continuous phase. Propanol and iso-propanol (and their acetate esters), ethanol and glycerol were evaluated as alcohol acceptor substrates. The esterase exhibited only limited activity with isopropanol, and isopropyl acetate as alcohol acceptors, indicating steric constraints on alcohol substrate recognition. Using glycerol as alcohol acceptor also resulted in little activity, indicating again that the enzyme is a true esterase, and not a lipase. For reactions with propanol and propyl acetate, 5- to 10-fold greater selectivity was observed for butanoic and hexanoic acids compared to longer chain length fatty acids. With ethanol as alcohol acceptor, fatty acid selectivity was diminished (although this may have been confounded by a general lack of activity in the esterase preparation used for this study), and 4-8 *n*-acyl carbon derivatives reacted at similar rates.

Using hexanoic acid as the acyl donor with multiple alcohol substrates indicated that the enzyme was most selective toward short chain length *n*-alcohols, (such as propanol, butanol, pentanol and hexanol), followed by benzyl alcohol, and then followed by isoamyl alcohol and *n*-octanol. Activity on phenyl, *sec*-butyl, 2-octyl, isobutyl, cyclohexyl and (-) menthyl alcohols was limited, again indicative of steric constraints on reactivity with alcohols.

The esterase was incapable of acyl transferase activity using acyl-CoA substrates (2-, 4- and 6- *n*-acyl carbon derivatives) and butanol as reactants, indicating the absence of alcohol acyltransferase-type activity, which is indigenous to tropical fruits and responsible for forming aromatic esters during ripening. The esterase was also incapable of thioester synthesis, using the reaction between butanoic and hexanoic acid and butanethiol as a model.

One dilemma that was encountered during the course of assessing ester synthesis capability of the esterase was the estimated 2-orders of magnitude difference in specific activity observed between different esterase preparations. We have not yet identified a feasible explanation to account for this anomaly. However, this anomaly may be responsible for the distinct patterns of selectivity observed in some cases (using propanol as acceptor for multiple fatty acid donors, and using multiple alcohol acceptors for hexanoic acid donor), and a lack of selectivity in others (using ethanol as alcohol acceptor for multiple fatty acid donors).

Combined results from this work indicate that esterase selectivity toward acyl constitutive groups is for short chain (2-8, and especially 4-6, acyl carbons) fatty acids. Selectivity toward the alcohol constitutive group is both for aromatic and short chain *n*-aliphatic alcohols (and some *iso*-derivatives), provided that no steric constraints are imposed by other functional groups of the alcohols.

The *Lb. helveticus* CNRZ32 esterase possesses definitive selectivities toward both alcohol and acyl constitutive groups in hydrolytic and synthetic mode. The esterase showed a sharp selectivity toward *n*-fatty acids of less than 10 carbons, and this trait could be useful for "biochemically fractionating" fatty acids derived from milkfat by using the enzyme in synthetic mode. The nature of esterase activity may also bear relevance to aging-related flavors in fermented dairy products, such as cheese, because of the ability to hydrolyze/synthesize aromatic esters that may possess flavor characteristics. The constitutive groups for these esters may be derived from lipid hydrolysis and amino acid catabolism during aging. Thus, the presence of this esterase in fermented dairy products may modulate the development of flavor and this may be useful tool from the standpoint of trying to understand some features of the molecular basis for flavor development in products such as cheese.

*FINAL REPORT*

# Studies of the influence of milkfat on the formation of flavor compounds in Cheddar cheese

**Personnel**

Robert C. Lindsay, professor, Dept. of Food Science; Norman F. Olson, professor, Dept. of Food Science; David Bogenrief, associate researcher, CDR; Qiaoling Zeng, graduate research assistant, Dept. of Food Science

**Funding**

Wisconsin Milk Marketing Board 93-8, Kraft-General Foods

**Dates**

October 1993 -December 1996

## Objectives

To investigate the basic physical and chemical influences of milkfat on the development of flavor compounds in Cheddar-type cheeses.

To use the information to devise strategies for manufacturing low fat Cheddar cheeses with flavors similar to traditional full fat cheese.

## Summary

In order to simulate more appropriately the conditions encountered in industry for the manufacture of Cheddar cheese, pilot-sized, mechanically-stirred cheese vats were installed and used throughout the experiments. Trials composed of the following lots of cheese were made: full fat (32 % fat); 25% reduced fat (25% fat); 33% reduced fat (18% fat); 50% reduced fat (12% fat); 75% reduced fat (3% fat) no fat skimmilk (defined as "0%" fat, but 1.2-2.5% fat).

Cheeses were manufactured with several culturing systems, established with industry collaboration to identify desirable traits. The cheeses in the trials were evaluated for flavor by the project team (both UW and Kraft) according to the following schedule based on achieved age of the cheese: 1-2, 3, 6, 9, and 12 months. When the cheese samples progressed to a point that the characteristics were beyond commercial or scientific value they were removed from the experimental design.

Flavor compounds in cheeses from the trials were analyzed according to the age-based sampling scheme, and included, where appropriate, the following analyses: $\alpha$-Dicarbonyls (glyoxal, methylglyoxal, diacetyl) by HPLC; neutral and sulfur headspace volatiles by GC; higher boiling flavor compounds by acetonitrile extraction; free fatty acids by alumina adsorption and GC; free amino acids and selected non-volatile compounds by HPLC.

A major advance in the understanding of cheese flavors was made by defining an essential contribution of volatile fatty acids to Cheddar cheese flavor. Milkfat was identified as the primary source of essential volatile fatty acids rather than microbial biosynthesis. Data were developed which showed that cell-associated lipases of starter cultures were responsible for the hydrolytic release of volatile fatty acids. It was established that butyric acid concentrations provided a means to index cheesiness flavor intensities in Cheddar cheeses.

Results revealed that the fat content greatly influenced the level of free fatty acids in cheese. Cheeses containing less fat than that obtained at a 33% reduction do not develop adequate levels of butyric acid and other free fatty acids to provide cheesy flavors. Thus, cheesiness was absent from reduced fat cheeses (>50%) because of insufficient formation of volatile fatty acids. Therefore, the challenge moves from defining the causes of a lack of cheesiness flavor to that of developing strategies to elevate the concentrations of volatile free fatty acids in substantially reduced-fat cheese.

Adding free lipase yielded excessive levels of free fatty acids. However, the key discovery—that cell-associated lipases of microbes in normal ripened cheese provide the required level of free fatty acids—opened the way to very viable strategies for reinstating volatile fatty acids in reduced-fat cheeses. Successful reinstatement of volatile fatty acids in reduced-fat cheese was accomplished to various degrees using a specially selected adjunct culturing organism, *Lactobacillus casei* Lila strain. This lactobacillus consistently elevated volatile fatty acid concentrations in reduced-fat Cheddar cheese previously characterized by a lack of cheesiness before altering the make strategy.

An improved acetonitrile extraction method for analyzing high boiling flavor compound in cheese was developed, and studies revealed that several postulated amino acid metabolites are present in the aging cheeses. Included are p-hydroxybenzoic acid, phenyllactic acid, p-hydroxyphenyllactic acid, and methyl p-hydroxyphenyllactic acid. These compounds are influenced by the conditions in full and reduced fat cheeses, and although the direct impact of compounds on desirable Cheddar flavor was not clear, they may exert a positive influence. More importantly, further information regarding their contibution to unclean type flavors in cheeses was developed.

2-Phenethylamine was shown for the first time to be an important flavor compound in some aged Cheddar cheeses where it provided distinctive rosy floral notes. However, 2-phenethylamine was not found to be essential for aged Cheddar flavor. When present in concentrations exceeding 100 ppm in Cheddar cheese, it caused lingering rosy flavors, and as a cautionary note, such levels could produce harmful biogenic amine side-effects on sensitive individuals.

Succinic acid produced in aged Cheddar cheese was found to provide a desirable complementary savory flavor to cheesy flavors, and although widely distributed in Cheddar cheese, pronounced flavor effects were not encountered due to concentrations. Because selection of starter cultures has not taken into account the potential importance of succinic acid production, modern Cheddar cheese does not appear to develop comparable levels found in cheeses produced at earlier times in history.

Some important flavor issues related to low fat Cheddar cheese flavor quality remain, and the sickening sweet, umami, meaty/brothy flavor is one which presents a major obstacle to wide acceptance of such cheeses. Cheeses that have 75% or greater fat reduction build up much greater levels of methylglyoxal than those containing higher levels of milkfat. Additionally, the levels of methylglyoxal and other dicarbonyls decrease during the later stages of ripening, and might be associated with the development of brothy, umami, and protein-like flavor defects. The chemical basis of the brothy, umami flavor remains to be discovered.

One of the major limitations in expanding markets for low fat Cheddar cheese is the development of off-flavors and the lack of development of typical desirable cheese flavors. Removing milkfat and selecting cultures by trial and error has produced only partially acceptable reduced fat cheeses. This research has unraveled the complex associations between free fatty acid essential flavor contributions and cheese flavor development. This discovery will result in greatly improved flavors in low fat cheeses through the use of appropriate adjunct culturing techniques. Such cheeses will greatly increase the demand for low fat cheeses and will allow the dairy industry to provide desirable contemporary cheeses that the consumer wants. The findings relative to unclean flavor development and savory flavor contributions provided by phenethylamine and succinic acid, respectively, also present pathways for strategies to use adjunct culturing to greatly enhance the flavor acceptability of low fat Cheddar cheeses. Thus, the discoveries accomplished in this project should have immediate impact on improving low-fat cheese flavors as well as providing basic knowledge to enhance reaching the ultimate goal of producing very consumer-acceptable low fat Cheddar cheese.

*FINAL REPORT*

# Physical chemistry of lipid mixtures: dairy based spreads

**Personnel**

R.W Hartel, professor, Dept of Food Science; RL Lindsay, professor, Dept of Food Science; B. Liang, research associate, Dept of Food Science

**Dates**

September 1994 - August 1996

**Funding**

Dairy Management Inc. HRL 95

## Objectives

To relate the specific triglyceride composition of milkfat fractions to phase and crystallization behavior of milkfat fractions with each other.

To relate the specific triglyceride composition of milkfat fractions to phase and crystallization behavior of mixtures of milkfat fractions and canola oil.

## Summary

Phase Behavior
A technique was developed for studying the solubility of hard milkfat fractions in low-melting fractions (liquid oils). This technique relies on measuring change in turbidity (absorbance) with increasing levels of addition of hard fraction (HMF) in the liquid fraction (LMF). In this technique, the liquid oil was maintained at constant temperature (25 - 40°C) in a jacketed beaker and a specific amount of hard fraction added as a liquid fat. The system was allowed to equilibrate for 5 days before measuring absorbance. Using this technique, a clear solution was obtained with essentially no change in absorbance until the point where the hard fraction crystallized separately from the liquid fraction. At this point, the absorbance increased dramatically with only slightly higher addition level of the hard fraction. The point of increased absorbance provides a measure of the intersolubility of one lipid in another. While this is not a true thermodynamic solubility, the intersolubility measured in this way provides an estimate of how much hard fraction can be accommodated in the liquid fraction before crystallization occurs.

A similar technique based on turbidity measurements was used to quantify a metastable zone width for mixtures of HMF with either LMF or canola oil. In this case, the two fats were mixed in the liquid form with known concentrations and held at the desired temperature under agitation for 7 days before measuring the turbidity of the mixture. In this case, the metastability limit of HMF in liquid fat was determined as the concentration where crystal formation was just observed.

Figure 1 shows the solubility of HMF (28S, melting point of 45°C) in either LMF (16L, melting point of 18°C) or canola oil. At low temperatures, the solubility of HMF in the liquid oils is quite low, but this increases dramatically as temperature increases. The solubility of HMF is higher in LMF than in canola oil, as might be expected from the closer similarity of triglyceride structure between the milkfat fractions.

In order to relate this solubility to the triglyceride (TAG) composition, the ratio of TAG in the two peaks of the acyl carbon number profile typical for milkfat was used. That is, the ratio of composition of TAG in the range of C46 to C52 to composition of TAG in the range of C36-C42 was calculated and the solubility curves replotted in terms of this ratio. Note that the supersaturation driving force for fats with different composition can be obtained from such a diagram. This allows prediction of crystallization kinetics based on the equilibrium driving

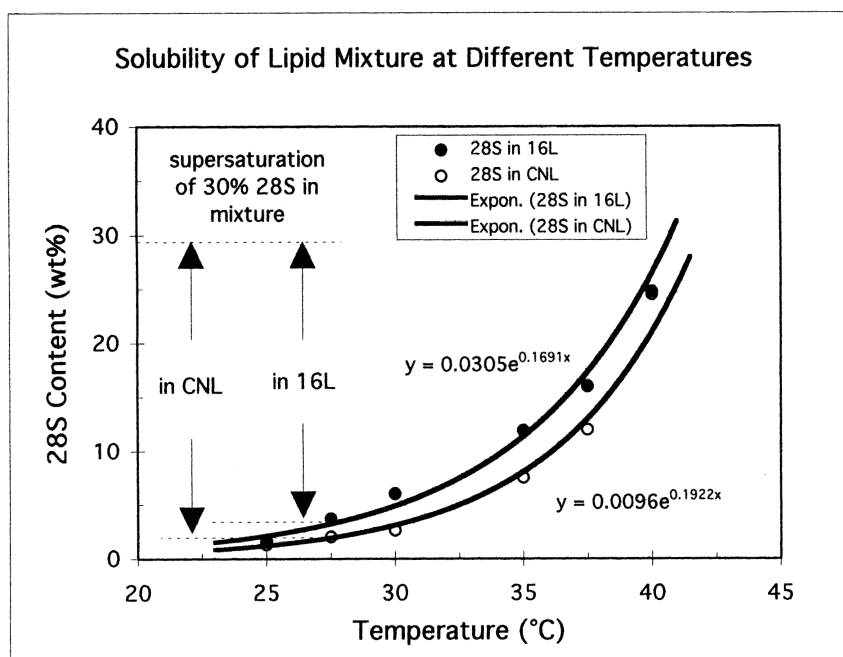


**Figure 1**

force, although the range of applicability of this approach is limited to temperatures above where massive crystallization occurs. However, this approach has merit for predicting crystallization kinetics during fractionation of milkfat.

The phase behavior of mixed lipid systems can be seen in isosolids diagrams, where solid fat content (SFC) is measured at different temperatures for the range of mixtures from 100% hard fraction to 100% liquid oil (either low-melting fraction or canola oil). These diagrams provide information about the compatibility of two lipids. Using a Bruker Minispec PC-120 NMR, the solid fat curves for mixtures of several hard and soft milkfat fractions were measured. In general, mixtures of HMF with either canola oil or LMF resulted in straight lines on the isosolids diagram, indicating complete compatibility between these fats. The shapes of these isosolid diagrams was somewhat dependent on the nature of the milkfat fractions used. As expected, milkfat fractions with higher melting point gave higher SFC in mixtures with any LMF or canola oil.

Differential scanning calorimetry (DSC) thermograms of mixtures of HMF with either LMF or canola oil further supported this compatibility, where the melting peaks associated with each component changed uniformly with increasing addition of one fraction to the other.

## Crystallization Behavior

Several techniques were evaluated for measuring crystallization kinetics of mixtures of HMF with either LMF or canola oil. Isothermal (from 10 to 35°C) crystallization studies were performed using both the DSC (in isothermal mode) and a turbidity technique. In the DSC, the fat sample was cooled quickly to crystallization temperature and held until a crystallization exotherm was observed. Mixtures of two HMF and three liquid fats (two LMF and canola oil) were studied with addition levels of the HMF at 30, 50 and 70% (w/w). In the spectrophotometer, the onset of crystallization was measured by the increase in turbidity. From these experiments, the induction time for onset of crystallization and the approximate rate of crystallization (based on the slope of the curve) were obtained. Some batch suspension crystallizer experiments were also carried out, with the change in crystalline mass with time used as a measure of crystallization rate. In these experiments, the lipid was agitated rapidly for a brief time and the crystals formed were then allowed to grow under static conditions.

The induction time for onset of crystallization increased dramatically as crystallization temperature increased for all mixtures. The induction time decreased as the concentration of HMF was increased. At the same temperature, HMF crystallized more rapidly out of canola oil than either LMF studied. Based on the difference in solubility of HMF in either LMF and canola oil (Figure 1), this can be explained by the difference in supersaturation of the high-melting TAG in the liquid oil. The supersaturation was higher in canola oil since the solubility concentration was lower. The nucleation rates of HMF (28S) were higher in canola oil (CNL) than LMF (16L) at either temperature. In all cases, nucleation rates were higher at 25°C than at 27.5°C despite comparing data at equivalent supersaturations.

The relative rates of crystallization, based on the slope of the turbidity data, for the different fat mixtures are shown in Figures 2 and 3. A certain minimum super-

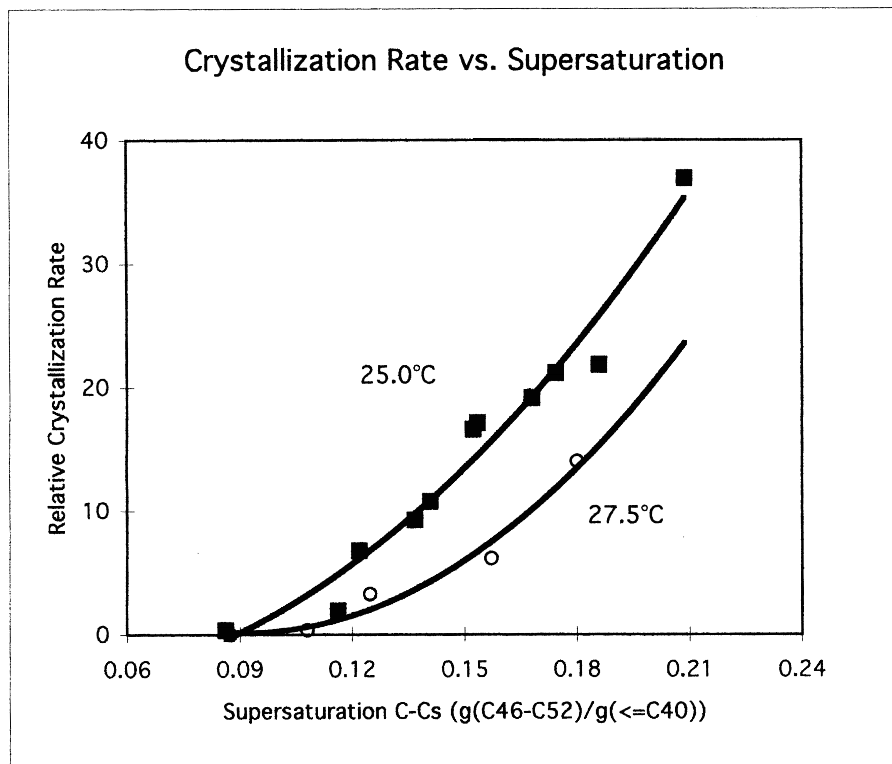


**Figure 2**

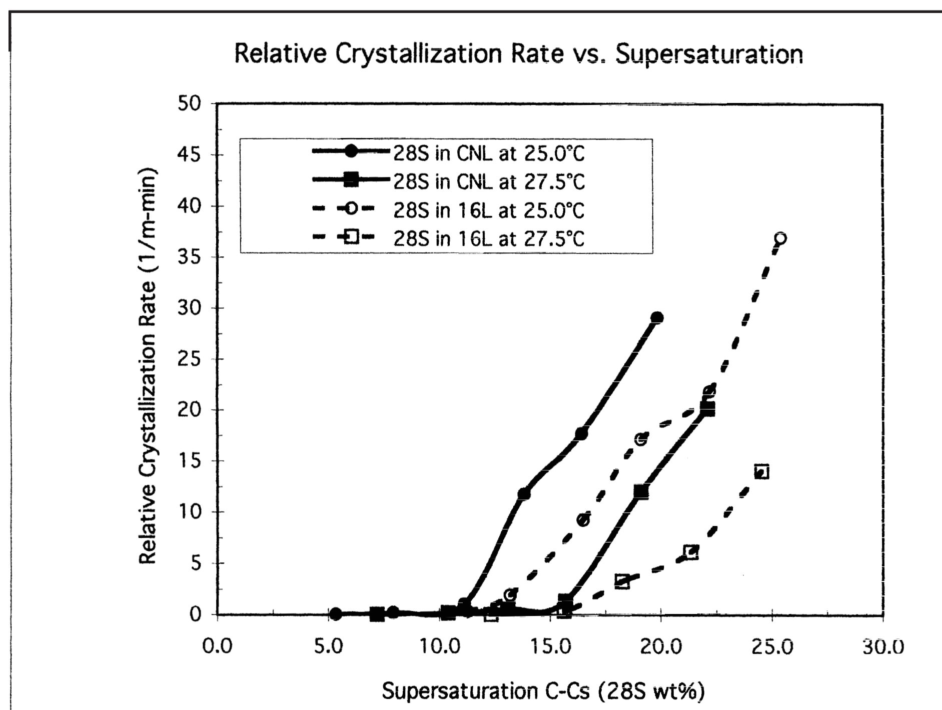


**Figure 3**

saturation was required in all cases before crystallization occurred. Figure 2 shows how crystallization changes with supersaturation expressed in weight % of the HMF (28S) for mixtures of HMF and either LMF (16L) or canola oil, whereas Figure 3 shows crystallization rate in terms of supersaturation based on TAG composition. In addition, the nature of the HMF affected the rate of crystallization. The HMF with higher melting point (28S) crystallized more rapidly than a fraction with lower melting point (20S). Again, this may be explained by the relative composition of the higher molecular weight TAG present in the HMF with higher melting point.

Note that X-ray diffraction analysis of the crystallized samples showed that b′crystals were formed under all conditions. However, there were cases when other forms also crystallized. At higher concentrations of HMF, the appearance of a crystals was sometimes observed, and crystallization of HMF from canola oil at lower temperatures (25°C) sometimes produced b crystals.

Increased utilization of milkfat and milkfat fractions in dairy-based spreads requires an understanding of how chemical composition influences physical behavior. In this study, the focus was understanding the effects of chemical composition on phase and crystallization behavior. The results of this study will lead to improved fractionation processes and applications of milkfat fractions in dairy-based spreads.

## Publications

Liang, B., R.W. Hartel and Y. Shi, Crystallization and Phase Behavior of Lipid Mixture Containing Milkfat Fractions and Canola Oil, paper presented at Annual AOCS Meeting, Seattle, WA (1997).

Liang, B., Y. Shi and R.W. Hartel, Solubility and Metastability of Hard Milkfat Fraction in Soft Milkfat Fraction and Canola Oil, poster presented at Annual AOCS Meeting, Seattle, WA (1997).

*INTERIM REPORT*

# Interactions of milkfat and milkfat fractions with confectionery fats

**Personnel**

R.W. Hartel, professor, Dept of Food Science, Julia Schmelzer, Dept of Food Science, Russell Tietz, Dept of Food Science

**Dates**

June 1997 - June 1999

**Funding**

Dairy Management Inc. BA14

## Objectives

Determine compositional differences in triglyceride and other lipid components between milkfats and milkfat fractions.

Understand crystallization kinetics and phase behavior of triglycerides and other lipid components in lipid mixtures.

Evaluate the effects of triglycerides and other lipids on the physical properties of confectionery fats.

## Summary

A winter milkfat and five fractions obtained from it were supplied by Grassland Dairy (Greenwood, WI) for analysis. The five fractions had melting points of 47.4, 42.2, 24.4, 28.4 and 18.5°C. The milkfat and milkfat fractions are currently being analyzed for chemical composition (triglyceride, fatty acid and minor lipids) and physical properties (melting profile, solid fat curve, melting point, etc.). Minor lipids are being analyzed using a column chromatography technique. Florisil was used as adsorbent for removal of minor lipids. The milkfat sample was dissolved in hexane, placed onto the Florisil and eluted with specific solvents. A solvent gradient was used, starting with pure hexane, then 2.5% ethyl ether in hexane with concentration steadily increasing to 50% ethyl ether in hexane. Polarity of the solvent was then increased by addition of 2% methanol in ethyl ether, then 4% acetic acid in ethyl ether and final cleaning with methanol. Both TLC and HPLC are being used to confirm the efficiency of separation and quantify differences in minor lipids between milkfat and the fractions.

Currently, our work is focusing on analysis of the physical properties of the milkfat and fractions. Analysis of triglyceride composition and fatty acid profile by GC documented the expected differences amongst milkfat fractions. Higher levels of long-chain, saturated fatty acids are present in the higher melting fractions, whereas the lower melting fractions contain higher levels of short-chain and unsaturated fatty acids. The two middle-melting fractions have some interesting differences in solid fat content and melting profiles due to the different fractionation procedures used to obtain them. One was the liquid fraction of a higher temperature fractionation step, whereas the other was the solid fraction of a lower temperature fractionation. Analysis of minor lipids for these components is currently underway.

Once analysis is completed, crystallization and phase behavior experiments will begin. Mixtures of these milkfat fractions, with and without the minor lipids, with cocoa butter and palm kernel oil will be studied. Crystallization rate is being studied by an absorption/turbidity technique where the cuvette for the spectrophotometer is held at constant temperature under agitated conditions. Preliminary results show that milkfat purified by column chromatography to remove minor lipids has faster crystallization rate than the original milkfat. The

nontriglyceride components serve to inhibit crystallization of milkfat. Once crystallization rates for the lipid have been determined, chocolates and coatings made with addition of these milkfat fractions will be evaluated.

Milkfat or milkfat fractions used in chocolate or coatings affect product texture and storage stability. However, the mechanisms of these effects are still not understood. Previous results have demonstrated significant differences between winter and summer milkfat in terms of bloom promotion in compound coatings even though their triglyceride and fatty acid distributions were not that different. We hypothesize that slight changes in composition (i.e., minor lipids) may have substantial influence on crystallization rates and thereby alter the crystalline microstructure in a product. This influences product quality, texture and shelf stability.

*INTERIM REPORT*

# Interactions of milkfat fractions in foods– Ice cream

**Personnel**

RW Hartel, professor, RC Bradley, Jr., professor, Rachel Adleman, Dept of Food Science

**Dates**

June 1997 - June 1999

**Funding**

Wisconsin Milk Marketing Board UW 9604

## Objectives

To understand the effects of changing composition of the fat phase in ice cream on emulsion characteristics and behavior.

Evaluate the potential for milkfat fractions to produce high quality, reduced fat ice creams.

## Summary

An experimental program to evaluate ice creams made with different milkfat fractions and emulsifiers is currently underway. Ice creams are being made with AMF, and three different fractions (low-melting, middle-melting and high-melting) obtained from Grassland Dairy (Greenwood, WI). The three emulsifiers are lecithin, mono- and diglycerides (MAG/DAG) and a commercial blend containing MAG/DAG with polysorbate 80. The development of overrun, air cell distribution and fat destabilization during freezing are being studied. Hardness and melt-down of each ice cream will also be measured. These results will be correlated to the interfacial tension measured for each fat-emulsifier blend.

We are measuring fat destabilization using the standard turbidity technique, but are also investigating the use of a laser particle size analyzer for this measurement. Some preliminary results suggest that we can correlate these two measurements during ice cream manufacture. An automated image analysis procedure has been developed to measure air bubble size in ice cream.

Some preliminary results of ice cream characteristics are available. Ice cream made with LMF had the highest level of fat destabilization, lowest overrun and were significantly softer, whereas ice cream made with HMF had the lowest level of fat destabilization, slightly higher overrun and were significantly harder.

The rate and level of fat crystallization during aging of ice cream mix has been studied. As expected, ice creams made with HMF crystallized more rapidly and had higher solid fat content (SFC) at aging temperature (4°C). As melting point of the AMF or milkfat fraction decreased, the rate of solidification and final SFC decreased.

The potential for application of milkfat fractions in ice cream has not been extensively evaluated. In this project, the interactions between lipid phase and emulsifier are being studied. This information will provide the background for optimizing emulsifier functionality in ice cream and other frozen desserts. Potential applications include development of ice creams with added functionality, such as a reduced-fat ice cream with similar physical characteristics as a full-fat product.

*FINAL REPORT*

# Incorporation of milkfat fractions in chocolates — Phase 2

**Personnel**

R. W. Hartel, professor, J. Bricknell, graduate research assistant, S. Metin, graduate research assistant, Dept. of Food Science

**Dates**

September 1994 - August 1996

**Funding**

Wisconsin Milk Marketing Board 94-02

## Objectives

To further study the fundamental aspects of incorporation of milkfat and milkfat fractions into chocolates.

To investigate the incorporation of milkfat fractions produced by the Tirtiaux pilot plant in both milk and dark chocolates.

To study processing techniques for improving the compatibility of milkfat fractions and cocoa butter in chocolates.

## Summary

It has long been known that milkfat and milkfat fractions, particularly the high-melting components, inhibit bloom in chocolates. Our past work has documented the kinetics of bloom formation under a variety of conditions and shown that significant bloom inhibition can be obtained by addition of certain components of milkfat. However, we don't know the mechanism of this bloom inhibition. It has been hypothesized that the bloom inhibition of milkfat is caused by inhibition of the polymorphic transition of cocoa butter to the most stable form. This polymorphic transition is thought to cause bloom in chocolate. However, no experimental evidence is available relating the effect of milkfat on polymorphic transitions in chocolate, and how this might influence bloom. The combined objectives 1 and 2 were designed to further our understanding of this complex phenomena.

In order to study the effects of milkfat fractions on bloom formation in chocolate, experimental chocolates were made with amorphous sugar. In regular chocolate, the presence of crystalline sugar interferes with the X-ray analysis of cocoa butter crystals and thus, one can not use X-ray to follow cocoa butter polymorphism in real chocolate. Our approach was to formulate a chocolate with amorphous sugar replacing the crystalline sucrose and then follow cocoa butter polymorphism during bloom formation.

A mixture of sucrose and 20 DE corn syrup was spray dried to make a powder. This powder was screened to provide a powder with particle size ranging from 44 to 88 μm. Normal chocolate has particle size in the range from 10 to 80 μm so this is a reasonable target. Defatted cocoa powder was also screened in the same way to generate a fine powder of cocoa solids. These powders were mixed and blended with the appropriate fats. Two types of cocoa butters were used—a hard butter (most likely from Malaysia) and a medium-melting butter (from Ivory Coast). To these cocoa butters, 2% of either milkfat (AMF) or milkfat fractions (high, middle and low-melting) from the Tirtiaux pilot plant was added. Total fat content was set to 33% in these chocolates and lecithin added at 0.3%. The milkfat fractions had the following dropping points (based on Mettler Drop Point analysis): AMF - 31.5°C; high-melting fraction (25S) - 45.0°C; middle-melting fraction (25L) - 26.0°C; low-melting fraction (16.4L) - 18.0°C. The high and middle-melting cocoa butters has drop points of 29.0 and 26.9°C, respectively.

Chocolates were tempered using a cyclo-thermic process, poured into molds and allowed to set for 24 hours at 13.5°C. The degree of temper was verified using a temper meter available in the lab. This temper meter documented that all chocolates had approximately the same degree of temper. Molded discs of each chocolate were placed in a cycling chamber, where temperature was cycled every 6 hours from 19 to 29°C. These temperatures were chosen to promote bloom formation in 3 to 4 weeks in the control chocolates. Samples were analyzed periodically for change in whiteness index (WI) as measured by Hunter Colorimeter. Samples were also analyzed every two days for the ratio of the two stable polymorphs of cocoa butter. A standard curve representing mixtures of these two polymorphs (called b V and b VI) was generated based on pure cocoa butter. The ratio of these two polymorphs in each chocolate was estimated (within 10 to 20%) by comparison of the entire X-ray spectrum with the standard curve. Attempts were made to take electron micrographs of the surfaces of these chocolates during onset of bloom formation. However, the lack of a cold-stage SEM prevented us from obtaining usable images of the surface.

Analysis of polymorphic changes in cocoa butter during storage of chocolates was extremely successful. This was the first documented study that demonstrated these changes directly in chocolates. However, our results came out slightly different than anticipated, leading us to develop new ideas about the mechanisms of bloom formation and the action of milkfat as a bloom inhibitor.

The results for chocolates made with the Ivory Coast cocoa butter are shown, although results were similar for chocolates made with the hard cocoa butter. X-ray analysis showed that the polymorphic transition from the b V form of cocoa butter (expected for well-tempered chocolate) to the most stable b VI form occurred as expected during storage. The cocoa butter control (MMCB) had the fastest conversion to b VI although the chocolates made with 2% replacement of either AMF (MMAMF), middle- (MM25L) or low-melting (MM164) milkfat fractions were not significantly different from the control. The high-melting milkfat fraction (MM25S) did indeed delay the onset of the polymorphic transition, as expected based on literature conjecture. The formation of visual bloom in these chocolates, as measured by whiteness index, did not directly follow the trend of the polymorphic changes. As the whiteness index increases, the surfaces of the chocolates become whiter and the extent of visual bloom increases. As seen in Figure 2, the control chocolate (MMCB) made with only cocoa butter did not bloom very rapidly. In fact, the control chocolate had about the same level of bloom formation as the chocolate made with 2% addition of the high-melting milkfat fraction (MM25S). Our past results document that the high-melting milkfat fraction should have substantially delayed visual bloom formation compared to the control. One can also see that the AMF and low-melting milkfat fraction did not inhibit visual bloom at all. The middle-melting milkfat fraction fell somewhere between. Thus, the control chocolate made with only cocoa butter did not follow the expected trends, and certainly did not track with the evidence for polymorphic transition.

In order to explain this result, we made another set of chocolates in the same manner, but with ground crystalline sucrose instead of the amorphous powder. Our goal was to ascertain if it was the technique of making chocolate or the structure of the sugar surface that was responsible for the contradictory results. This chocolate was only analyzed for changes in whiteness index since it could not be evaluated by X-ray spectroscopy. The control chocolate bloomed most rapidly, the addition of AMF provided some inhibition of bloom and the high-melting milkfat fraction provided substantial bloom protection. These results are consistent with previous studies. Thus, we conclude that the structure of the surface of the sugar particles plays an important role in the rate of visual bloom formation and that the polymorphic transition of cocoa butter is not the only mechanism of bloom formation. One can speculate that the interaction between the lipid, emulsifier and sugar particle surface plays an important role in the mechanism of bloom formation in chocolate. Further work will be necessary to clarify this complex issue.

It is also widely known in the confectionery industry that processing conditions for tempering need to be modified when adding milkfat to chocolate. Typically, addition of milkfat requires tempering for longer times at lower temperatures. However, very little quantitative data showing the extent of inhibition of cocoa butter crystallization by milkfat is available in the literature, and the effects of milkfat fractions have not been studied at all. In this study, we evaluated the effects of milkfat and milkfat fractions on the crystallization kinetics of cocoa butter to aid in future development of

processing techniques for chocolates made with milkfat fraction incorporation.

Crystallization kinetics of cocoa butter were studied using two calorimetric techniques. In the first, an isothermal technique was employed, where the fat sample was quickly brought down to crystallization temperature and enthalpy changes associated with crystallization were measured. In addition, a scanning technique was utilized where the melted fat was cooled slowly in a Differential Scanning Calorimeter (DSC) and enthalpy changes measured as crystallization occurred. These techniques give different information about the impact of milkfat and milkfat fractions on crystallization kinetics of cocoa butter. The enthalpy curves from both scanning and isothermal calorimetry were analyzed according to the Avrami equation. This model is often used to describe solidification events, and the parameters obtained related back to potential mechanisms of solidification (nucleation and growth). Note that these experiments were all carried out under static conditions, although commercial tempering units involve substantial agitation. Thus, these results must be used carefully when designing tempering systems. Nevertheless, the relative effects of different milkfat fractions can easily be seen using the techniques in this study.

To supplement the calorimetry data, fat crystals were analyzed by X-ray spectroscopy for polymorphic form. In addition, mixtures of different milkfat fractions and cocoa butters were studied using Nuclear Magnetic Resonance (NMR). Fat samples were tempered to ensure complete solidification, and the solid fat content (SFC) obtained at different temperatures using the NMR. SFC curves for different mixtures of fats were analyzed to yield iso-solids diagrams. These diagrams show phase compatibility between fats and are indicative of crystallization problems when two fats are mixed together.

Milkfat fractions were obtained by a lab-scale, isothermal fractionation technique. Molten AMF was cooled rapidly to crystallization temperature and allowed to stabilize for 24 hours. The resulting crystal slurry was vacuum filtered to yield solid and liquid fractions at a given temperature. Sequential fractionation of the liquid fractions at lower temperatures was done to generate a set of milkfat fractions with melting points ranging from 12 to 49°C. These fractions were added to cocoa butter at 5 or 10% addition levels for crystallization studies.

The incompatibility of milkfat and cocoa butter at addition levels above 30% was clearly shown in the iso-solids diagrams. The SFC at a given temperature for mixtures of AMF and cocoa butter decreased dramatically as the addition level of AMF to cocoa butter increased above 30%. High-melting milkfat fractions were much more compatible with cocoa butter, as expected based on the greater concentration of trisaturated triglycerides. In fact, at some levels, high-melting fractions caused a slight increase in SFC of the mixture as co-crystallization was mutually enhanced. However, SFC at lower temperatures was generally decreased as the more liquid-like triglycerides present even in this milkfat fraction were incompatible with cocoa butter. Middle-melting milkfat fractions behaved in much the same way as the original AMF, giving a eutectic incompatibility at levels of 30% and higher. Low-melting milkfat fractions initially caused a dilutional softening of cocoa butter at low levels of addition, but then exhibited a moderate eutectic incompatibility at levels above 60%. These results suggest that the differences in triglyceride composition between milkfat fractions and cocoa butter cause problems with co-crystallization between these two fats. These results express the incompatibility of the most fully crystallized state of the fat mixtures. The remaining studies describe the incompatibilities caused by milkfat as cocoa butter crystallizes.

When considering the induction times, based on isothermal calorimetry, for onset of crystal formation in the pure fats (cocoa butter, AMF and milkfat fractions) at several temperatures we noted that AMF and all milkfat fractions crystallize more rapidly than cocoa butter at all temperatures. Interestingly, all of the components of milkfat crystallize rapidly at the temperatures studied. On the other hand, cocoa butter crystallizes quite slowly at 20°C, as compared to crystallization at 15°C. Cocoa butter did not crystallize in the three hour duration of these experiments at 25°C. Cocoa butter crystallizes more slowly than milkfat and exhibits a strong temperature dependence which is probably associated with the higher degree of polymorphism in cocoa butter.

When 5 and 10% of AMF or milkfat fractions were added to cocoa butter, the induction times for cocoa butter crystallization generally increased. Increases in induction time for 5% addition of milkfat factions were relatively small, although 10% addition caused significant delays in crystallization. Only minor, and somewhat inconsistent, differences were observed among the

different milkfat fractions. Nevertheless, it is clear that addition of 10% milkfat or milkfat fractions to chocolates caused a significant delay in crystallization of cocoa butter. On a chocolate weight basis, this would be about a 3% replacement of cocoa butter by milkfat fraction, well within the range allowed by the Standard of Identity. We found that 10% addition of the 23S fraction caused nearly a 5-fold increase in induction time. This is a substantial decrease in crystallization kinetics and tempering processes must be adjusted accordingly. However, it is somewhat surprising that there were no obvious trends in inhibition of cocoa butter crystallization according to the nature of the milkfat fraction utilized. One might expect that the differences in triglyceride composition between fractions would cause significantly different effects on rate of cocoa butter crystallization, especially since the iso-solids diagrams indicate some differences in the final amount crystallized. Apparently, these composition differences are not sufficient to cause differences in cocoa butter crystallization kinetics. Thus, one would expect that tempering of chocolates made with any of the milkfat fractions should be nearly similar to tempering conditions for the same amount of AMF.

The isothermal crystallization curves were analyzed according to the Avrami equation. The Avrami parameter, n, is often used to suggest potential mechanisms for crystallization. For cocoa butter itself, an n-value of about 4 was found, as suggested by previous research on lipid crystallization. Based on the value of 4, people have suggested that cocoa butter crystals form via a heterogeneous nucleation mechanism that occurs throughout the duration of the experiment and grow as spherulitic crystals. For pure AMF, the Avrami exponent decreased to about 3. This suggests that nucleation occurred only during the early stages of crystallization and that these nuclei grew as spherulites. Milkfat fractions had even lower Avrami exponents, with values varying from a low of 1.6 (for the highest melting fraction) to a high of 3.3 . In general, the Avrami exponent increased as the melting point of the fat decreased. These values suggest that the mechanisms of crystal formation and growth was different for the different fats used in this study.

Addition of 5 or 10% AMF or milkfat fractions to cocoa butter also caused changes in the Avrami exponent for crystallization of the mixture. However, addition of AMF or milkfat fractions to cocoa butter caused the Avrami exponent to increase, despite the lower values for n found for the individual fats. Avrami exponents increased to between 4.2 and 6.5, as compared to about 4 for pure cocoa butter, when milkfat fractions were added. In addition, the half-time for crystallization of the blends increased significantly as compared to the half-time for crystallization of pure cocoa butter. These results again document and quantify the inhibition of cocoa butter crystallization upon addition of AMF or milkfat fractions.

Scanning calorimetry was also used to demonstrate and quantify the inhibition of cocoa butter crystallization by milkfat fractions. The temperature at which crystallization began was dependent on the cooling rate and the type of fat being studied. In general, higher-melting milkfat fractions actually caused an increase in the temperature at which crystallization began. This is most likely due to phase separation of these high-melting triglycerides from the fat matrix as they crystallized separately. Lower-melting milkfat fractions had little effect on crystallization temperature. A modified Avrami analysis (Ozawa equation) was applied to these scanning results. However,the results were significantly different from those obtained from analysis of the isothermal study. This was most likely due to the polymorphic effects associated with crystallization during cooling. Our analysis strongly suggested that the analysis of scanning crystallization data was not satisfactory as assumptions of the equation were generally not fulfilled.

In summary, these results document for the first time the quantitative effects of milkfat fractions on crystallization behavior of cocoa butter. The results are necessary for optimal design of commercial tempering units for chocolates made with added milkfat fractions.

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presented at AOCS Annual Meeting, San Antonio, TX (1995).

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*FINAL REPORT*

# Characterization of creaming and aeration functionalities of milkfat fractions for cakes, cookies, and butter cream icings

**Personnel**

Robert C. Lindsay, professor, Dept. of Food Science; Kerry Kaylegian, associate Researcher, CDR; Johanna Setiabudhi, graduate research assistant, Dept. of Food Science

**Dates**

July 1993 - June 1996 (Extended December 1996)

**Funding**

Dairy Management Inc.
LS394

## Objectives

To devise means to overcome limitations of poor creaming and aeration properties in milkfat in cakes, cookies, and icings to expand opportunities for using milkfat and milkfat fractions in the bakery industry.

To develop fundamental data on physico-chemical properties of milkfat that govern creaming and aeration by investigating interactions that occur between milkfat and other constituents in cake batters, cookie doughs, and butter cream icings.

To validate the fundamental data for improved creaming and aeration functionalities for selected milkfat ingredients by testing them in selected model cake, cookie, and icing formulas.

## Summary

Cake volume is governed by the creaming and aeration properties of shortenings or fats in cake batters. Creaming results from fat particles incorporating and stabilizing air bubbles in cake batter, and the entrapped air subsequently provides a means for expansion of cake volumes during baking. Generally higher cake volumes are considered desirable, providing other structural features of the cake remain within acceptable parameters. The type of shortening employed in the preparation of cake batters greatly affects the creaming functionality, and specialty vegetable shortenings manufactured specifically for this application provide superior functionality to intact butter.

Measurement of the specific gravity of cake batters provides an index of the degree of aeration achieved in batters, and measurement of the volume of finished baked cakes provides the means to assess the final functionality of the shortening in relation to air retention. Butter pound cakes were chosen as the model for studies on cakes, and initial studies documented the performance of anhydrous milkfat and butter compared to commercial vegetable shortenings. In these studies, it was found that the vegetable shortenings performed superior to butter or anhydrous milkfat as is generally recognized in the bakery industry.

Investigations of the effects of mixing temperatures for cake batters upon the creaming revealed that relative creaming functionality or efficiency of milkfat and butter compared to shortenings improved as the temperature was decreased from 29°C to 13°C. The solid fat content profiles of the fats revealed that the vegetable shortening contained a low, but wide profile which resulted in a wide plastic range. Butter and anhydrous milkfat exhibited solid fat contents that contained large (over 30%) amounts of solids at lower temperatures (ca 10°) which reduced plasticity of the mass, and inhibited the formation of small fat crystals that stabilize trapped air cells in cake batters.

Texturizing or physically reducing the size and increasing the numbers of milkfat crystals was found to greatly improve the creaming functionality of anhydrous milkfat. The principal effects were increasing cake volumes and decreasing the instrumentally-measured firmness of cakes. The effect was a more moist finished cake, and further research should be carried out on this aspect in future projects.

Several emulsifiers were evaluated for their ability to improve the creaming and aeration capabilities of anhydrous milkfat. The use of lecithin yielded only marginally acceptable cakes, and over-emulsification was frequently encountered which resulted in destabilization and collapse of the foam structure during baking. However, the incorporation of 1 percent of mono- and di-glycerides significantly improved cake volumes and revealed that this emulsifier system was highly compatible and functional in anhydrous milkfat. Several distilled monoglycerides were investigated for their functionality in pound cakes prepared with anhydrous milkfat, and it was found that the medium melting monoglycerides gave the greatest enhancement of cake volumes and improved crumb softness.

Studies with milkfat fractions revealed that texturized middle and higher melting fractions containing monoglycerides performed comparable to vegetable shortening in pound cakes. However, lower melting milkfat fractions performed inadequately in pound cakes because they lacked suitable solid fat crystals to stabilize air bubbles in batters. Further research is recommended with mixtures of milkfat fractions for cake applications to overcome aeration limitations in low melting fractions and lessened flavor intensities in higher melting milkfat fractions.

The performance of anhydrous milkfat and milkfat fractions in butter cookies was characterized by degree of spread of dough during baking, and a range of spread is desired for various cookie products. Fundamental characteristics of the solid fat content of milkfats governed the degree of spread, and milkfat fractions that possessed notable amounts of solid crystals between 23°-35°C gave a high degree of spread. Milkfats that lacked this property, including both low- and high-melting fractions, did not provide high degrees of spread. Thus, combinations of selected milkfat fractions will allow the development of ingredients that provide controlled spreading of cookies according to the desire of the baker.

Buttercreme icings prepared with milkfat fractions have been characterized for volume, stability and sensory characteristics. The data indicate that the higher melting fractions provide improved icings compared to intact butter and lower melting milkfat fractions.

Butter and anhydrous milkfat have been replaced by vegetable shortenings for many applications in the baking industry. Bakers often state that the poor performance of butter in bakery products compared to specialty vegetable shortenings offsets the benefits of the image and flavor of butter. The development of fundamental and applications data in this project will provide needed information for the newly initiated commercial milkfat fractionation industry in the U.S., and many of the bakery applications for butter and milkfat can be recaptured as well as expanding the use in new baking applications.

*APPLICATIONS PROGRAM REPORT*

# Milkfat applications research program


**Personnel**

Kerry E. Kaylegian, researcher, Barbara H. Ingham, associate researcher, Orville N. Harris, research specialist, Christopher Johnson, research specialist, Christiaan Kirk, research specialist, Center for Dairy Research



**Funding**

Wisconsin Milk Marketing Board UW A9701, Dairy Management Inc. UW 133-AM77


**Dates**

July 1996 - June 1997

## Objectives

Provide technical support on butter and milkfat fractions to the dairy, bakery, confectionery, and food industries

Coordinate the Milkfat Fractionation pilot plant program operation and activities, including the Milkfat Fractionation Consortium (MFC)

Conduct applied research in butter and milkfat fractions, and investigate potential new applications for milkfat fractions

Coordinate technical transfer activities in conjunction with CDR's Communication Team on UW research projects relating to milkfat

Implement an electronic database on milkfat fractionation technology and applications, and maintain database with up-to-date information

Technical training for staff in areas related to the manufacture, analysis, or use of milkfat and milkfat fractions, or other topics related to job duties

## Summary


The Milkfat Application Program at CDR aims to be a world-class program supporting all aspects of milkfat fractionation technology and applications. We provide samples, access to equipment, product analysis, applications evaluation, and general technical support to the food industry and research community. A big focus of the program this year was, and continues to be, supporting the food industry in their efforts to successfully commercialize milkfat fractionation.

Technical support was provided to the food industry and universities through interaction regarding many aspects of the production and use of milkfat fractions, availability of fraction samples, as well as general butter and other dairy-related questions. Several presentations were given at scientific meetings on the production and use of milkfat fractions and dairy ingredients in bakery and chocolates. The Milkfat as a Food Ingredient short course was implemented to teach the industry about the functional properties and use of milkfat in foods, and will be an annual course offered at the UW. The milkfat group assisted with numerous tours at CDR and PR efforts for CDR, DMI, and WMMB at trade shows.

The pilot plant and applications program had a very eventful year. We experienced success with fraction and specialty butter production, and our applications lab. Milkfat fraction production protocols were refined, and the relationships between the old vacuum filter and current pressure filter were evaluated. Some milkfat fractions samples were produced through the end of 1996.

The Gerstenberg & Agger texturizer pilot plant was temporarily installed at Systems Bio-Industries at the end of 1996, until the site at Babcock Hall was ready. Initial evaluations were made on processing conditions for recombined butter,

spreadable butter, and pastry butter. This unit was moved and reinstalled in Babcock Hall Fall 1997, and is presently operating.

The applications laboratory construction was completed in February 1997 and the lab was operational by March. Methods for the evaluation of specialty milkfat ingredients in puff pastries, croissants, and chocolates were refined. Several evaluations of experimental ingredients were conducted for Consortium members. Trade show samples (chocolates, butter cookies) were made for CDR, DMI, and Consortium members.

Milkfat Fractionation Consortium activities included an annual member meeting and quarterly newsletters. Special requests were handled on an individual basis, and included custom fraction and specialty butter samples, evaluation of experimental ingredients in bakery products, and production of trade show chocolate samples.

The demands of the pilot plant program curtailed activities on the applied research and database development objectives. Some applied research was conducted as part of methods development for the production of milkfat fractions and specialty butters. CDR also provided samples of milkfat fractions to UW researchers for ongoing research projects.

The milkfat program staff had continuous on-site training in the area of pilot plant operations, analytical methods, and product evaluation methods. Some of the training was initial job training for new staff and some was to improve their own knowledge and better refine our techniques. The staff also attended several off-site workshops and short courses covering dairy food production, analysis of raw materials and finished products, and traveled to scientific meetings to attend technical lectures and discuss research with colleagues.

Fractionation provides an opportunity to tailor milkfat for specific applications that may benefit from the flavor of milkfat, but the physical properties milkfat hinder its use. The fractionation of milkfat combined with blending and texturization processes to produce specialty ingredients will increase the value of milkfat and expands its use in the food industry. The CDR Milkfat Group provides much needed research facilities, data, and technical support to assist manufacturers and users of milkfat fractions and specialty milkfat ingredients, particularly with the recent commercialization of milkfat fractionation in the U.S.

## Publications and Presentations

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Kaylegian, K.E. Milkfat Fractions Innovative Food Ingredients. Invited speaker at the Midwest Food Processing Conference, Regional IFT meeting. LaCrosse, WI, 10/96.

Kaylegian, K.E. The Use of Milkfat Fractions in Bakery Products. Invited speaker at IBC's Fat and Cholesterol Reduced Foods Conference, Orlando, FL, 3/97.

Kaylegian, K.E. Milkfat Fractions in Chocolates. Invited speaker at the Pennsylvania Manufacturing Confectioner's Association Annual Meeting, Hershey, PA, 4/97.

Kaylegian, K.E. Manufacture of Specialty Milkfat Ingredients. Symposium Chair, Functional and Nutritional Attributes of Milkfat, at the American Dairy Science Association Meeting, Guelph, Ontario, 6/97.

*INTERIM REPORT*

# Development of reduced fat, dairy-based spreads

### Personnel

Kerry E. Kaylegian, researcher, Center for Dairy Research; Kirk L. Parkin, professor, Wade N. Schmelzer, research assistant, Department of Food Science

### Dates

January 1996 to December 1998

### Funding

Dairy Management Inc. UW 133-BA16

## Objectives

Screen for formulations that yield stable, dairy-based, reduced-fat, water-in-oil emulsions.

Optimize formulations and processing protocols for preparing dairy-based, reduced-fat table spreads on a pilot scale.

## Summary

The initial phase of this project focused on standardizing analytical and experimental techniques required for screening formulations for suitability for pilot-scale trials. These developmental activities are being applied to 60% fat spreads, with a control or standard spread, containing commercial soft margarine and distilled monoacylglycerols (MAG), to serve as a reference for comparison of the dairy-based spreads. Homogenization speed, time and temperature have been evaluated and optimized within narrow ranges for each parameter.

Relationships between reducing interfacial surface tension and % MAG included in the oil phase are currently being investigated to determine optimum MAG levels required for each type of formulation (including the reference and dairy-based emulsions). Preliminary experiments have shown that the reference formulation behaves in a classical fashion with regard to increasing % MAG, in that a decrease in interfacial surface tension takes place until the (apparent) critical micelle concentration (CMC) is reached, and after that point there is little effect of increasing % MAG further. In contrast, when butteroil is used as the continuous phase or when butteroil-derived MAG fractions are used as the emulsifying agent, this clear transition in effect of increasing % MAG is not noted. The reasons for this are subject to ongoing studies.

Once the experimental procedures are developed for preparing and analyzing reduced-fat emulsion systems, our efforts will focus on testing dairy-based systems (at 40 and 60% fat levels) for suitability for pilot-scale evaluation with a Gerstenberg & Agger texturizer.

Table spread products constitute an established and expanding global market. Much of the current focus on developing these products is on reduced-fat formulations, however, they are also being considered as vectors for delivering "nutraceuticals" and other health-promoting ingredients. Butteroil holds advantages over other fats and oils in this type of product because of inherent flavoring properties and low *trans* fatty acid content relative to hydrogenated vegetable oils. The objective of this project is to develop entirely dairy-based, reduced-fat table spread formulations, with the ultimate goal of trying to expand the use of milkfat. The approach taken in this project is to optimize the use of butteroil, butteroil fractions and functional derivatives of butteroil in table spread products.

VISITING SCIENTIST REPORT

# Effect of temperature on the behavior of a calf pregastric esterase (PGE) immobilized on microporous polypropylene hollow fibers

**Personnel**

Hugo S. Garcia, Depto. Ing. Quimica y Bioquimica, Instituto Tecnologico de Veracruz
Veracruz, Mexico

**Funding**

Wisconsin Milk Marketing Board HLL 97

**Dates**

July 1996

## Objectives

One research project in the summer of 1997 involved a study of the effect of the reaction temperature on the rate of hydrolysis of a low-melt fraction of butterfat in the presence of calf pregastric esterase (PGE). This enzyme is one that dairy flavor manufacturers use extensively to produce lipolyzed butteroil (LBO). Another study attempted to determine if the loading and activity of the immobilized form of a partially purified form of the enzyme on polypropylene fibers could be increased. Extents of lipolysis were monitored using methods reported previously. The reactor, associated peripheral equipment, and procedures used were the same as those employed in previous studies.

## Summary

Solutions from a single batch of PGE were prepared by a protocol involving membrane microfiltration of the crude enzyme solution followed by ultrafiltration of the permeate. The polypropylene fibers were loaded with the enzyme by recirculation of these solutions through both the shell and tube sides of the reactor. A single batch of PGE was used to study the effect of temperature on reaction rates and fatty acid specificity. HPLC analyses indicated that optimum hydrolytic activity was obtained near pH 6 and an operating temperature of 30˚C. The kinetic data were fit to a 2-parameter model which provides a good description of the performance of the reactor over the entire range of space times investigated (0.4 to 12 h).

Calculation of key fatty acid ratios in the effluent stream (e.g., C4/C8, C4/C6, C4/C12) indicated statistically significant differences between the LBOs prepared at 30˚C and those prepared at either 22 or 40˚C. Relative to an LBO produced under similar conditions using a PGE from kid goats, the product obtained using the calf PGE contained a lower concentration of butyric acid (C4). Conversely, the concentration of the long-chain fatty acids in the LBO produced using the calf PGE was about an order of magnitude greater than that contained in the product generated with the kid PGE. Moreover, key fatty acid ratios (i. e., C4/C8, C6/C8 and C4/C12) in the product LBO's were different for the two immobilized enzymes. Hence they are characterized by different flavor profiles.

Conversion rates obtained in the reactor containing the immobilized calf PGE were sufficiently high to encourage further work in this area with a view towards ultimate commercial implementation of this technology.

Two generic types of experiments were carried out to incorporate conjugated linoleic acid (CLA) into the triacylglycerides which constitute butteroil. In the first class of experiments, four commercial enzyme preparations (free *Candida cylindracea*, free *Pseudomonas sp*., immobilized *Mucor miehei* and immobilized *Candida antarctica*) were tested for their ability to catalyze the acidolysis reactions responsible for incorporation of CLA into butterfat. The butterfat to CLA ratio was

10:1 and reaction time was 72 hours. Substantially complete incorporation of the free CLA into butteroil glycerides was achieved using either of the two immobilized lipases. The second class of experiments involved the use of an immobilized *C. antarctica* lipase in a reaction medium which contained no organic solvent. The effect of two butteroil:CLA (w:w) ratios (7:1 and 4:1) was studied. Analyses for both total and esterified fatty acids were conducted by gas chromatography in Dr. Michael Pariza's laboratory. These measurements provided information on the amount of CLA present in esterified form in the triacylglycerides of butterfat. After 52 hours of reaction, the CLA present in esterified form in butterfat was increased from the native level in which the residues were less than 1% (w/w) CLA to a situation in which the residues were greater than 15% CLA. For the butteroil:CLA ratios of 7:1 and 4:1, the percentages of the CLA present in esterified form were 68 and 56%, respectively.

There are two principal results derived from the investigations conducted in the summer of 1997 that benefit both CDR's research program and the dairy industry in general. The use of a low melting butterfat fraction as the feedstock for the immobilized PGE reactor provides a suitable substrate that allows the lipolysis reaction to be carried out at temperatures below 40°C. Operation at a lower temperatures enhances enzyme stability while maintaining both an appropriate rate of hydrolysis and retention of the product in a liquid state to facilitate subsequent processing operations. The data generated will be useful to dairy flavor manufacturers since it expands both the knowledge base concerning the selectivities of immobilized pregastric esterases and the range of process conditions under which continuous production of lipolyzed butter oils (LBO) has been demonstrated. On the basis of the information generated in our laboratory in recent years, one manufacturer is in the initial phases of a pilot plant study which will implement continuous production of LBO.

The experiments involving incorporation of CLA into butterfat demonstrate the technical feasibility of our proposed approach. The results obtained thus far are very encouraging. However, additional appropriate quantitative data on the kinetics of this reaction are necessary for purposes of process design and assessment of process economics. Results obtained to date support our belief that a practical technology for enrichment of butterfat in CLA can be achieved in a reasonable length of time.

*INTERIM REPORT*

# Use of immobilized lipases to prepare dairy products enriched in conjugated linoleic acid (CLA)

**Personnel**

Charles G. Hill, Jr., professor, Dept. of Chemical Engineering; Dr. Hugo S. Garcia, visiting scientist, associate professor, Department of Food Technology, Centro de Graduados, Instituto Tecnologico de Veracruz, Veracruz, Mexico; Prima Sehanputri, research assistant, Department of Chemical Engineering; Colin Crowley, research assistant, Department of Chemical Engineering, Jose Arcos, postdoctoral fellow, Department of Chemical Engineering.

**Dates**

July 1996 - June 1999

**Funding**

Dairy Management Inc. Hll 97

## Objectives

To effect the synthesis of glycerides containing residues of conjugated linoleic acid (CLA) using immobilized lipases (e.g., *Candida* sp. or *Rhizomucor miehei*). Both batch and continuous flow reactor configurations will be employed to bring about these reactions. Two synthetic routes are being investigated, *viz*., a) direct synthesis of the glyceride via the reactions between CLA and glycerol to obtain mixtures of monoacylglycerides (MAG), diacylglycerides (DAG) and triacylglycerides (TAG), and b) direct interesterification (acidolysis) of butteroil or butteroil fractions with free CLA.

To generate the experimental data necessary to characterize the rates of the reactions of interest over a limited range of conditions. The resulting rate expressions will be employed to develop mathematical models for process simulation, optimization and economic analysis. Such information will be necessary to conduct a preliminary assessment of the commercial feasibility of producing butteroils enriched in CLA. The resultant butteroils could be used in the formulation of dairy products designed for consumers seeking foods with both nutritional and medical/health benefits.

To assess whether the results obtained in this preliminary study indicate that more comprehensive studies are merited. The expanded work would encompass such aspects as studies of expanded ranges of experimental conditions (e. g., type of reactor, enzyme source, temperature, pH), nutritional/animal feeding work, determination of physical and functional properties, and engineering/economic analyses. The future studies would provide the information necessary for implementation of this technology for commercial production of dairy products containing glycerides enriched in CLA residues.

## Summary

Our efforts to date have focused on three primary activities:
Generation of the kinetic data necessary to characterize the rate of release of linoleic acid from corn and safflower oils. These data permit us to determine the appropriate process conditions necessary to accomplish the production of linoleic acid in high yield and high selectivity from natural products using esterases immobilized in a hollow fiber reactor. Our data indicate that for corn oil, good yields and selectivities can be obtained at pH 7.0, an operating temperature of 30° C, and reactor space times of a few hours. Additional work needs to be accomplished establishing the degree of purity of the linoleic acid product stream which is most efficacious (both technically and economically) for use in the subsequent bioconversion process.

Studies of the production of CLA from linoleic acid using a bioconversion process involving *Lactobacillus ruteri*. To date we have demonstrated that we can accomplish this isomerization reaction in a manner which gives high yields of the desired biologically active cis-9, trans-11 isomer of CLA. These studies have also

been successful in identifying conditions under which dramatic increases in the yield of CLA have been achieved. Work in this area is continuing with a view towards identification of optimum process conditions for free cells, to be followed by studies (with concomitant economic implications) involving immobilized cells. Additional work also needs to be accomplished with respect to the degree of purification necessary to produce a CLA stream which can be converted in high yield to glyceride forms for incorporation in dairy products.

Studies of the kinetics of the reactions of CLA with glycerol and butterfat in the presence of immobilized enzymes in order to determine the reaction conditions and types of reactors that will be most useful for commercial implementation of this technology. Preliminary studies of these reactions indicate the technical feasibility of both routes for obtaining glycerides that can be incorporated in any dairy product that contains milkfat. However, much more quantitative data are necessary to obtain a reliable assessment of the technical and economic viability of this approach to producing nutraceutical type dairy products. Conversations with representatives of several major corporations have been encouraging.

A recent trend in food purchases has involved increased consumer interest in purchasing foods which confer health benefits as well as nutritional content. There is increasing evidence that ingestion of particular foods leads to reduced incidence of certain diseases. In this context, nutraceuticals (any substance that can be considered a food or part of a food that provides medical or health benefits, including the prevention and treatment of disease) have become an attractive option. Examples of dairy related nutraceuticals include not only those products which could readily be marketed as alternative forms of traditional dairy products e. g., modified milk (high in ß-casein, low in ß-lactoglobulin), but also products which could be readily derived from milk, e. g., lactoperoxidase (antimicrobial), lactoferrin (bacteriostat and antioxidant), as well as products which provide ideal vehicles for incorporation of nutraceuticals in the human diet, e. g., any dairy products containing milkfat into which CLA has been introduced in the form of glycerides. CLA has antioxidant and antimicrobial properties and exhibits anticarcinogenic properties *in vivo*.

In the light of the growing demand for nutraceuticals and the increasing health awareness of consumers, dairy products containing milkfat enriched with CLA present an intriguing marketing option for the dairy industry. These products could partially counteract the negative image that milkfat has developed in recent years because of its relatively high proportion of saturated fatty acids, particularly those that have demonstrated hypercholesterolemic effects on humans. In practice, any dairy product that may be formulated using milkfat as an ingredient could be a potential product of the technology on which our proposal is based; in particular, butter, butterine, butteroil, and reconstituted dairy products prepared from skim milk and anhydrous (modified, CLA-rich) milkfat. Fluid milks, cream, cheese and frozen products represent very attractive marketing options. The modified milkfat products thus have significant dietary implications with respect to both nutrition and potential anti-cancer activity. Using immobilized enzyme and bioconversion technology offers the intriguing possibility of being able to produce specially designed foods for selected segments of the population. A particular segment are those individuals who are especially health conscious from a dietary aspect or are high risk candidates for cancer, atherosclerosis, hypertension, or other health problems. These products represent a very significant long term marketing opportunity for the dairy industry.

## Presentations

"Hydrolysis of Naturally Occurring Fats and Oils by Esterases Immobilized in a Hollow Fiber Reactor," by C.G. Hill, Jr., H.S. Garcia, K.E. Rice, F.X. Malcata, L. Lessard, and S. Ghannouchi, invited paper presented at the 6th Biochemical Engineering Conference, September, 1996 at the Korea Advanced Institute of Science and Technology

"Membrane Reactors: Ceramic Modules and Immobilized Enzyme Systems," by C.G. Hill, Jr., F. Tiscareño-Lechuga, F.X. Malcata, K.E. Rice and M.A. Anderson, invited paper presented at the October, 1996 meeting of the Congreso Mediterráneo de Ingeniería Química.

"Rapid Enzymatic Production of Glycerides from Conjugated Linoleic Acid and Glycerol in a Solvent-Free System," by J. A. Arcos, and C. G. Hill, Jr., paper submitted for presentation at the 1998 national meeting of the Institute of Food Technologists

"Enrichment of Butteroil in Conjugated Linoleic Acid via Enzymatic Interesterification (Acidolysis) Reactions," by H. S. Garcia, J. M. Storkson, M. W. Pariza, and C. G. Hill, Jr., paper submitted for publication in Biotechnology Letters.

*INTERIM REPORT*

# Use Of immobilized esterases/lipases to modify the composition of milkfat

**Personnel**

Charles G. Hill, Jr., professor, Dept. of Chemical Engineering; Dr. Hugo S. Garcia, visiting scientist, associate professor, Department of Food Technology, Centro de Graduados, Instituto Tecnologico de Veracruz, Veracruz, Mexico; Julio Vinay, visiting scientist, Department of Food Technology, Instituto Tecnologico de Veracruz, Veracruz, Mexico; Louis Lessard, research assistant, Department of Chemical Engineering; Souheil Ghannouchi, research assistant, Department of Chemical Engineering.

**Funding**

Dairy Management Inc.
MF07

**Dates**

July 1997 - June 1999

## Objectives

Generate the experimental data necessary to characterize the rates of the various reactions constituting the reaction networks of interest. Determine the effects of temperature and pH on both the overall rate of lipolysis and the reaction specificity for each esterase of interest.

Use these kinetic data to develop both uniresponse and multi-response mathematical models of the reaction network, which can be used for process design and simulation, control, and optimization.

Establish the nature of the dependence of the composition of the lipolyzed dairy product on the process conditions (reactor space time, pH, temperature, source of enzyme).

Assess the commercial viability of proposed processes in terms of technical and economic considerations.

## Summary

This project is an extension of an earlier project funded in part through the Center for Dairy Research and in part through a grant from the National Science Foundation. Recent efforts in our laboratory have focused on the use of pregastric esterases derived from the salivary glands of suckling animals (calf, kid goat and lamb) to effect the lipolysis of butteroil. We have developed experimental protocols for partial purification of these enzymes, beginning with the crude preparation generously supplied by Systems BioIndustries. Subsequent immobilization of these enzymes in a hollow fiber reactor provides a vehicle for obtaining lipolyzed butteroil products with significantly different sensory attributes than either typical commercial products or the effluent from a reactor containing an immobilized *A. niger* lipase. The three pregastric esterases give products which also differ from one another.

HPLC analyses of the product streams indicate that all three pregastric esterases have high specificities for release of butyric (C4) and caproic (C6) acid residues, but lower specificities for caprylic (C8) and capric (C10) acid residues. None of these enzymes released significant amounts of intermediate length or long chain fatty acids. While lamb and kid lipases give a higher acid release ratio of the C4 to C6 residues, calf lipase gave more even proportions of these acids. These results suggest that reactors containing immobilized lipases from different sources could be used to tailor-make lipolyzed butter oils with specific flavor notes. For example, high values of C4/C6 and C4/C8 correspond to intense, but desirable flavors. By contrast, low values of C4/C12 can be used as indicators of soaplike (undesirable) flavors. In studies with an immobilized kid goat lipase we varied the buffer pH and the reactor space time to manipulate the composition of the reactor effluent. In several cases, we were able to approximate the C4-C10 fatty acid content of commercial lipolyzed butteroils while reducing the C12-C18 content by an order of

magnitude or more. Manipulating the pH and temperature at which the reactor operates permits additional tweaking of the chemical composition (and hence the apparent flavor notes) of the product mixture. Results obtained to date clearly demonstrate that our immobilized pregastric esterase reactor allows us to tailor the product composition for specific applications by selecting operating conditions and the source of the enzyme.

This research project addresses that component of the 1996 National Milkfat Plan which is intended to create new uses for milkfat, modified milkfat and/or its components. Specifically, it focuses on enzymatic modification of milkfat to produce lipolyzed butteroils and/or diacyl- and monoacyl-glycerides which can be employed as food grade emulsifiers.

This research project is intended to establish a rational scientific basis for employing immobilized enzyme technology for the manufacture of lipolyzed dairy products with specified free fatty acid profiles and unique sensory and functional characteristics. It has direct implications for producing lipolyzed dairy products which find applications as flavoring agents within the food industry. In addition, this immobilized enzyme technology has far-reaching implications with respect to the development of processes for the production of designer foods with specific physiological consequences for those segments of the population that are high risk candidates for cardiovascular, hypertensive, or other health problems, or who are particularly health-conscious. There is a growing awareness of the role of foods in human health, and hydrolysis and interesterification reactions can be used to produce food products known as nutraceuticals, which confer both nutritional and medicinal benefits on the consumer. By using appropriate combinations of immobilized enzyme and conventional technologies, we can manipulate the chemical composition of the milkfat precursor to produce tailor-made foods for specific segments of the population. These value added products represent a very significant long term marketing opportunity for the dairy industry.

## Publication/Presentations

"Modification of the Composition of Butteroil Using Immobilized Enzyme Technology," by C. G. Hill, Jr., H. S. Garcia, and J. C. Vinay, paper presented at the VII Simposium Internacional Avances en Ciencia Y Tecnologia de Aliementos, Veracruz, Mexico, September, 1997 (invited plenary lecture).

"Effects of pH and Temperature on Product Distribution for Lipolysis of Butteroil Over an Immobilized Calf Pregastric Esterase," by H. S. Garcia, J. C. Vinay, and C. G. Hill, Jr., paper submitted for presentation at the 1998 national meeting of the Institute of Food Technologists.

"Effects of pH on the Rate of Lipolysis of Anhydrous Milkfat Over a Calf Pregastric Esterase Immobilized in a Hollow Fiber Reactor," by Louis P. Lessard and C. G. Hill, Jr., paper submitted for presentation at the 1998 national meeting of the Institute of Food Technologists.

"Effects of pH and Temperature on Product Distribution for Lipolysis of Butteroil Over an Immobilized Calf Pregastric Esterase," by H. S. Garcia, J. C. Vinay, and C. G. Hill, Jr., paper submitted for publication in Biotechnology Letters.

*INTERIM REPORT*

# Determination of caloric bioavailability and apparent lipid digestibility of liquid milkfat fractions

**Personnel**

Denise Ney, associate professor, Nutritional Sciences

**Funding**

Wisconsin Milk Marketing Board UWA9606

**Dates**

July 1996- June 1997

## Objectives

To determine growth, apparent lipid digestibility, and the concentrations of cholesterol and
triacylglycerol in liver and plasma of weanling rats fed diets containing liquid milkfat fractions, intact milkfat or corn oil, Study 1.

To determine the caloric bioavailability of liquid milkfat and intact milkfat with a bioassay method based on the growth of weanling rats fed a basal diet supplemented with corn oil (caloric standard), Study 2.

## Summary

Delays in setting up the CDR milkfat fractionation pilot plant have prevented the production of milkfat fractions for this study. We anticipate beginning the study in 1998.

# chapter 2

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*INTERIM REPORT*

# Improved quality of shredded cheese—antimycotics, oxygen scavengers and modified atmosphere packaging

**Personnel**

J. Russell Bishop, professor, Center for Dairy, Joseph E. Marcy, associate professor and Tina Moler Grove, graduate student, Dept. of Food Science & Technology, Virginia Polytechnic Institute & State University

**Funding**

Wisconsin Milk Marketing Board UW9506

**Dates**

June 1997-December 1999

## Objectives

To determine the effect of natamycin on shelf-life of low fat and reduced fat shredded cheddar cheeses in combination with MAP packaging and after opening.

To determine the effect of natamycin application method (water spray of cellulose dispersal) on antimycotic effect.

Oxygen absorbers were added to cellulose and tested separately and with natamycin for mold inhibition in shredded cheeses.

## Summary

A 4 month shelf-life study has been completed. Commercial reduced fat and low fat cheddar cheeses were evaluated for shelf-life as measured by sensory quality and mold growth. The experiment sampled shredded cheese at one month intervals and then again at weekly intervals after the initial opening of the container. This gave a measure of the shelf-life in the commercial MAP conditions and an estimate of the shelf-life in the consumer's refrigerator.

Natamycin was applied as a 300 ppm slurry in a water spray and dispersed with cellulose. Test results indicate there was no differences caused by application method. We are presently comparing these test results with the data for regular Cheddar shelf-life trials.

### Our Next Steps

The test results indicate natamycin is less effective with reduced fat cheese. Since natamycin is entirely insoluble in water, the ability to have the active compound dispersed is questioned.

The next round of shelf-life tests will continue to evaluate natamycin, but at much higher levels of use. These higher levels of natamycin use are similar to those found in the cheese industry. We are currently working on methods to better disperse natamycin on shredded cheeses to improve the antimycotic effect.

We are proposing to evaluate natamycin in a form which is water soluble. We are also proposing to evaluate compound which will have a synergistic effect with natamycin in mold control.

The biggest issue is finding ways to have the active mold inhibitor uniformly covering the shredded cheese. We are currently working to have a water-soluble form of natamycin. Our success in obtaining this altered natamycin will affect the future research plans. We are looking at alternative ways to improve the distribution of natamycin.

*INTERIM REPORT*

# Minimizing the watering-off of unripened lower fat and no fat Mozzarella cheese

**Personnel**

Carol M. Chen, researcher, Mark E. Johnson, senior scientist, Amy L. Dikkeboom, research specialist, Bill Hoesly, food production assistant, Kristen Houck, research specialist, John J. Jaeggi, assistant researcher, Juan Romero, associate researcher, William A. Tricomi, assistant researcher, Matt G. Zimbric, research specialist

**Funding**

Dairy Management Inc.
CJH96

**Dates**

January 1996 - June 1998

## Objectives

To evaluate how manipulations to cheese milk, different manufacturing protocol or the addition of specific casein hydrolyzing enzymes can minimize the watering off of unripened high moisture lower and no fat Mozzarella cheese. Specifically, manipulations will focus on water-to-protein interactions to maximize the water absorption capability of the cheeses.

## Summary

The amount of serum expressed from cheese upon centrifugation is called expressible serum and is an indirect method to determine the ability of water to accumulate on cheese when shredded (the latter is called watering off).

All cheeses made during this investigation, regardless of fat level (1.5-10%), moisture level (52-63%), milk pasteurization temperature (164˚F/16s-184˚F/16s), temperature of storage (45˚F- 56˚F), or method of salt addition (salt in whey, direct salt, brine) had very limited if any expressible serum after 8 days. However, high temperature pasteurization of milk (184˚F/16s) greatly increased the level of expressible serum at one day after manufacture (3-10 x the expressible serum of cheeses made from milk pasteurized at 164˚F/16s). Different methyl cellulose-based fat mimetics (Methocel-Dow Chemical) were also evaluated since they have the capacity to absorb moisture. Methocel was added to the cheese milk after pasteurization but before coagulant addition. Methocel does not absorb water until after it is first heated. Upon subsequent cooling Methocel absorbes water. One type of Methocel, (A 4M), completely eliminated expressible serum in both lower fat and no fat Mozzarella cheese by one day after manufacture. Additionally, the use of Methocel improved the quality of no fat Mozzarella cheese and resulted in a cheese of high acceptability when baked on a pizza.

The addition of trypsin to cheese milk was attempted (specific β-casein hydrolysis may increase the rate of water absorption) but due to a cooler malfunction the trial had to be redone. Three additional trials were completed but the cheeses have not yet been analyzed completely. The analytical work is continuing.

*INTERIM REPORT*

# Optimizing the standardization of milk in the manufacture of 50% reduced fat Cheddar cheese

**Personnel**

Carol M. Chen, researcher, Mark E. Johnson, senior scientist, Brian Gould, senior scientist, Amy L. Dikkeboom, research specialist, Bill Hoesly, food production assistant, Kristen Houck, research specialist, John J. Jaeggi, assistant researcher, Juan Romero, associate researcher, William A. Tricomi, assistant researcher, Matt G. Zimbric, research specialist

**Funding**

Dairy Management Inc.
CJG97

**Dates**

July 1996 - June 1998

## Objectives

To determine the maximum levels of condensed skim (40% solids) and reconstituted NDM (20% solids) and initial milk solids content in the standardization of whole milk for the standardization of 50% reduced fat Cheddar cheese.

To evaluate levels of denatured whey protein incorporated by the different standardization methods and the correlation between denatured whey protein levels, cheesemaking parameters and cheese quality.

To evaluate the economic impact of standardizing milk with condensed skim or NDM in the manufacture of 50% reduced fat Cheddar cheese.

## Summary

The cheesemaking experiments conducted in the first year of this project evaluated the standardized milk to a casein:fat ratio of 1.70 and 10% total solids for the manufacture of 50% reduced fat Cheddar cheese. Whole milk was standardized with either condensed skim (40% solids) or reconstituted NDM (20% solids), and skim milk or cream. It was necessary to add water to obtain the 10% solids. Gilles and Lawrence (1981) reported that full fat Cheddar cheese made solely from low-heat, reconstituted NDM with anhydrous milk fat tended to have quality defects such as poor milk clot formation, slow whey expulsion, slow cheese flavor development, excessive acid flavor and a firm crumbly body. Currently, we are evaluating cheesemaking observations, fat and nitrogen component recovery, yield, chemical analysis and sensory characteristics. From these experiments, a maximum level of condensed skim or reconstituted NDM will be recommended. The second phase of the experiment will focus on determining maximum milk solids content for the initial milk used in cheesemaking. Lastly, an economic evaluation will be conducted using cheese trial results.

Table 1 summarizes the milk standardization treatments and defines abbreviations for experimental treatments. On the first cheesemaking date for each type of standardization, all coagula were cut at the same firmness. For milk standardized with reconstituted NDM and condensed milk, an additional 15 minutes were required to reach the same coagulum firmness as the control vat. The resulting cheeses were higher in moisture content (1.5 - 2.0%). Thus for subsequent cheesemaking trials setting times were decreased, so the final cheeses would have more similar moisture contents.

A complete mass, fat and nitrogen analysis was conducted. The percentage of fat recovered in the cheese was significantly lower for all experimental treatments (range 85.8-86.9%) as compared to the control (88.3%). Standardization of whole milk with condensed skim did not affect the percentage of nitrogen recovered in the cheese. However, when milk was standardized with reconstituted NDM the percentage of nitrogen recovered significantly increased from 74.9 to 76.1. The

Table 1. Milk standardization treatments for cheesemaking experiments.

| Treatment [1] | whole (lb) | reconstituted NDM or condensed skim (lb) | skim (lb) | cream (lb) | water (lb) |
|---|---|---|---|---|---|
| Reconstituted NDM (20% solids) | | | | | |
| control | 511 | | 789 | | |
| -25.0% rNDM | 518 | 263 | 214 | | 305 |
| -12.5% rNDM | 517 | 306 | 101 | | 376 |
| rNDM | 518 | 351 | | | 431 |
| Condensed skim (40% solids) | | | | | |
| control | 504 | | 796 | | |
| -12.5% CS | 529 | 131 | 100 | | 540 |
| CS | 529 | 150 | | | 621 |
| +12.5% CS | 423 | 169 | | 11 | 697 |

[1] Mean of three milk blends. On one cheesemaking day the milk was either standardized with reconstituted NDM or condensed skim. There were 3 cheesemaking days where the milk was standardized with reconstituted NDM and condensed skim. Eight vats of cheese or duplicates of each treatment were conducted on each date.

Table 2. Percentage of fat and nitrogen recovery, R value and cheese yield for 50% reduced-fat Cheddar cheese made from milk standardized with reconstituted NDM and condensed skim.

| Treatment | % Fat Recovery | % Nitrogen Recovery | R value | Actual Yield | Adjusted[1] Yield |
|---|---|---|---|---|---|
| Reconstituted NDM | | | | | |
| control | 88.19 | 74.88 | 1.13 | 7.97 | 8.05 |
| -25% NDM | 86.82 | 75.53 | 1.138 | 7.93 | 7.99 |
| -12.5%NDM | 86.37 | 75.91 | 1.14 | 7.95 | 7.93 |
| NDM | 86.59 | 76.05 | 1.142 | 7.82 | 7.9 |
| Condensed Skim | | | | | |
| control | 88.36 | 75.3 | 1.143 | 7.86 | 7.93 |
| -12.5% CS | 86.98 | 75.64 | 1.141 | 7.86 | 7.8 |
| CS | 86.05 | 75.4 | 1.13 | 7.7 | 7.68 |
| +12.5% CS | 85.75 | 75.82 | 1.14 | 7.84 | 7.75 |

adjusted cheese yield (47% moisture, 1.7% salt) was lower for all experimental treatments.

Proteolysis in the cheese was assessed by 12% TCA soluble nitrogen at 2, 6, 12, and 24 weeks of aging. Cheeses made with milk standardized by rNDM resulted in 10-20% less proteolysis at all sampling points as compared to the control. Proteolysis for -12.5% rNDM and -25% rNDM did not differ from the control. Standardization of milk with condensed skim did not affect the levels of 12% TCA soluble nitrogen.

Descriptive taste panels were conducted to evaluate the flavor and texture of the 50% reduced fat Cheddar cheese. Cheeses standardized with rNDM had less Cheddar flavor and were more firm than the control, -25% rNDM, and -12.5% rNDM. Standardization of milk with condensed skim did not affect the flavor and texture.

The cost of manufacturing reduced fat Cheddar cheese is greater than that of whole milk Cheddar. There are two reasons for this; low yield and the expense of standardization. Standardization costs fluctuate due to the current price received for cream or the cost of condensed skim and NDM. Use of the latter two may be economically advantageous over cream removal due to their actual cost and increased yields. To take advantage

of this, cheesemakers need to know the optimum usage levels and if the cheese produced is of high quality. This study is evaluating cheesemaking, cheese quality, cheese yield and its economics when standardizing milk with reconstituted NDM and condensed skim.

## Presentations/Publications

Comparative study of milk standardization methods for milk used to manufacture 50% reduced- fat Cheddar cheese. C.M. Chen, A.L. Dikkeboom, J.J. Jaeggi, M.E. Johnson, W.A. Tricomi, and M.G. Zimbric. July 1997. 93nd Annual ADSA Meeting, Denver, CO.

## Reference

Gilles J, Lawrence RC. The manufacture of cheese and other fermented products from recombined milk. N.Z. J. Dairy Sci. Technol. 1981; 16:1-12.

*INTERIM REPORT*

# Pizza cheese II: Shelf-life evaluation and tailor manufacturing of pizza cheese

**Personnel**

Carol M. Chen, researcher, Mark E. Johnson, senior scientist, Amy L. Dikkeboom, research specialist, Bill Hoesly, food production assistant, Kristen Houck, research specialist, John J. Jaeggi, assistant researcher, Juan Romero, associate researcher, William A. Tricomi, assistant researcher, Matt G. Zimbric, research specialist

**Funding**

Wisconsin Milk Marketing Board UW9701

**Dates**

July 1997 - June 1999

## Objectives

To determine the minimal and maximal aging for optimal physical and sensory characteristics of pizza cheese when used on pizzas.

To outline manufacturing protocols for tailor-made Pizza cheese applications: oven conditions, physical properties and composition.

## Summary

Pizza cheese is an ingredient cheese that has a similar composition, melt and stretch to LMPS Mozzarella, but is whiter, does not brown during baking in a convection oven, and has less oiling off. When pizza cheese was developed, the cheese was initially tested on pizzas baked in a stacked shelved convection oven. However, in a commercial setting, pizzas are being baked in forced air ovens (Impinger), and the cheese has a tendency to burn (blisters form). This is due to excessive moisture loss. Initial experiments focused on increasing the fat level of the cheese and producing a cheese that does not blister as readily. Both steps should decrease burning. Increasing the fat level of the cheese decreased burning, but final experimental results are pending.

Future cheese making experiments include:
Evaluating the effect of milk coagulant level on the chemical and physical characteristics of pizza cheese. Specifically, the concentration of intact proteins during the aging of the cheese will be measured and correlated to the stretch and meltability of the cheese.

Evaluating the effect of different type of acidulants on the composition and physical characteristics. We are interested in the pliability, softening and flow of the melted cheese. We will be using new melt methods developed by the Biological Systems Engineering group, headed by Sundaram Gunasekaran. In past experiments, we noted that there was no difference in the thermal and microwave meltability, but did note that the LMPS Mozzarella flowed off the crust and the pizza cheese did not.

Evaluation of different starter cultures systems (both mesophilic and thermophilic cultures). Browning and cheese flavor intensity issues will be addressed.

Incorporation of denatured whey proteins and/or further manipulations to the manufacturing protocol will be used to increase the moisture content of Pizza cheese to greater than 47%. Denatured whey proteins may be included by use of selected starter culture media or addition of whey protein-based fat mimetics (Dairy-Lo; Cultor Foods, or Simplesse; NutraSweet Kelco Co.) The effect of incorporation of denatured whey protein on the physical properties of the Pizza cheese will be evaluated.

This technology has generated much interest on a state, national and international level. Working with cheese companies on scaling-up this technology from 500

pound research batches to 30,000 to 50,000 pound batches has generated many questions concerning ingredient usage and milk standardization, shelf-life stability and tailor-making pizza cheese. We propose to address these issues by providing a research base that CDR staff can use and effectively communicate, both to cheese makers to resolve scale-up issues and to end users regarding product physical properties.

*APPLICATIONS PROGRAM REPORT*

# Cheese applications research program

### Personnel

Carol Chen, researcher, Amy Dikkeboom, research specialist, Bill Hoesly, food production assistant, Kristen Houck, research specialist, John Jaeggi, assistant researcher, Mark Johnson, senior scientist, Matt Zimbric, research specialist

### Funding

Wisconsin Milk Marketing Board
UWA9705

### Dates

January 1997- December 1997

## Objectives

Provide technical support for the use of commodity and specialty cheeses in food application systems through consultations, pilot plant trial runs, applications lab evaluation, and plant visits.

Conduct industry directed cheese applications research—modifying manufacturing processes or ingredients during cheesemaking to produce a functionally specific cheese.

Direct contact with industry to meet informational needs.

## Summary

In 1997, the Cheese Ingredient Applications Program worked with 32 Wisconsin-based cheese manufacturers, ingredient suppliers, and cheese end users. Of these companies, 26 are cheese manufacturers (all sizes), 4 are ingredient suppliers and 2 are cheese end users. We adapted commodity cheeses (Cheddar, Swiss) for specific applications, tailor-made cheeses (Muenster, Pizza cheese) for distinctive physical characteristics, and developed new cheese making protocols (Tvarog, Swiss-type, Yoghurt cheese, cold pack cheese food). See Table 1.

One of our most rewarding projects has been with pizza cheese. Pizza cheese is a ingredient cheese that has a similar composition, melt and stretch to LMPS Mozzarella, but is whiter, does not brown during baking and has less oiling off. When pizza cheese was developed, the cheese was initially tested on pizzas baked in a stacked shelved convection oven. However, in a commercial setting, pizzas are being baked in forced air ovens (Impinger). Baking conditions in this type of oven are extreme and cheeses tend to skin, blister or burn. We are currently working on alterations to the pizza cheese manufacturing process that result in a cheese more suited for Impinger-type ovens. We have learned that through alterations to key manufacturing steps, Pizza cheese can easily be altered so the resulting cheese has specific physical properties.

In summarizing the activities of the first year of the Cheese Ingredient Applications Program, we are pleasantly surprised by the wide variety of cheese projects. Our interactions tell us that the Wisconsin cheese industry is growing and finding new and innovative cheese applications. The number of interactions demonstrates the commitment between the Wisconsin Center for Dairy Research and the Wisconsin cheese industry.

The Cheese Ingredient Applications program works directly with Wisconsin cheese manufacturers, ingredient suppliers and cheese end users as a technical resource for cheese ingredient applications, cheese technology topics and the tailor manufacturing of cheese for specific end uses.

## Publications and Presentations

Members of the Cheese Ingredient Applications Program team also provided technical information at several national and regional meetings/conferences. Staff play an important role in the Cheese Technology Short course held in March and October, 1997. Throughout the year the CDR provides tours for various journalists, councils and industry groups. Pizza cheese and/or reduced-fat Cheddar technology has been shown at 5 of these tours.

Cheese Applications Program. Carol Chen. April 3, 1997. Wisconsin Cheese Industry Conference, Green Bay, WI.

Influence of Methocel™ on the physical characteristics frozen-thawed Mozzarella. A.L. Dikkeboom, C.M. Chen, K.B. Houck, J.J. Jaeggi, and M.E. Johnson. June 22-25, 1997. 92nd Annual ADSA Meeting, Guelph, Canada.

Manufacturing lower fat Mozzarella cheese by adding milk coagulant at different pH values. C.M. Chen, J.J. Jaeggi, and M.E. Johnson. June 22-25, 1997. 92nd Annual ADSA Meeting, Guelph, Canada.

Manufacturing Lower fat and Skim milk Mozzarella cheese. Carol Chen. September 18, 1997. 34th Marshall's Italian Cheese Seminar, Madison, WI.

The Power of Pizza. Carol Chen. November 5, 1997. Wisconsin Regional IFT Meeting, Madison, WI.
Strategies for Enhancing Cheese Functionality: Impact of cheese manufacturing on physical and functional properties. Carol Chen. December 10, 1997. DMI Technology Forum, Chicago, IL.

Table 1. Cheese Ingredient Applications Program Technical Support

| | | |
|---|---|---|
| Cheesemaking | * | Worked with 12 companies |
| | * | 23 cheesemaking dates (79 vats/batches of cheese) |
| | * | Manufactured a wide variety of cheeses including: Cheddar, Cheddar-type, Club, Cottage, Muenster Parmesan, Pizza cheese, Swiss, Swiss-type, Tvorag, and Yoghurt. |
| Laboratory | * | Worked with 11 companies |
| | * | Applications work included various physical properties: melt, slice, browning. Evaluated various cheeses in under different cooking conditions (forced air vs convection). |
| | * | Chemical analysis on cheese |
| | * | Sensory evaluation on cheese |
| CDR visits or Onsite visits | * | Worked with 11 companies (13 visits) |
| | * | Types of topic covered during visits: scale-up of current CDR cheese technologies, milk standardization, cheese quality, cheese technology. |
| Phone Conversations | * | Worked with 12 companies on topics that were independent of cheesemaking, laboratory or visits. |
| | * | Wide variety of topics: nutrient claims, ingredient addition, cheese technology, milk quality, cheese ingredient applications. |

*FINAL REPORT*

# Implications of consumer nutrient concerns for the consumption of dairy foods

**Personnel**

Brian W. Gould, senior scientist,
Wisconsin Center for Dairy Research

**Funding**

Wisconsin Milk Marketing Board
UWA9608

**Dates**

June 1996 - June 1997

## Objectives

Analyze the role of consumer health concerns in determining nutrient intake

Determine changes in consumption patterns of U.S. dairy products

Determine the impact of nutrition knowledge and attitudes on dairy product purchase decisions

## Summary

Since the mid-1950's, public and private efforts to improve the U.S. diet have focused on increasing the awareness of the link between dietary fat intake and coronary heart disease. The effectiveness of these education efforts can be measured by both the level of awareness and the impact it has on the consumption of specific foods. An examination of household level data indicates a relatively successful effort in increasing awareness.

There is ample evidence that food manufacturers have responded to this increased awareness by increasing the supply of "nutritionally improved" foods. Between 1988 and 1995 there was a 300% increase in the number of improved nutrient content claims by new food introductions. These nutritionally improved foods represent an important growth area for the food industry, accounting for over 55% of the increase in the value of sales in the associated food types.

Putler and Frazao (1991, 1994) found that although the public is becoming more aware of the impact of dietary fat intake on health, this increased awareness has not resulted in a reduction in the consumption of total fat, saturated fat or cholesterol. They refer to this phenomenon as a dietary "balloon" effect. That is, increased awareness reduces fat intake from one food group such as red meat while at the same time increasing fat intake from another food group such as dairy products, the net result being little overall change in fat intake. They attribute this result to consumers "having difficulties making effective food substitutions in their diets, perhaps due to insufficient knowledge about the relative fat content of different food groups."

To better understand the nature of U.S. household demand for dairy products we undertook a series of analyses based on household level data during the 1997 fiscal year.

Within the Wisconsin Center for Dairy Research is a database developed from the 1989/91 USDA Continuing Survey of Food Intakes of Individuals (CSFII) and companion Diet and Health Knowledge Survey (DHKS). These surveys are used by federal food/nutrition policy analysts along

with researchers across the U.S. to provide a snapshot of the status of the healthiness of the typical U.S. consumer as well as the state of knowledge of the linkage between nutrient intake and health knowledge. With previous support of the Wisconsin Milk Marketing Board, we have used the CSFII to analyze nutrition knowledge and consumer demand for a variety of dairy products.

Using the five years of data, we developed a series of fat and cholesterol awareness variables based on survey respondents answers to a series of questions about their knowledge of the impact of dietary fat and cholesterol intake. Table 1 presents a listing of the questions used for the 1994 data. For each problem type the respondent was asked whether or not he(she) had heard of problems associated with excessive fat (cholesterol) intake. The number of correct answers were summed to generate a "fat (cholesterol)" knowledge index. We compared the values of these indexes over the 1989-1995 period for the main meal planner and found very little difference.

Table 1. Hypothetical Health Problems from Dietary Fat and Cholesterol Intake Used to Generate Fat (and Cholesterol)- Disease Indices

| Question Number | **Type of Health Problem** |
|---|---|
| 1 | arteriosclerosis, atherosclerosis, clogged arteries, coronary disease, hardening of the arteries, heart problems, heart attack |
| 2 | arthritis |
| 3 | breathing problems |
| 4 | cancer |
| 5 | colon problems, constipation, digestive problems, diverticulosis, irregularity |
| 6 | cavities, caries, tooth problems |
| 7 | diabetes, high blood sugar |
| 8 | fatigue, lack of energy, tiredness |
| 9 | blood pressure, hypertension |
| 10 | hyperactivity |
| 11 | kidney disease, renal disease |
| 12 | bone problems, rickets, osteoporosis |
| 13 | overweight, obesity |
| 14 | stroke |

Based on the correct answers to a subset of the questions relating the health problems shown in Table 1 to dietary fat and cholesterol intake, we developed "disease awareness" variables. Questions 1, 3, 9 and 13 were used for the fat awareness variable and 1 and 9 were used for the cholesterol awareness variable. Using the fat and cholesterol awareness variables as dependent variables we estimated a series of PROBIT equations using the 1989, 1990, 1991 and 1994 datasets. Table 2 provides an overview of the significance of household and individual characteristics on whether or not an individual is classified as being aware of the health implications of excessive dietary fat and cholesterol intake in determining fat or cholesterol intake.

Table 2. General Results Obtained From Awareness Probit Results

| Variable | Sign of Impact on Probability of Being Aware |
|---|---|
| Individual Age | - |
| Ratio of Household Income to Poverty Threshold Income | + |
| Percent of Household Members < 18 yr. old | 0 |
| Diagnosed with High Cholesterol | + |
| On a Lowfat Diet | + |
| Black | - |
| Hispanic | - |
| Asian | - |
| High School Dropout | - |
| Graduate School Degree | + |
| Smoker | 0 |

Besides attempting to explain the important determinants of disease awareness, we used the disease awareness variables as explanatory variables in several multivariate regression models of total fat, saturate fat and cholesterol intake using the CSFII data. The surprising result obtained from these analyses was the consistent lack of statistical significance of the fat awareness and cholesterol awareness variables.

### Factors Influencing the Timing of Cheese Purchases

The increasing availability of scanner data has made it possible to study the dynamics of the food purchase process at the household or individual level. The ability to match these purchase histories with individual and household characteristics makes such panel data especially useful for economic and public policy analysis. For the last decade, the Wisconsin Center for Dairy Research has had a continuing research effort focusing on understanding the determinants of the consumer market of U.S. and Wisconsin dairy products. A database containing detailed dairy product purchases of a panel of U.S. consumers has been developed. This database has been used to answer a series of questions about the impact of pricing and promotion policies on quantity consumed and the determinants of the adoption of new reduced fat varieties of traditional dairy products.

We continued developing this database to analyze the impact of nutrition and health concerns on consumer demand for dairy products. Thus, we obtained data for a 12 month period for 12,000 households for the 1994/95 period. When combined with the previous weeks of data the Wisconsin Center for Dairy Research has a detailed household dairy product consumer panel encompassing a 222 week panel observed over the March 1991-June 1995 period. Weekly data is available for each household in the panel. On each purchase occasion a panel member records: date, dairy product UPC code, expenditures, coupon use, the existence of in-store promotions, and quantity purchased. This recording process is conducted at home using a hand held UPC scanner.

Prior to obtaining the new 52 week update, we conducted several analyses to understand the important determinants of the timing and consumption of dairy products. The primary focus of this analysis was quantifying the impact of coupon-based promotion programs on the purchase of cheese. We estimated a series of duration models for four cheese classifications: all, processed, natural cheddar, and cottage cheese. We incorporate within these duration models household demographic and purchase characteristics that allow for the distribution of interpurchase times to vary across households. The results of likelihood ratio tests indicate that these characteristics are statistically significant factors influencing the distribution of interpurchase time.

A likelihood ratio test of the null hypothesis that coupon use has no impact on the timing of cheese purchases is clearly rejected. As hypothesized, the use of coupons result in reduced interpurchase times for all cheeses. This impact, however varies across cheese type, especially when considering the type of household doing the purchasing.

### Factors Influencing the Timing of Butter, Margarine and Blend Purchases

We examined the impact of household and purchase characteristics on the timing of purchases of butter, margarine and butter/margarine blends. The model we used is an extension of previous market research analyses that focused on the phenomenon of brand switching. The present analysis is an extension that examines the dynamics of the purchasing of foods that are substitutes for one another.

Although concerned with commodity definitions that are fairly broad, the methodology used here can be used by a variety of analysts such as those involved in evaluating the effectiveness of brand specific as well as generic advertising/promotion programs. In our example we simulated the impacts of a long term coupon based promotion program on purchase behavior. Although not available in the data used in this analysis, similar analysis can be used to examine the impacts of increased media exposure (e.g., print, radio, TV, etc. advertisements) on purchase dynamics. Since our focus is on food purchased for home consumption, this analysis could also be used to differentiate the purchase dynamics of households that spend a significant share of their food budget for consumption away from home compared to households that tend not to purchase food away from home.

We find that both purchase and household characteristics influence the profile of commodity purchases. We also find that the relative impact of these characteristics on switching behavior varies across switching regime. This information is important to market analysts who want to determine which households are the least likely to switch from one commodity to another regardless of promotion effort/price cut.

One shortcoming of the current analysis is a lack of good information about the level of health knowledge/awareness and its impact on switching behavior. To overcome the shortcomings of having to use a time trend variable as a proxy for health knowledge is to supplement the panel data with another data set such as USDA's Diet and Health Knowledge Survey.

This research effort is an initial step to develop dynamic models of household purchase behavior. The next step will be to incorporate the quantity purchased and to develop a dynamic variant of the traditional Heckman sample selection model. Then, we will be able to ask questions about the primary effect of commodity promotion— for example, is it a reduction in interpurchase time with little overall impact on commodity demand, e.g., a stockpiling effect?

### Impact of Household and Purchase Characteristics on the Timing and Quantity Decisions of Purchases of Cheese

To meet the third objective of the research project we attempt to link the nutrition information contained within the 1994 CSFII/DHKS with the food choice data contained within the Nielsen Dairy Product Consumer Panel. Under this objective we focus on the interaction of nutrition knowledge and food choice. Specifically, we show how knowledge concerning the fat content of foods influences the choice between varieties of a specific commodity differentiated by fat content. We are concerned with the role of dietary fat characteristics given that the purchase of reduced/fat free products are the most popular healthy food alternatives.

The commodity we analyzed is three varieties of fluid milk: whole milk (W), 2% milk (2%) and milk with less than 2% fat (<2%). We use milk in our empirical application given that the structural change in the demand for fluid milk in the U.S. has paralleled the increasing awareness of the health implications of excessive dietary fat intake. In 1970, whole milk accounted for more than 81% of per capita total fluid milk disappearance. In 1987, per capita disappearance of reduced fat milks exceeded that of whole milk for the first time. By 1995, whole milk represented less than 37% of total disappearance (USDA, 1996).

Our analysis improves upon previous research by allowing for the simultaneous determination of the level of nutrition knowledge and food choice, investigating the impact of this knowledge on the demand for specific foods and using a methodology that incorporates independently collected data sets.

For this study, we used the 1994 USDA Diet and Health Knowledge Survey (DHKS) to obtain information about individual nutrition knowledge status. Previous analysis of the role of health and nutrition knowledge on fat and cholesterol intake have used the companion Continuing Survey of Food Intake of Individuals (CSFII) to obtain estimates of the impacts of such knowledge on nutrient intake. There are obvious problems with using a 2 or 3 day food intake diary to provide an accurate representation of typical intakes. For example, intake may vary depending on the days encompassed (e.g. weekday versus weekends), there may be seasonal differences in intakes, there may be differences in intakes depending on household food stocks, etc. To overcome these shortcomings, we improve previous research by using the 1994 component of the Nielsen Dairy Product Consumer Panel which contains estimates of annual fluid milk purchases. Although the DHKS and the purchase data are collected independently, we adapt an econometric model which uses both to examine the linkage between nutrition knowledge status and food choice.

Previous analyses of the role of health knowledge in determining nutrient intake have differentiated the roles of knowledge, awareness of health related problems and nutrition attitudes in determining nutrition intake. Our present analysis focuses solely on the role of a specific measure of nutrition knowledge, however, the model developed here can easily be extended to incorporate other definitions of health knowledge and awareness. Our analysis pertains to fluid milk purchases, and we measured nutrition knowledge relative to the respondent's knowledge of fat content of foods. A series of 17 questions were used as the basis of our health knowledge indicator. Table 3 provides a listing of the questions used and the percent of the DHKS respondents who provided correct answers. An individual is considered to be "knowledge" for this analysis if the individual had greater than the median number of correct answers to the above 17 questions.

From the results of the milk choice component we obtained statistically significant nutrition knowledge coefficients.That is, the greater the likelihood of being classified as knowledgeable, the less likely a household is to purchase whole and more likely to purchase <2% milk.

With the exogenous variables set at their sample means, we calculate the elasticity of a change in knowledge probability on milk purchase probabilities (Table 3). The effect of a change in the nutrition knowledge probability generates an inelastic negative response on the unconditional probability of purchasing whole milk and positive inelastic response on the <2% milk purchase probabilities. Elastic responses were obtained in the conditional probabilities of purchasing only one milk type for whole (-1.02) and <2% (1.04) milk.

Combining 2% and <2% milk into a single "reduced fat" milk category we see that an increase in the nutrition knowledge probability generates a positive and relatively large increase in the probability of a household purchasing not purchasing whole milk.

## Publications/Presentations

Brian W. Gould, 1997. The Role of Nutrition Knowledge in Food Purchase Decisions:
Evidence from Two Complementary Data Sets, submitted for publication in the American Journal of Agricultural Economics

Table 3. Elasticity Impacts of Changes in Knowledge Probabilities on Fluid Milk Choice

| Choice Probability | Elasticities |
|---|---|
| Purchase Whole Milk | -0.620 |
| Purchase 2% Milk | -0.063 |
| Purchase <2% Milk | 0.273 |
| Purchase Only Whole Milk | -1.021 |
| Purchase Only 2% Milk | -0.201 |
| Purchase Only <2% Milk | 1.041 |
| Purchase Only Reduced Fat | 0.656 |

Note: All elasticities are evaluated at the means of the exogenous variables.

Brian W. Gould, 1997. Factors Affecting the Timing of Purchases of Butter, Margarine and Blends: A Competing Goods Analysis, American Journal of Agricultural Economics, 2nd revision.

Brian W. Gould, 1997. Consumer Demand For Butter, Margarine and Blends and The Role of Purchase and Household Characteristics, Canadian Journal of Agricultural Economics, accepted for publication.

Brian W. Gould, 1997. Consumer Promotion and Purchase Timing: The Case of Cheese, Applied Economics, 29: 445-457.

B.W. Gould, 1997. Cheese Demand in the U.S.: An Analysis Using Household Purchase History, paper presented at the 1997 American Agricultural Economics Association Annual Meeting, Toronto Canada, July,1997.

*INTERIM REPORT*

# Structure and function relationships during melting and cooling of lower fat cheeses

**Personnel**

S. Gunasekaran, associate professor, Ag Engineering, R. Subramanian, research assistant, Department of Food Science, and N. F. Olson, professor emeritus, Department Food Science

**Funding**

Wisconsin Milk Marketing Board UW9504

**Dates**

December 1995 – December 1998

## Objectives

Determine and evaluate the changes in the microstructure quantitatively using the confocal laser scanning microscopy and digital image processing techniques.

Measure fundamental rheological parameters via transient and dynamic viscoelastic experiments.

Determine melt and flow characteristics via objective rheological tests and empirical methods.

Develop hypotheses to explain changes in functional properties in terms of microstructural and rheological properties, compositional factors and chemical changes in the cheese.

## Summary

Regular (32.3% fat) and reduced-fat (19.4% and 10.2% fat) Cheddar cheese blocks were vacuum packaged in plastic bags and ripened at 6-8˚C. Rheological measurements were taken from 2 to 6 weeks after manufacture. In addition, we also used two commercial cheeses —a low-moisture, part-skim Mozzarella and a process cheese. At the time of measurement, disk-shaped samples (mean thickness of 2 - 3 mm and diameter of 20 mm) were cut from refrigerated cheese blocks using a borer and a cutter. The linear viscoelastic properties were studied using a Bohlin constant-stress (CVO) rheometer with a 20-mm diameter parallel plate measuring system. Semi-coarse sandpaper was glued to the upper plate to prevent sample slippage during measurement. Also, the exposed sides of the sample were coated with mineral oil to minimize moisture and drying during measurement.

Stress sweep measurements were performed at a frequency of 9.43 rad/s to obtain the linear viscoelastic range. This is crucial because all subsequent measurements (dynamic and transient) have to be performed within the linear viscoelastic range. Frequency sweep (dynamic) measurements were made over two decades of frequency (0.628 to 62.8 rad/s). Creep and recovery (transient) measurements were performed for 240 s and 180 s, respectively. Heating and cooling ramp measurements were made at a frequency of 9.43 rad/s and at a rate of 3˚C/min. The samples in the rheometer were heated from a starting temperature of 10˚C up to 40˚C for regular-fat Cheddar and process cheeses and reduced-fat process, and up to 60˚C for reduced-fat Cheddar and low-moisture, part-skim Mozzarella cheese. They were then held at that temperature for 30 min before cooling to 10˚C. In addition to this experiment, both Cheddar and Mozzarella cheese samples were also heated to 80˚C and 100˚C in a convection oven and cooled to room temperature (25˚C). Both methods (rheometer temperature ramps and convection oven) were performed to mimic actual conditions of heating and cooling. The linear

viscoelastic properties of convection oven heated and cooled samples were then compared to the rheometer heated and cooled samples to study the effect of heating and cooling.

For both Cheddar and Mozzarella cheeses, the linear viscoelastic range of shear stress decreased with increasing age and temperature. Also, the linear viscoelastic range increased with decreasing fat level for all types of cheeses studied. The linear viscoelastic properties (both dynamic and transient) for regular- and reduced-fat Cheddar cheese and low-moisture, part-skim Mozzarella cheese decreased with age, indicating cheese softening due to proteolysis. The linear viscoelastic properties of process cheese were lower than both Cheddar and Mozzarella cheese because of weaker protein-mineral network structure. Rheological measurements at 25˚C showed that, for both Cheddar and Mozzarella cheese, convection oven heated and cooled samples has lower dynamic moduli ($G'$ and $G''$) and higher creep and recovery shear compliance than rheometer heated and cooled samples. This indicates that samples heated and cooled in the convection oven were softer than samples heated and cooled in the rheometer.

As far as convection oven heated and cooled samples are concerned, the room temperature linear viscoelastic properties of samples heated to a temperature of 100˚C were higher (and hence harder) than those heated to a temperature of 80˚C. This is probably due to a higher loss of fat from samples heated to 100˚C than those heated to 80˚C. This trend was observed for regular- and reduced-fat Cheddar cheese as well as low-moisture, part-skim Mozzarella cheese.

*INTERIM REPORT*

# Machinability of reduced and lowfat cheeses

**Personnel**

S. Gunasekaran, associate professor, Biological Systems Engineering, Department of Food Science, K. Muthukumarappan, research associate, Department of Food Science, Biological Systems Engineer, and N. F. Olson, professor emeritus, Depart,ment Food Science

Funding
Dairy Management Inc. GNO96

**Dates**

July 1995 – June 1998

## Objectives

Most of the cheese used as a food ingredient is in one of the following machined forms: shredded, diced, grated, or sliced. Cheeses manufactured in large blocks are cut into smaller pieces for direct sales as table cheese or for other process operations such as shredding. In 1995, shredded cheese was the fastest growing of all categories of natural cheese market (about 10%) accounting for nearly on billion dollars in sales. However, information on mechanical properties of cheeses relative to the machining operations is very limited. The specific objectives of this study are to characterize the following mechanical properties of Cheddar cheese as a function of composition (fat: 0-35.5% and moisture: 44-55%) and age (1-12 wk):

Tensile fracture stress using uniaxial tension and three-point bending tests

Apparent compression modulus using uniaxial compression test

Specific fracture energy using wire-cutting test

## Summary

Cheddar cheese samples of varying fat contents (8.0, 14.5 and 35.5%) and varying moisture contents (44, 47, 50 and 55% wet basis) were manufactured in the University of Wisconsin dairy plant. They were tested at 1, 3, 6, 9 and 12 wk after manufacture. We used uniaxial compression, tension, wire cutting, and bending tests. All the experiments were conducted in an Instron (Model 1130) Universal Testing machine equipped with a 222.4 N load cell and a data logger. All the experiments were conducted at room temperature (21˚C). The crosshead speed for all tests was 5.1 cm/min to characterize the mechanical properties of cheeses. Statistical significance of the data was tested by a multifactor analysis of variance. The chemical composition of the cheese samples is presented in Table 1.

Table 1. Chemical composition of Cheddar cheeses

| Sample | pH | Fat, % | Moisture, % | Salt, % |
|---|---|---|---|---|
| 1 | 5.17 | 35.5 | 38.4 | 1.18 |
| 2 | 5.22 | 14.5 | 50.3 | 1.68 |
| 3 | 5.12 | 8.0 | 51.8 | 1.48 |
| 4 | 5.41 | 5.3 | 44.2 | 2.33 |
| 5 | 5.21 | 5.1 | 47.4 | 2.24 |
| 6 | 5.17 | 3.8 | 50.0 | 2.15 |
| 7 | 5.13 | 0.0 | 54.9 | 2.55 |

Uniaxial Compression Test - Cheese samples with dimensions of 19.96 (dia.) x 19.96 mm (height) were prepared. Apparent compression modulus (kPa) was determined by the tangent method.

Uniaxial Tension Test - Cheese samples with dimensions of 60 x 7 x 7 mm (length x width x thickness) were prepared. Apparent tensile fracture stress (kPa) was calculated at the fracture point.

Three-Point Bending Test - Cheese samples of dimensions 51 x 21 x 17 mm were prepared. Critical tensile fracture stress (kPa) was calculated at the fracture point.

Wire-Cutting Test - Cheese samples of dimensions 25.4 (Length) x 25.4 (Height) x 12.5, 18.7, 25.4, 31.6, 37.9 mm (Breadth) were prepared. Stainless steel spring-tempered wires of different diameters (0.46 to 1.4 mm) were used. Specific fracture energy (J/m2) was estimated from the force-deformation data

The variations in apparent modulus with moisture, fat and age are presented in Figure 1.

The uniaxial compression test showed that the apparent modulus determined by the tangent method increased (0.09 to 1.06 MPa) with decreased fat content (35.5 to 8.0%) and decreased (2.46 to 0.13 MPa) with increased moisture content (44 to 55%) of cheese. In general, the modulus decreased during maturation (1-12 wk).

Figure 1. Apparent modulus of Cheddar cheese as a function of fat, moisture content (MC), and age.

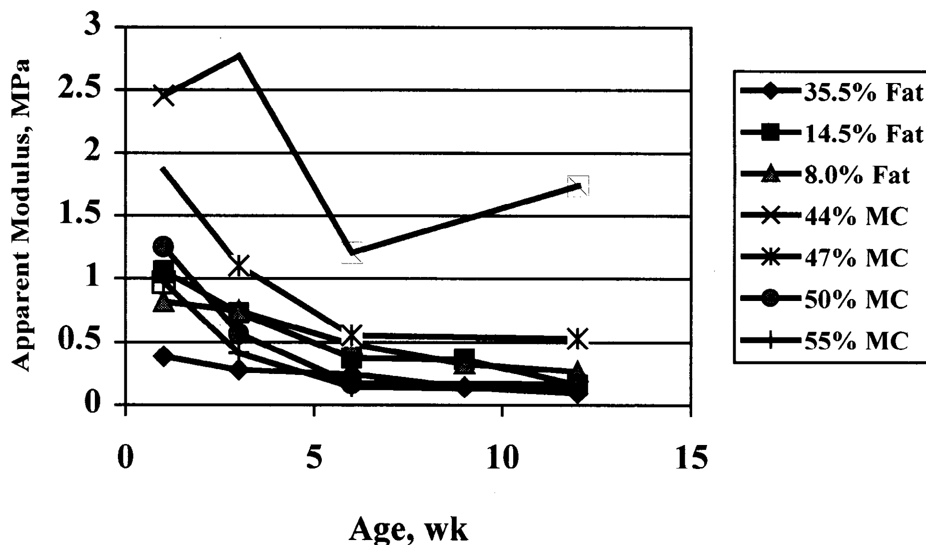
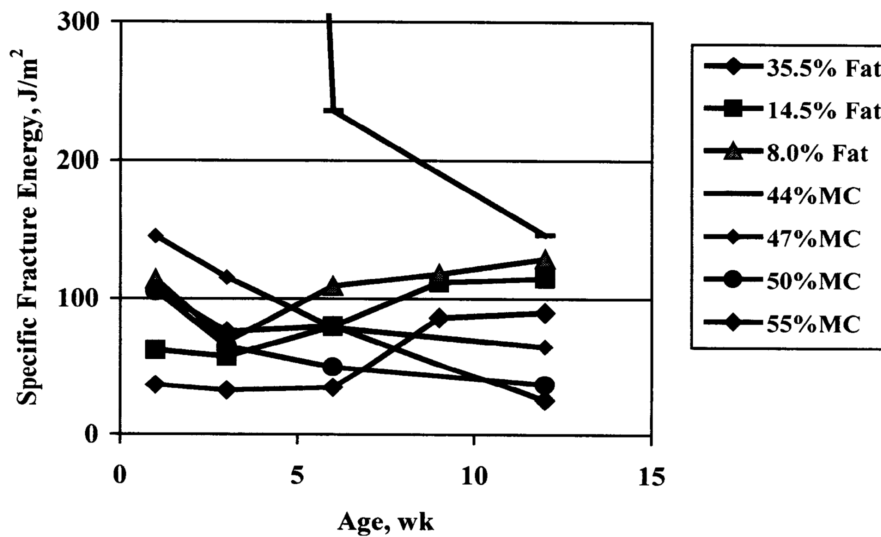



Figure 2. Specific fracture energy of Cheddar cheese as a function of fat, moisture content (MC) and age.




The variations in specific fracture energy (wire cutting test) are presented in Figure 2. The specific fracture energy increased (32.6 to 128.7 J/m2) with decreased fat content and decreased (1366 to 25.3 J/m2) with increased moisture content of cheese. The trend was mixed during maturation.

The three-point bending test showed that the tensile fracture stress at failure increased (72 to 165.7 kPa) with decreased fat content and decreased (633.8 to 68.4 kPa) with increased moisture content of cheese (Tables 2 &3). In general, the fracture stress decreased during maturation (1-12 wk) for all the cheeses except for 44%MC and 35.5% fat cheeses.

The uniaxial tensile test showed that the tensile fracture stress at failure increased (28.1 to 74.9 kPa) with decreased fat content and decreased (136.7 to 77.3 kPa) with increased moisture content of cheese (Tables 4 & 5). The fracture stress did not vary significantly during maturation.

Table 2. Variations of tensile fracture stress of Cheddar cheeses with moisture content and age (Three point bending test)

| Age, wk | 44%MC | 47%MC | 50%MC | 55%MC |
|---|---|---|---|---|
| 1 | $562^{g}$ | $310^{f}$ | $126^{d}$ | $103^{c}$ |
| 3 | $543^{g}$ | $172^{e}$ | $116^{c}$ | $128^{d}$ |
| 6 | $634^{h}$ | $206^{e}$ | $89^{b}$ | $68^{a}$ |
| 12 | $277^{f}$ | $99^{c}$ | $76^{a}$ | $71^{a}$ |

Table 3. Variations of tensile fracture stress of Cheddar cheeses with fat and age (Three -point bending test)

| Age, wk | 35.5%Fat | 14.5%Fat | 8%Fat |
|---|---|---|---|
| 1 | $72^{a}$ | $148^{e}$ | $166^{f}$ |
| 3 | $78^{a}$ | $121^{d}$ | $132^{d}$ |
| 6 | $78^{a}$ | $112^{c}$ | $109^{c}$ |
| 9 | $77^{a}$ | $86^{b}$ | $77^{a}$ |
| 12 | $77^{a}$ | $88^{b}$ | $75^{a}$ |

Table 4. Variations of tensile fracture stress of Cheddar cheeses with moisture content and age (Uniaxial tension test)

| Age, wk | 44%MC | 47%MC | 50%MC | 55%MC |
|---|---|---|---|---|
| 1 | $124^{a}$ | $99^{b}$ | $77^{c}$ | $102^{b}$ |
| 3 | $137^{a}$ | $127^{a}$ | $108^{b}$ | $127^{a}$ |
| 6 | $102^{b}$ | $112^{b}$ | $99^{b}$ | $90^{b}$ |
| 12 | $129^{a}$ | $132^{a}$ | $104^{b}$ | $82^{c}$ |

Table 5. Variations of tensile fracture stress of Cheddar cheeses with fat and age (Uniaxial tension test)

| Age, wk | 35.5%Fat | 14.5%Fat | 8%Fat |
|---|---|---|---|
| 1 | $36^{a}$ | $47^{b}$ | $70^{c}$ |
| 3 | $43^{a}$ | $57^{b}$ | $60^{b}$ |
| 6 | $39^{a}$ | $49^{b}$ | $53^{b}$ |
| 9 | $28^{a}$ | $75^{b}$ | $70^{b}$ |
| 12 | $38^{a}$ | $54^{b}$ | $38^{a}$ |

*INTERIM REPORT*

# Effect of water distribution on physical properties of pizza cheese and LMPS Mozzarella cheese during early stages of maturation and freezing and thawing

**Personnel**

S. Gunasekaran, associate professor, Biological Systems Engineering. K. Muthukumarappan, associate researcher, Biological Systems Engineering, M.E. Anderson, associate instrument researcher, Biological Systems Engineering. C. Chen, researcher, CDR, M.E. Johnson, senior scientist, CDR,

**Funding**

Dairy Management Inc. BJ23

**Dates**

September 1997 – August 1999

## Objectives

Quantify the amount of free moisture (expressible serum) and its distribution in LMPS Mozzarella and pizza cheeses during the early stages of maturation;

Study the redistribution of water in cheese protein matrix during freezing and thawing

Study the effects of freezing and thawing on various physical properties of block and shredded forms of LMPS Mozzarella and pizza cheeses during early stages of maturation and up to 6 wk of aging.

Evaluate interrelationships among the cheese type, composition, water distribution, age, and freezing and thawing.

## Summary

In young Mozzarella cheese, accumulation of water in large columns of void space contributes to poor water-holding characteristics. Thus, during early stages of maturation (~ first 10 days post manufacture) the Mozzarella cheese exudes free moisture at the block and freshly cut surfaces, making it unsuitable for shredding and melting. Soon after its manufacture, the block and/or shredded Mozzarella cheese is frozen to improve cheese production and handling. The distribution of water phase in the cheese during freezing and thawing plays a major role in altering melt/flow and shredding characteristics of the cheese and affects the oiling-off and blister formation when used on a pizza pie. Closely monitoring the water phase in the cheese, its redistribution during early stages of maturation and freezing/thawing, and the concomitant changes in cheese properties will enable us to formulate methods to optimize the desirable end-use properties of the cheese.

We will quantify the amount of free moisture (expressible serum) in LMPS Mozzarella and pizza cheese during early stages of maturation. We will then investigate the distribution/redistribution of water in the protein matrix during early stages of maturation and during freezing and thawing using the MRI techniques. We will evaluate a number of physical properties important for end-use applications of cheeses. They include meltability, stretchability, shreddability, oiling-off, and blister formation. These properties will be evaluated using both block and shredded forms of cheeses. The changes in physical properties will be evaluated in terms of amount and distribution of water in the cheese matrix, cheese age (up to 6 wk), and freezing and thawing. We will manufacture LMPS Mozzarella and pizza cheeses of varying composition (three levels of fat and moisture) and use in all experiments. In conjunction with another DMI

project, we are investigating the effect of adding denatured whey protein, a water binding agent, on water distribution and physical properties. The factors we will consider will be comprehensive review of all major factors affecting physical properties of young LMPS Mozzarella and pizza cheese.

*INTERIM REPORT*

# Investigating reasons for hardening of reduced-fat Cheddar cheese during heating


### Personnel

S. Gunasekaran, associate professor, Biological Systems Engineering, N. F. Olson, professor emeritus, Dept. of Food Science C. Chen, researcher, CDR, M. E. Johnson, senior scientist, CDR, and S.Y. Kim, research assistant, Biological Systems Engineering



### Funding

Dairy Management Inc. CW25


### Dates

September 1997 – August 2000

## Objectives

To evaluate the effects of heat treatments on the nature and extent of different protein interactions: hydrophobic interactions, hydrogen bonding, and ionic bonding.

To evaluate effects of heat treatments on water binding (free water vs. bound water) in the cheese structure with the help of NMR (nuclear magnetic resonance) techniques.

To evaluate the usefulness of certain emulsifying agents (KCl and Na-Citrate) and a surfactant (Tween 20) in reducing hardening of the lower-fat cheeses.

To evaluate if the experimental approaches proposed in this project will help in alleviating the skin formation which occurs when very low-fat cheeses are heated.

To evaluate possibility of independently controlling the meltability and firmness of the cheese by combined use of chymosin and the enzyme from *C. parasitica.*

## Summary


We have determined that the viscosity of melted reduced-fat Cheddar cheeses increases when the softened/melted cheese is held at high temperatures before it is allowed to flow. The increase in viscosity of melted cheese, which seems to depend on the length of holding time at the melting temperature, is definitely an unfavorable change in texture for consumers. Apparently some temperature-induced physicochemical changes occur during the holding time contributing to the increased toughness of the cheese.

Currently, we are investigating a number of possible physicochemical changes that might occur during heating and melting of cheeses in order to explain the undesirable toughness in reduced-fat cheeses. We will investigate the effect of relative hydrolysis of $\alpha_{s1}$- and ß-casein in independently controlling meltability and firmness of Cheddar cheeses.

*INTERIM REPORT*

# Temperature profiles of cheeses during melting in convection and microwave ovens

**Personnel**

S. Gunasekaran, professor, Biological Systems Engineering, K. Muthukumarappan, research assistant, Department of Food Science, and L. T. Marschoun, Biological Systems Engineering

**Funding**

Dairy Management Inc. GNM97

**Dates**

July 1996 – June 1997

## Objectives

To determine thermal (heat capacity, thermal conductivity, thermal diffusivity) and dielectric (dielectric constant and loss factor) properties of cheeses as a function of cheese composition and age.

To compare the experimentally determined thermal properties against composition based thermal properties predictive models.

## Summary

Cheddar cheeses of various compositions (Table 1) were manufactured at the UW Food Science Department Dairy Plant.

Thermal conductivity (k) was determined by the line heat source method and thermal diffusivity (a) was determined by the Dickerson method. The necessary probes and instrumentation designed and fabricated at the Biological Engineering Department's machine shop. Heat capacity (C) data were collected using a DSC (Netzsch) available in the Chemical Engineering Department. In addition, density (r) of the samples was determined to calculate a using the experimental k and C values.

The following models were developed for predicting thermal properties of Cheddar cheese based on its composition:

$$k = 0.112+0.673\ X_W + 0.197X_F - 0.075\ X_P$$

$$a = (2 - 4.74\ X_W - 0.335\ X_F - 4.64\ X_P + 7.78\ X_W X_P - 12.1\ X_F X_P) \times 10^6$$

$$C = 9.74 + 15.1\ X_W + 17.8\ X_F - 98.9\ X_P - 101\ X_F^2 + 159\ X_P^2 + 216\ X_F^3$$

Where $X_W$, $X_F$, $X_P$ are mass fractions of water, fat, and protein respectively.

Table 1. Composition of different Cheddar cheeses used.

| Moisture (%) | Fat (%) | Protein (%) | Salt (%) | pH |
|---|---|---|---|---|
| 44.7 | 5.3 | 39.6 | 2.33 | 5.41 |
| 49.5 | 5.1 | 35.8 | 2.24 | 5.21 |
| 52.8 | 3.8 | 33.3 | 2.15 | 5.17 |
| 56.3 | 0.0 | 33.6 | 2.55 | 5.13 |
| 37.6 | 32.2 | 24.8 | 1.30 | 5.12 |
| 44.9 | 19.7 | 29.0 | 1.33 | 5.11 |
| 49.5 | 10.5 | 32.8 | 1.52 | 5.12 |
| 46.1 | 21.7 | 26.2 | 1.70 | 5.25 |

These models are to be fully validated with new set of cheeses before they can be generally applied. As we get new cheeses for other projects, we plan to collect thermal properties data for validation and possible improvement of the models.

The dielectric properties, dielectric constant (e') and loss factor (e'') were determined using the open-ended probe technique using a Hewlett Packard Network Analyzer series 8753 with HP85046 S-Parameter Test Set. The samples (3 x 3 x 6 cm) were equilibrated to room temperature (21- 24˚C). The data were collected at the two common microwave frequencies – 915 and 2450 MHz.

In general, we have observed that e' and e'' increased with moisture content. But the dielectric properties decreased with fat content due to low dielectric activity of the fat. At low levels of protein (up to 35%) the positive correlation between total protein and dielectric properties is observed indicating the surface charge effect is dominant. At high levels of protein, molecular folding dominates the total dielectric activity of cheese resulting in a significant effect of total protein on the dielectric properties of Cheddar cheese.

*INTERIM REPORT*

# Characterization of melt and flow properties of cheeses

**Personnel**

S. Gunasekaran, professor, Biological Systems Engineering, A. J. Giacomin, M.E. Johnson, senior scientist, CDR, Y-C. Wang, and S. Tariq

**Funding**

Wisconsin Milk Marketing Board UW9602

**Dates**

July 1996 – June 1998

## Objectives

To develop method(s) to measure softening, melting and flow properties of cheeses in terms of fundamental engineering principles, and

To investigate the physico-chemical and technological reasons that govern softening, melting, and flow of cheeses.

## Summary

A device to objectively measure the melt and flow behavior of cheeses at different temperatures was designed and developed. It consists of a temperature-controlled heater, an LVDT (linear variable differential transformer), and a personal computer with a data acquisition system. This device, named UW-Meltmeter, operates in the squeeze-flow configuration and can either be operated alone at a constant force or in conjunction with a uniaxial compression device at a constant deformation rate (i.e., constant cross-head speed). Flow of melted cheese under two constant forces (0.7 and 0.9 N) and two constant deformation rates (0.5 and 2.5 cm/min) were studied. Biaxial extensional strain rate and biaxial extensional viscosity were calculated. Mozzarella cheeses of two fat contents (14 and 43%) were studied at two temperatures (40 and 60˚C). The results supported the expected trend of increased meltability at higher fat levels and at higher temperatures.

A modification of the above device and test procedure was used to determine the softening point of the cheese. We defined the softening point as the point of transition at which the viscosity of the cheese when continuously heated changes from low to high.

We also studied the non-linear viscoelasticity of cheese using large amplitude oscillatory shear (LAOS) tests. The first specimens to be studied were processed Mozzarella cheese singles obtained commercially. Both fat free and full fat Mozzarella were evaluated. LAOS tests were conducted for strain amplitudes ranging from 0.25-11, test temperatures of 30˚C and 35˚C, and a test frequency of 0.25 Hz. The cheese melted at about 40˚C. Results were obtained in the form of stress versus rate-of-strain loops and amplitude spectra for increasing strain amplitudes were also plotted. Comparisons were made to the Lodge rubber-like liquid theory to establish the amount of nonlinearity. The discrete relaxation spectra for the cheese were obtained from dynamic data using IRIS. Future work on process Mozzarella cheese will include developing constitutive equations governing the LAOS behavior.

*FINAL REPORT*

# Influence of lipolytic reactions in cheese on flavor and texture development


### Personnel

Sithian Pandian, adjunct professor, Universite Laval, Quebec, Canada; Mark E. Johnson, senior scientist; Carol Chen, researcher; John Jaeggi, associate researcher; Bill Tricomi, assistant researcher; Marianne Smukowski, research apecialist, Center for Dairy Research.



### Funding

Wisconsin Milk Marketing Board 92-14


### Dates

March 1993 - February 1995

## Objectives

(Wisconsin Objectives)
To prepare pilot lab-scale cheeses by adding lipase producing lactobacilli and to follow the course of development of the organoleptic properties.

## Summary


Full fat and 50% reduced fat Cheddar cheeses were made. The variables tested were control (no added Lactobacilli), freeze-shocked (2% addition) or direct culture addition (.002%) of either a Lactobacilli with a high esterase activity (wild strain) or its mutant with a low esterase activity. Three vats of each variable as well as control vats (no added lactobacilli) were made at each fat level. The cultures were grown in MRS broth (Difco) at 37˚C for 12-14 hr. Cells for direct culture addition were not grown under pH control nor were they frozen but were used directly after the growth period.

The cell stock for freeze-shocked addition were allowed to grow to pH < 5.0, were then neutralized with ammonium hydroxide to pH 6.5 -7.0 and allowed to grow once again to pH < 5.0, and were then neutralized as before. After the second base addition the cells were allowed to grow without further neutralization (final pH < 5.0). The cells were harvested by centrifugation and washed twice with cold .1 M phosphate buffer. The cell pellet was resuspended in .1 M phosphate buffer and frozen at -20˚C for 2 days. The cells were thawed just prior to addition to the cheese milk. The 2% addition refers to the amount of MRS media in which the cells were grown based on 2% of the weight of milk used for cheese making. Thus for 227 kg of milk, all the cells harvested from 10.4 kg MRS broth and added to the milk. The amount of cells added to the cheese milk were as follows: for direct addition 5 ml per vat (1-3 x $10^8$ per ml) for both strains and for frozen cells 500 ml per vat (2-4 x $10^{11}$ per ml).

The small differences in manufacture and in cheese composition that could not be attributable to the added Lactobacilli. As expected, TCA and PTA soluble nitrogen increased with time and increased faster in cheeses with added Lactobacilli. Descriptive taste panels on the full fat Cheddar cheeses showed that there were no differences between cheeses made with wild or mutant cells. Experimental cheeses had distinctly more Cheddar cheese flavor intensity and slightly softer body than the control cheeses. However, the smoothness of the cheeses was not affected by the added cells. Also there was a trend that the cheeses made with frozen cells had more Cheddar flavor intensity than cheeses made with direct culture addition. No rancidity nor sulphide flavors were noted in full fat Cheddar cheese. Body and texture and flavor preference scores were similar for all cheeses with the one exception that at 6 months the cheese made with the viable wild Lactobacilli cells had a lower flavor preference score.

In the 50% reduced fat Cheddar cheeses the experimental cheeses had more Cheddar flavor intensity, but a slight rancid flavor (this trend continued through 6 months), and more atypical flavors than the control cheeses. However, there were no differences in overall flavor preference between any of the cheeses. At 6 months there was a slight increase in the body and texture preference scores for all experimental cheeses over the control cheeses. There were no differences in attributes between cheese made with wild or mutant cells frozen or by direct addition.

The addition of the lipase positive *Lactobacillus casei pseudoplantarum* had a positive effect on the flavor of full fat Cheddar cheese. Although the addition of the Lactobacilli increased Cheddar flavor in reduced fat Cheddar, there was a slight decrease in the overall quality of the flavor. Most of the decrease is due to off-flavors such as unclean flavors rather than rancidity since the intensity of the rancid flavor was very slight. The reasons for an increased Cheddar flavor intensity is not known. It could be due to the limited release of free fatty acids, an increase in proteolysis or to an unrecognized metabolic pathway. Chemical analysis was unable to verify that an increase in fatty acid level was responsible for the increase in Cheddar flavor.

Recent experiments in an unrelated project using the wild strain in the manufacture of 50% reduced fat Cheddar cheese and a different starter culture has been extremely promising. The new starter (same one used in the full fat Cheddar manufacture) rapidly utilizes the residual sugar in the cheese and this appears to influence the effectiveness of *Lactobacillus casei pseudoplantarum*. There was a substantial decrease in off-flavors but a retention in the increase in Cheddar flavor intensity. In another study (Wisconsin-Utah collaboration) it was clearly shown that there is a synergism between the starter culture and adjunct Lactobacilli in the development of off-flavors in cheese. (the strain used in that study did not include *Lb. casei pseudoplantarum*). We believe that there is great potential for the strain used in this study to improve the flavor of reduced fat cheese and we plan to investigate the metabolism of this strain in detail. It is our plan to eliminate the negative metabolic activity of the strain but retain the desired attributes.

*INTERIM REPORT*

# Lower-fat Swiss cheese: evaluation of free fatty acid concentration on the development of flavor

**Personnel**

Mark E. Johnson, senior scientist, Carol Chen, researcher, Wisconsin Center for Dairy Research

**Funding**

Dairy Management Inc. 133-A296

**Dates**

July 1997 - December 1998

## Objectives

To develop manufacturing protocols for lower fat Swiss cheese (25% and 50% reduced-fat), with goals of mimicking the flavor and body of full fat Swiss cheese. The main focus will be to describe manufacturing techniques required to obtain an optimal balance of moisture, protein and minerals that allow for development of typical eye and flavor development for various fat reductions in Swiss cheese.

## Summary

A manufacturing protocol for 25% and 50% reduced fat Swiss has been established. The flavor of 25% reduced fat Swiss was similar to “full-fat” Swiss, but the 50% reduced fat Swiss was bland. Therefore, we decided to concentrate our efforts on the 50% fat reduced Swiss. Further cheese making has been delayed but cheese making is planned for early Spring.

Developing cheese making protocol for the manufacture of a lower fat Swiss cheese will be essential for assisting the cheese industry in manufacturing a quality lower fat Swiss cheese. Over the past several years, individual cheese manufacturers have contacted researchers at the Center for Dairy Research for expertise on lower fat Swiss cheese. This project allows researchers to effectively communicate and meet the needs of the cheese industry. In addition, monitoring volatile acids and free fatty acids in lower fat Swiss cheese will help to give insight as to why lower fat cheese often lacks the flavor development of the reference full fat counterpart.

*FINAL REPORT*

# Cheese making properties of milk from cows of different genotype


### Personnel

Robert Bremel, professor, Josie Lewandowski, research specialist, Department of Animal Science, Mark Johnson, senior scientist, Carol Chen, researcher, Amy Dikkeboom, research specialist, Bill Tricomi, assistant researcher, John Jaeggi, assistant researcher, Matt Zimbric, research specialist, CDR



### Funding

Dairy Management Inc. HLL 97


### Dates

June 1995 - June 1997

## Objectives

Select Holstein cows with the AA and BB kappa-casein genotype.

Manufacture cheese with segregated milks.

Sensory and chemical analysis of the cheese.

## Summary


In a herd of over 250 cows we found six cows that had the BB kappa casein genotype. Thirty cows had the genotype AA for kappa casein. Cheddar cheese was manufactured from milk obtained from Holstein cows with the AA or BB kappa-casein genotype. The milks were pooled from 3-6 individual cows and from at least two separate milkings. Five vats of cheese were made from each type of milk over three months. Only one vat of cheese was made from each milk each day. The average composition of the milks and cheeses are in Table 1. The milk from cows with the AA genotype were higher in protein, casein and fat than milk from cows with the BB genotype. The casein to fat ratio of the milk from the BB cows had a higher C/F ratio indicating more casein in relation to the milk from the AA cows. The higher casein and fat in milk from the AA cows resulted in higher cheese yield. The milk from cows with the BB genotype had slightly higher non-protein nitrogen as a percent of the total nitrogen than milk from cows with the AA genotype but the % casein as a percent of the true protein was higher (81.42 vs 80.43%). There was a slight trend towards more nitrogen recovery (protein) in milk from BB genotype cows. Fat retention was higher in cheese made from milk from BB genotype cows (91.3 vs. 88.5). This translated into higher FDM in cheese made from milk of the BB cows even though the C/F was higher in milk from BB genotype cows. Milk from cows of the BB genotype clotted faster and reached the desired firmness at cutting much faster than milk from cows with the AA genotype (19 vs 33 min). There was a trend towards higher moisture in cheeses made from milk from cows with the BB genotype even though the manufacturing schedules of all vats were similar.

Sensory analysis of the Cheddar cheeses indicated no differences between the cheeses that could be attributed to the genotype of the cow. Similarly thermal melt tests did not indicate any differences between cheeses that could be attributed to the milk from which the cheese was made. A new test that measures the softening point of the cheese indicated a trend towards a slightly higher softening point in cheeses made with milk from cows of the AA genotype. The significance of this, if any, has not yet been elucidated.

Low moisture part skim mozzarella was made on three separate occasions using milk from Holstein cows with AA, AB, and BB genotype for kappa casein. Mozzarella cheese was also manufactured from milk obtained from Brown Swiss cows with the BB kappa casein genotype (referred to as DD

milk). The milks from the AA cows clotted slower than the milks from the other cows (Table 2). The difference between the clotting times of AA and BB milks is much less than when Cheddar cheese was made because the pH at coagulant addition was much lower when making Mozzarella (6.55 for Cheddar and 6.35 for Mozzarella). No differences in any of the sensory attributes among the cheeses could be directly attributable to the type of milk from which the cheese was made. There is a trend towards a softer body in the cheese made from BB milk and a trend towards a less chewy and better elastic stretch when cheese made from BB milk was baked on a pizza.

In conclusion, lower solids in the milk from BB cows used in this work resulted in lower cheese yields compared to the higher solids milk from AA cows. In order to justify the breeding of cows with the BB kappa casein genotype, the casein and fat of the milk would have to be increased. At equal casein and fat levels of milk to that of cows with other kappa-casein genotypes, the milk from BB cows would be preferred for cheese making because of the higher fat retention. Milks from cows of different kappa-casein genotypes do not appear to offer any positive or negative attributes or characteristics to the cheese made from them.

Table 1. Composition analysis of milk and Cheddar cheese.

| | AA Milk | BB Milk |
|---|---|---|
| Standardized Milk | | |
| % Total Protein[1] | 3.26 ± .06 | 3.16 ± .05 |
| % True Protein[2] | 3.06 ± .06 | 2.96 ± .05 |
| % Casein | 2.49 ± .07 | 2.42 ± .02 |
| % Casein/%Total Protein | 75.57 ± .67 | 76.26 ± .73 |
| % Casein/% True Protein | 80.43 ± .36 | 81.42 ± .96 |
| % Fat | 3.59 ± .88 | 3.39 ± .09 |
| Casein/Fat | .69 | .72 |
| Make Sheet Information | | |
| Coagulant addition to cut[3] | 33 ± 3 min. | 33 ± 3 min. |
| pH at cut | 6.58 ± .03 | 6.52 ± .03 |
| Cheese Information | | |
| % Moisture | 38.40 ± .80 | 38.60 ± .60 |
| % Cheese Yield | 9.89 ± .41 | 9.60 ± .20 |
| % FDM | 52.10 ± 2.0 | 52.50 ± .70 |
| % Fat Recovery in Cheese | 88.50 ± .80 | 91.30 ± 1.50 |
| % Nitrogen Recovery in Cheese | 74.10 ± .40 | 74.60 ± .70 |

[1] % Total nitrogen X 6.35
[2] % Total nitrogen- non protein nitrogen X 6.35
[3] Coagulae cut at the same firmness

Table 2. Composition analysis of milk and Mozzarella cheese.

| | AA Milk | AB Milk | BB Milk | DD Milk |
|---|---|---|---|---|
| Standardized Milk | | | | |
| % Total Protein[1] | 3.18 ± .07 | 3.08 ± .06 | 2.91 ± .05 | 3.21 ± .04 |
| % True Protein[2] | 2.97 ± .07 | 2.87 ± .04 | 2.68 ± .03 | 2.97 ± .04 |
| % Casein | 2.33 ± .07 | 2.17 ± .15 | 2.05 ± .11 | 2.29 ± .11 |
| % Casein/%Total Protein | 73.19 | 70.62 | 70.27 | 71.22 |
| % Casein/% True Protein | 78.45 | 75.60 | 76.25 | 77.17 |
| % Fat | 2.63 ± .02 | 2.56 ± .22 | 2.52 ± .14 | 2.45 ± .12 |
| Casein/Fat | .88 | .85 | .81 | .93 |
| Make Sheet Information | | | | |
| Coagulant addition to cut[3] | 21 ± 3 min. | 19 ± 2 min. | 14 ± .2 min. | 15 ± .8 min. |
| pH at cut | 6.42 ± .02 | 6.41 ± .05 | 6.37 ± .02 | 6.40 ± .07 |
| Cheese Information | | | | |
| % Moisture | 45.95 ± 1.60 | 45.74 ± .86 | 45.77 ± .36 | 45.72 ± .37 |
| % Fat | 25.47 ± 1.05 | 24.97 ± 1.38 | 25.49 ± 1.8 | 24.10 ± 1.18 |
| % Protein | 24.85 ± .73 | 24.43 ± .84 | 23.63 ± .38 | 26.59 ± 1.16 |
| % FDM | 46.18 ± .94 | 46.05 ± 3.24 | 47.00 ± 3.14 | 44.40 ± 2.00 |

[1] % Total nitrogen X 6.35
[2] % Total nitrogen- non protein nitrogen X 6.35
[3] Coagulae cut at the same firmness

*INTERIM REPORT*

# Growth of nonstarter lactic acid bacteria in reduced fat Cheddar cheese

**Personnel**

Mark E. Johnson, senior scientist, Wisconsin Center for Dairy Research, James Steele, Department of Food Science, University of Wisconsin, and Jeff Broadbent, Food Science Department, Utah State University, Logan, Utah.

**Funding**

Dairy Management Inc. CW11

**Dates**

July 1997 - December 1999

## Objectives

To establish the population dynamics between starter, nonstarter, and adjunct bacteria during ripening of 50% reduced fat Cheddar cheese.

To construct derivatives of the adjunct *Lactobacillus casei* subsp. pseudoplantarum that are unable to co-metabolize citrate and lactate and to test the influence of the loss of this metabolism on the ability of the adjunct to grow in cheese.

To establish the impact that adjunct bacteria have on the sensory attributes of reduced fat Cheddar cheese by monitoring the relationship between growth of starter, adjunct and nonstarter bacteria and flavor attributes during aging of cheese with added adjunct bacteria.

## Summary

DNA primer sequences have been identified for the starter Lactoccocus strains and adjunct Lactobacillus sp. that will be used in cheese manufacture. This will allow us to identify each strain of added bacteria from non-starter bacteria. We will then be able to follow the individual growth and death of specific bacteria in cheese.

Experiments are being conducted on *Lactobacillus casei* strains to establish specific enzyme activity (L and D-lactate dehydrogenases), racemase activity, and citrate utilization. These strain (s) will be used in later cheese making experiments.

Cheese making is planned for the Fall-1998.

According to Objective 1 of the National Plan this proposal addresses Goal 1.1 via Tactic 3. We will be establishing a knowledge matrices relating flavor and the role of adjunct and non-starter microorganisms. We will do this by investigating the impact of adjunct bacteria on the growth of non-starter bacteria and flavor in reduced fat cheese.

*INTERIM REPORT*

# Identification of potential gas-forming bacteria in cheese

**Personnel**

Mark E. Johnson, senior scientist, CDR, John Luchansky, associate professor, Food Research Institute, Jeff Christenson, research assistant, Dept. of Food Science, Kristen Houck, research specialist, CDR

**Dates**

July 1996-June 1997

**Funding**

Dairy Management Inc. JLC 97

## Objectives

To determine the presence of gas forming bacteria in cheese using genus and species specific primers and polymerase chain reaction (PCR) amplification.

To test the validity of existing enumeration techniques for determining the presence of viable gas forming bacteria.

## Summary

The development of slits, undesirable eye or hole formation, and puffy packages due to gas formation by bacteria remains a problem for the cheese industry. Several genera of bacteria have been implicated including coliforms, propionibacteria, clostridia, leuconostoc, and facultative and obligate heterofermentative lactobacilli. Classical microbiological plating techniques have been used to differentiate or at least enumerate all of these bacteria when the causative organism was the dominant bacteria in a cheese. However, if the culprit organism is not the dominant bacteria, or is not viable, the problem of isolation becomes much more difficult. Classical methods may be inadequate to detect potential gas forming or heterofermentative bacteria including heterofermentative lactobacilli.

There is another method that is used to detect the presence of bacteria. It is called PCR amplification. Each genus, species, and serotype of bacterium have certain base sequences within their DNA that are unique for that particular bacterium. The first step prior to PCR amplification is to identify and reproduce that sequence in vitro and to use a portion of that sequence as a primer to make additional copies of the entire sequence. In PCR amplification, the primer is added to a mixture of DNA where it attaches to regions in the DNA that exactly match the base sequence of the primer. Under controlled conditions multiple (millions) copies of the primer are produced, i.e. amplification. The presence of the amplified primer can now be detected by electrophoresis and is conclusive proof that the particular bacterium is present in the sample. Using this technique, identification of bacterial strains can be accomplished. PCR amplification can be used to verify the presence or absence of all the other known potential gas formers in cheese.

DNA sequences for 16s rRNA were obtained for target species through an Entrez word search of the National Center for Biotechnology Information web site. Sequences were obtained for *Lactococcus lactis* ssp. *lactis, Lc. lactis* ssp. *cremoris*; *Leuconostoc mesenteroides*; *Pediococcus acidilactis*, *Pd. pentosaceus*, *Propionibacterium jensenii*, *Pb. thoenii*, *Lactobacillus acidophilus*, *Lb. helveticus*, *Lb. gasserii*, *Lb. brevis*, *Lb. buchneri*, *Lb. casei*, *Lb. paracasei*, *Lb. delbrueckii* ssp. *lactis*, *Lb. fermentum*, *Lb. plantarum* and *Lb. pentosus*. Downloaded sequences were grouped by species and compiled using the Genetics Computer Group programs to produce a consensus sequence showing perfectly conserved regions of the 16s rRNA for a given species. The resulting 16s rRNA consensus sequences were then compiled to determine regions of variability between species. Primers for PCR were developed from these

variable regions and used to search DNA sequence databases through the NCBI web site to determine the uniqueness of the proposed primers. All acceptable species specific primer pairs were ordered through the Gibco BRL custom primer web site.

Experimental determination of primer specificity was performed in duplicate with all the bacterial species listed above. Chromosomal DNA was extracted from pure cultures and screened using PCR with all species specific primers sets. The DNA products of the PCR (if any) were electophoresed, sized and quantified. The results of these screens were tabulated and indicated that the primers were specific for the targeted bacterial species (see Table 1). In some instances, primers not developed for a given species resulted in a PCR product However, the pattern of PCR products obtained allowed unambiguous identification of a given species, with the exception of the pediococci and propionibacterium. For example, if chromosomal DNA of an unknown cheese isolate is screened with the complete set of species specific primers, and the only product obtained is with the *Lb. brevis* primer set, the isolate would be identified as *Lb. brevis* However, if DNA bands are obtained with primer sets for *Lb. gasseri* *Lc. lactis* and *Leu. mesenteroides*, the isolate would be identified as *Lb. gasseri* on the basis of the known cross-specificity of this group of primers for the identified species (see Table 1, *Lb. gasseri* column) Optimization of PCR reaction conditions by altering concentrations of magnesium chloride or adjusting the PCR reaction temperatures is expected to elevate some of the variable results. Primers for Clostridia have been identified and are now being tested. Tests are now underway to isolate bacterial DNA directly from cheese. This would circumvent the need to grow bacteria on agar plates and it would verify the efficacy of traditional isolation techniques for gas forming bacteria.

Cheeses with gassy cheese defects have limited use —most go to processing. This represents a significant monetary loss to the cheese maker or cooperative. In order to prescribe appropriate control procedures, the bacteria responsible for the problem must be identified. In the past, there have been cases where the bacteria could not be identified. Now, the PCR technique can be used to identify not only individual species of bacteria but also individual strains or serotypes within a species. Consequently, their ecological niche (including bacteria in the starter, milk, or dairy plant environment) can be found. PCR amplification procedures would not be a routine test completed by individual cheese makers, but it is a methodology that commercial testing laboratories could use. This is a powerful tool that can be extremely useful to determine the bacteria that cause gassy defects in cheese. Also, it is more efficient than conventional plating—preliminary results are ready within two days. This is especially true in situations where classical microbiological methods are unable to provide answers after several days or, at best, give ambiguous results.

| Primers | Bacterial Species: *Lb. helveticus* | *Lb. gasseri* | *Lb. brevis* | *Lb. buchneri* | *Lb casei* | *Lb. paracasei* | *lb. delbruckeii* | *Lb. fermentum* | *Lb. plantarum* | *Lc. lactis* | *Leu. mesenteroides* | *Pd. acidilactis* | *Pd. pentosaceus* | *Pb.jensenii* | *Pb. thoenii* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Lb. acidophilus/helveticus* | ++ | | | | | | | | | | | | | | |
| *Lb. acidophilus/gasseri* | | ++ | | | | | +/- | | | | | | | | |
| *Lb. brevis* | | | ++ | | +/- | + | | | +/- | +/- | +/- | | | | |
| *Lb. buchneri* | | | | ++ | +/- | + | | | | | | | | | |
| *Lb casei/paracasei* | | | | | ++ | ++ | | +/- | | | | | | | |
| *Lb. delbruckeii ssp. lactis* | | | | | | | ++ | | | | | | | | |
| *Lb. fermentum* | | | | | | | | ++ | | | | | | | |
| *Lb. plantarum/ pentosus* | | | | | | | | | ++ | | | | | | |
| *Lc lactis ssp.lactis/cremoris* | | + | | | | | | | | ++ | +/- | | | | |
| *Leu. mesenteroides* | | ++ | | | +/- | | | | +/- | +/- | ++ | | | | |
| *Pd. acidilactis* | | | | | | ++ | | | +/- | +/- | +/- | ++ | ++ | | |
| *Pd. pentosaceus* | | | | | | ++ | | | +/- | +/- | +/- | ++ | ++ | | |
| *Pb.jensenii* | | | | | | | | | +/- | | | | | ++ | ++ |
| *Pb. thoenii* | | | | | | | | | | | | | | ++ | ++ |

**Table 1.**
Determination of primer specificity with bacterial species

++ Indicates a strong positive result from PCR
+ Indicates a weak positive result from PCR
+/- Indicates a variable result from PCR

*FINAL REPORT*

# Mechanisms for production of cheese flavor compounds

**Personnel**

Robert C. Lindsay, professor, Dept. of Food Science; Christine Nowakowski, graduate research assistant, Dept. of Food Science

**Funding**

Dairy Management Inc. LD294

**Dates**

July 1993 - December 1996

## Objectives

To chemically define mechanisms for the formation of Cheddar cheese flavor compounds from interactions of alpha-dicarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) with other cheese constituents(amino acids and peptides).

To investigate interactions between the alpha-dicarbonyl and the aromatic amino acid flavor systems in the development of Cheddar cheese flavors.

To relate findings from the research to the selection of lactic cultures used in the production of Cheddar-type cheeses.

## Summary

Isolates capable of producing alpha-dicarbonyls from earlier studies and additional isolates from a range of Cheddar cheese samples were evaluated for production of alpha-dicarbonyl compounds. Although the concentrations produced were variable, the ability to produce these compounds was found quite widespread in the lactic acid bacterial flora of Cheddar cheese. Thus, quantitative analyses of cultures of lactic acid bacteria documented the general relative abilities of these organisms to produce glyoxal, methylglyoxal, and diacetyl.

A specially developed method utilizing bisulfite as a trapping agent showed that substantial amounts of dicarbonyls react very rapidly with amino compounds after their production in culturing or cheese media. Dicarbonyls do not accumulate intracellularly, and are transported out of cells. Using this procedure, cultures of lactic acid bacteria have been selected for further experiments based on their relative abilities (low, medium, and high) to produce the respective dicarbonyls.

Survey studies showed that the dicarbonyls, especially methylglyoxal and glyoxal, increase in young Cheddar cheeses (1 month), and then steadily decline through 9 months aging. This period corresponds to that for aged cheese flavor development in full fat cheese, but it also coincides with the development of meaty-brothy off-flavors in reduced fat cheeses.

Since it would be desirable to control levels of precursors and the microbial population in model cheese system studies, the capability for eliminating active microbe populations in aging cheeses using gamma-irradiation at dry ice temperatures was investigated. This approach, employing sufficient exposures, effectively eliminated microbes, but some radiation-induced flavor compounds were simultaneously formed which limits the application. High-pressure sterilizations carried out by a service laboratory provided a alternate means to avoid some of the off-flavors encountered in irradiation treatments. Additionally, model Cheddar cheese slurry model systems containing introduced alpha-dicarbonyls were developed, and these systems exhibited enhanced formation of cheese flavor compounds.

Slow Maillard-type reactions initiated by the combination of alpha-dicarbonyls and free amino acids or peptides are involved the ultimate formation of important cheese flavor compounds. Methods were developed to quantitatively measure concentrations of the free alpha-dicarbonyls present in cultures and cheeses. However, because of the very reactive nature of the alpha-dicarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) with amino compounds in media and cheese, only surplus concentrations, and not total concentrations, have been measured. Using bisulfite as a competitive binding compound for amino acids, it has been possible to add sufficient levels of this compound to tie-up alpha-dicarbonyls and still permit growth of lactic acid ripening organisms. In this manner, it was possible to study rates of actual production of alpha-dicarbonyl compounds by lactic organisms.

In combination with this work, studies were carried out on the rate of reactions between selected amino acids (including among others, methionine, lysine and glycine) and alpha-dicarbonyls. Great variation was documented for reaction rates, and methional was found to be produced readily in Cheddar cheese. While this carbonyl exhibits a cheesy note, probably more correctly called cheese cracker note, its role appears to be supportive of other cheese flavor notes.

Flavor chemistry studies on the secondary browning flavor compounds that result from initial amino acid and dicarbonyl reactions showed that furanones were another group of compounds that formed as cheese ripened. Furanones are produced in all Cheddar cheese to some degree, including reduced-fat cheeses, and it has been suggested that they are related to the development of browned or brothy flavor defects in low-fat cheeses. However, results of sensory analysis of spiked cheese systems revealed that the furnanones contribute to unique pineapple-like off-flavors when present in high concentrations, but did not specifically contribute brothy flavors. Furthermore, Furaneol was found to contribute to the blending of flavor notes in aged Cheddar cheese. High concentrations of alpha-dicarbonyl production in cheeses appears to be responsible for elevated levels of furanones.

Research included characterization of alpha-dicarbonyl production by lactic acid bacteria, use of model cheese systems for studying flavor compound formation upon introduction of ripening microbes and flavor precursor compounds, improved analytical methods for measurement of alpha-dicarbonyl compound production in cultures and cheese, and all of these approaches were used to establish a role for slow Maillard-type reactions in Cheddar cheese flavor development during ripening.

Cheese flavor development is key to providing quality cheeses and ingredients to consumers and the food industry. In order to develop and practice culturing and manufacturing procedures that yield consistent and desirable flavors, it is essential to understand the basis of their formation. This research has provided fundamental and applied cultural and technological information that will permit the development of adjunct culturing techniques to improve cheese flavors and increase the demand for cheese and cheese ingredients.

*INTERIM REPORT*

# Developing a graphical paradigm for organizing and delivering technical information about cheese

**Personnel**

John P. Norback, professor, Dept. of Food Science, Candelaria Barcenas, research assistant, Dept. of Food Science, J. Rusty Bishop, professor, Center for Dairy Research

**Funding**

Wisconsin Milk Marketing Board UW A9506

**Dates**

January 1997 - June 1997

## Objectives

To build a software program that allows convenient and friendly access to technical information about Cheddar cheese. This program, which uses the flow of materials diagram, will also provide the user with computational information about immediate and finished product costs, and the coefficients constraints for optimization models. The system will allow users to identify and justify cheese research needs when no information exist about connecting a problem observed or a desired quality with manufacturing process.

## Summary

During this period our efforts have been focused on gathering, organizing and incorporating more information into the system. Also, we have analyzed the organization of the information and the friendly use feature as a first step to evaluating the system. Some changes have been made to the organization and structure of first prototype, specifically those features related to building the flow chart and the menu options.

Our work has involved defining the type of information to deliver, organizing and displaying this information and coding all the changes from the first step evaluation. After implementing the changes, the software shows that it is feasible to provide information to cheese makers in a production environment. We can provide the type of information that will improve the quality of the finished product by displaying information about an operation or material as well as connecting quality parameters to the production process.

The first type of search, when the user seeks information about an operation, raw material and/or intermediate product, is done by clicking on that point of the manufacturing process. A menu is displayed and the user then chooses between technical information (controls), decisions or expert opinions for problems that are likely to happen at that specific point of the production process.

If the user is trying to solve a quality problem, the software provides information to help him/her understand how this parameter is affected at each point of the cheese making process. In other words, by clicking on a quality parameter (i.e. flavor) a window is displayed with all characteristics that define the parameter (rancid, cheese, etc.). The user then clicks in the desired characteristic and the operations where the product's characteristic may be modified or affected will be highlighted in a different color. By clicking on the highlighted operation(s), or raw material(s), a window will open with choices such as references (article or books), expert opinions and ongoing projects connecting the quality problem with the operation or raw material. A customized report of the search is also available. The user can choose to fully or partially print the search.

The software will be presented to several CDR investigators and professors dedicated to cheese production (cheese experts). The objective of this meeting is to evaluate the usefulness of the software to help people at the production plants. After implementing the recommendations obtained in this meeting, the software will be presented to some cheese makers from industry. This project will focus on defining the easiest way to update the system so there is no repetition of information. The actual data base will be increased by gathering expert opinions and information from ongoing projects to incorporate them into the system.

*APPLICATIONS PROGRAM REPORT*

# CDR specialty cheese applications program

**Personnel**

Jim Path, outreach specialist, John Jaeggi, assistant researcher, JoAnn Gauthier, program assistant

**Funding**

Wisconsin Milk Marketing Board, UWA 9703

**Dates**

July 1997 - July 1998

## Objectives

Continue deveoping the Artisan workshops, a module of the Master Cheese Maker program.

Provide technical support to cheese makers, including workshops, consulting, and on site manufacturing trials.

Manage the Master Cheese Maker program.

Develop a cheese database.

## Summary

On April 3, 1997, the first Certified Wisconsin Master Cheese Makers graduated and were honored at the CDR/WCMA ceremony in Green Bay Wisconsin (class of 1997). Four persons received this honor and it has been widely covered in the media.

Four new Wisconsin Master Cheese Makers (class of 1998) were certified by the Master Cheese Maker Board on December 3, 1997. They will be officially recognized at the CDR/WCMA ceremony in Madison, Wisconsin in April of 1998. The class of 1999, which includes nine cheesemakers, have all completed the second round of cheese sample testing in the apprenticeship phase of the program. The board received 10 new applications and approved 9. These nine, (class of 2000) have completed the oral exam and plant visit phase of the apprenticeship.

A Polish Cheese seminar was held on April 22-24, 1997. Instructors came from the Olsztyn University of Agriculture and Technology in Olsztyn, Poland to teach this seminar. They presented a discussion of cheese usage and the manufacture of Polish cheeses.

A French Cheese seminar, with a focus on soft cheese, was held on November 4-5, 1997. Three experts in the field of cheese technology were invited to speak. They included Josef Hubatschek, equipment and soft cheese expert from Germany, Jean Chataud, culture and soft cheese expert from France and Steve Wright, yogurt expert from the USA.

On Feb 25-26, 1997 the third Wisconsin Process Cheese course was held as part of the Wisconsin Master Cheese Makers curriculum. This is the only course of it's type in the USA and fills rapidly.

On May 6-7, 1997 the second Wisconsin Dairy Plant Water & Waste Mgmt short course was held in Madison, a joint effort between CDR and UW-Ag Ext.

On April 8-9, 1997 the first Wisconsin Whey and Whey Utilization short course was held in Madison, another joint effort between CDR & UW-Ag. Ext.

*FINAL REPORT*

# Identification of microbial enzymes and metabolites involved in the development of low-fat cheese and Cheddar cheese flavor (Phase I and Phase II)


### Personnel

James L. Steele, associate professor, UW-Madison Food Science, Mark E. Johnson, senior scientist, Center for Dairy Research, Jeff Broadbent, assistant professor, Utah State Univ., Bart Weimer, assistant professor, Utah State Univ., Kristen Houck, research specialist, UW-Madison Food Science,Song Gao, research assistant, UW-Madison Food Science, Ed Dudley, research assistant, UW-Madison Bacteriology,
Jeff Christensen, research assistant, UW-Madison Bacteriology


### Dates

June 1994 - June 1997


### Funding

Wisconsin Milk Marketing Board
UW9401


## Objectives

Please note this report contains information from a collaborative project with funding from the WMMB and NDPRB (through the Western Center for Dairy Protein Research and Technology). The principal investigators include Dr. Jeff Broadbent and Dr. Bart Weimer from Utah State University and Dr. Mark Johnson and Dr. Jim Steele from The University of Wisconsin-Madison.

Stage 1:

A systematic characterization of metabolic properties of starter cultures and flavor adjunct cultures.

Low-fat cheese (50% reduced) will be manufactured using various combinations of three starter cultures and six flavor adjuncts.

Detailed sensory and chemical analysis of the low-fat cheese manufactured for objective 2.

Gene banks will be constructed of the selected starter and starter adjunct bacteria.

Stage 2: (The following are thought to be the most likely targets for the second stage; however, these targets will be altered in response to the results obtained in stage 1.)

To evaluate the role of primary proteolysis on cheese flavor development.

To characterize the influence of individual peptidases from starter cultures and flavor adjuncts on cheese flavor development.

To characterize amino acid degradation pathways in starter cultures and flavor adjuncts and how they influence cheese flavor development.
To characterize the influence of -dicarbonyl production by starter cultures and flavor adjuncts on cheese flavor development.

## Summary


The starter culture and flavor adjuncts to be used in cheese trials were characterized with regard to their intracellular general aminopeptidase activities and intracellular esterase/lipase activities. Significant variation was observed among these strains for both of these activities. The proteinase specificity was determined for the three starter cultures employed. *Lactococcus lactis* S1, SK11, and S3 were determined to have PI, PIII, and PI/PIII specificity, respectively. Previous investigators have

suggested that cultures with PI specificity were more likely to produce bitter cheese. Gene banks have been constructed of the selected starter and starter adjunct bacteria.

Forty-two vats of low fat Cheddar cheese were manufactured. A grid of three different starter cultures and six different adjunct cultures (two strains each of *Lactobacillus helveticus, Lactobacillus casei, and Brevibacterium linens*) was employed. Sensory analysis (both a small expert panel and a large consumer panel) of these cheeses indicated that both the starter culture and the flavor adjunct had a significant impact on the quality of the final product. Both strains of *Lb. helveticus* and *B. linens* increase the "Cheddar flavor intensity" and reduced bitterness and off-flavors. Cheeses made with *Lb. casei* were more bitter and had elevated levels of off-flavors. Cheeses made with *Lc. lactis* S3 were consistently very bitter.

Capillary electrophoresis, preparative HPLC, peptide sequencing, and mass spectroscopy have been utilized to identify seven distinct peptides from the Cheddar cheese samples. The peptides which accumulated in specific cheeses are directly related to the specificity of the proteinase of the starter culture. A strong positive correlation ($r^2 = .90$) between the presence of $s_1$-CN(1-9) and the level of bitterness in the cheese was observed. It is not possible to determine at this point to determine whether or not this is a direct effect of this peptide or simply an indicator of proteinase specificity.

To determine which enzymes are most important in *Lb. helveticus* CNRZ32's demonstrated ability to debitter cheese when used as a starter adjunct, mutants lacking specific peptidases have been constructed. Specifically, mutants lacking the X-prolyl dipeptidyl aminopeptidase, a general aminopeptidase designated PepC, or another general aminopeptidase designated PepN have been constructed. Additionally, all possible double and triple mutants of these three enzymes have been constructed. Next, these mutants will be examined in a cheese slurry system to determine the relative contribution of each enzyme to CNRZ32's ability to debitter cheese.

The catabolism of aromatic amino acids is believed to play a significant role in the development of unclean flavors in cheese. It will be important to examine the interactions between the starter cultures, non-starter lactic acid bacteria, and adjunct cultures in order to understand how aromatic amino acid catabolites are formed in cheese. To initiate studies in this area, we have chosen to focus on characterization of the aromatic amino acid catabolism by lactococci, which are used as starter cultures in many ripened cheese varieties. Screening of lactococcal strains for enzymes know to initiate catabolism of tryptophan was completed and a broad specificity aromatic amino acid transaminase was detected in all eight lactococcal strains examined. The variation between strains for the level of this activity was at least ten fold. Metabolites produced from tryptophan by cell-free extracts of *Lc. lactis* S3 were indolepyruvic acid, indoleacetic acid, and indole-3-aldehyde. Indoleacetic acid and indole-3-aldehyde can form spontaneously from indolepyruvic acid under the conditions employed. A model system was developed to determine if the aminotransferase(s) was expressed and which metabolite(s) accumulate under conditions which simulate those of ripening Cheddar cheese. The results indicated that the aminotransferase(s) was expressed and stable in the model system. The tryptophan metabolites which accumulated were determined to be strain specific. These results indicate that the aromatic amino acid catabolic pathways present in the starter culture may influence whether or not unclean flavor compounds accumulate in cheese. Finally, the aromatic aminotransferase was purified to electrophoretic homogeneity and characterized. The results indicate that the enzyme would remain active under cheese ripening conditions; therefore the production of off-flavor compounds due to the spontaneous decomposition of products of the aromatic aminotransferase is a possible pathway for the development of unclean flavors in ripened cheeses. Similar studies have been conducted at Utah State University with various lactobacilli. All of the lactobacilli examined initiated aromatic amino acid catabolism via an aromatic aminotransferase and accumulate the actetate or lactate metabolite (i.e. indole lactate from tryptophan).

This project is a collaborative study between researchers at the University of Wisconsin-Madison and Utah State University. This collaboration brings together the expertise of the PIs in cheese manufacture, physiology of lactic acid bacteria, and the genetics of lactic acid bacteria in an attempt to solve the problems of lack of flavor and off-flavors in low-fat (50%) Cheddar-type cheeses. The expanded expertise and systematic approach is likely to yield a significant advance in our understanding of low-fat cheese flavor development. The enhanced understanding of the basic biochemistry of cheese flavor development will greatly facilitate the development of starter systems for the manufacture of

high-quality, low-fat cheese. Low-fat cheese with the organoleptic qualities of full-fat varieties will increase consumer acceptance of low-fat dairy products and expand the demand for these goods to individuals that avoid cheese for reasons of diet and the absence of high quality low-fat alternatives.

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"Aromatic amino acid catabolism by lactococci". At the International Dairy Federation's symposium on "Ripening and Quality of Cheeses." February 1996.

"Influence of proteolysis and amino acid catabolism cheese flavor development." Southeastern Dairy Center. March 1996.

"Impact of lactic acid bacteria on cheese flavor development: proteolysis." Symposium on "Microbial Generation of Flavors and Pigments" at the 1996 American Society of Microbiology Annual Meeting. May 1996.

"Influence of proteolysis and amino acid catabolism cheese flavor development." Systems Bio-Industries. May 1996.

"Low-fat cheese flavor development." Kraft-General Foods. June 1996

"Proteolytic enzymes of lactic acid bacteria and their importance in cheese flavor development". Symposium on "Recent Advances in Lactic Acid Bacteria" at the 1996 IFT Annual Meeting. June 1996.

"How to reduce bitterness in low-fat cheese"at the Twelfth Biennial Cheese Conference. August 1996.

"Impact of lactic acid bacteria on cheese flavour development: proteolysis and amino acid catabolism" at the University of College Cork, Cork, Ireland. September 1996.

"Identification of microbial enzymes and metabolites involved in the development of low-fat Cheddar cheese flavor" at the Dairy Ingredients Division of Rhône-Poulenc. September 1996.

"Development of flavor through starter adjuncts." Wisconsin Cheese Industry Conference. April 1997.

"Influence of starter cultures on Cheddar cheese flavor development." Symposium on "Cheese flavor: starters and starter adjuncts" at the 1997 ADSA Annual Meeting. June 1997.

"Starter culture attributes which affect cheese flavor development." At the LACTIC 97 Symposium. September 1997.

"Production of cheese flavor compounds by amino acid metabolism." Dairy Management, Inc's National Cheese Technology Forum. December 1997

*INTERIM REPORT*

# Glutathione and Cheddar cheese flavor development

**Personnel**

James L. Steele, associate professor, UW-Madison Food Science, Bart Weimer, associate professor, Utah State Univ., Debbie Mikesell, research assistant, UW-Madison Food Science

**Funding**

Dairy Management Inc. UW 133-BM88

**Dates**

July 1997 - June 1999

## Objectives

Construct derivatives of *Lactococcus lactis* 1228 lacking either -glutamyl transpeptidase activity or the ability to transport glutathione.

Evaluation of the ability of *Lc. lactis* 1228 derivatives lacking either -glutamyl transpeptidase activity or the ability to transport glutathione to produce volatile sulphur compounds in a defined medium which simulates Cheddar cheese ripening conditions.

Determine if starter cultures ability to transport glutathione influences the production of volatile sulphur compounds in cheese slurries.

Determine if starter culture encoded -glutamyl transpeptidase activity influences the production of volatile sulphur compounds in cheese slurries.

## Summary

*Lc. lactis* 1228 was determined to be capable of obtaining glutamic acid, an essential amino acid, from glutathione. Strains of lactococci which lacked either -glutamyl transpeptidase activity or the ability to transport glutathione were not capable of obtaining glutamic acid from glutathione. Therefore, we plan to use the ability to obtain glutamic acid from glutathione as a way to screen for 1228 derivatives lacking either -glutamyl transpeptidase activity or the ability to transport glutathione. The temperature sensitive integration vector pGH9::ISS*1* has been introduced into 1228 by electroporation. Integration of the vector following propagation at the non-permissive temperature was demonstrated to occur randomly into 1228 chromosomal DNA by Southern hybridizations utilizing a probe derived from ISS*1*. We are currently screening 1228 pGH9::ISS*1* integrants for the inability to obtain glutamic acid from glutathione.

Glutathione is likely the primary source of cysteine in the cheese matrix and cysteine has been shown to play a critical role in the production of volatile sulphur compounds involved in the development of Cheddar cheese flavor. We propose to evaluate characteristics of starter cultures which are responsible for the level of glutathione-derived cysteine in the cheese matrix. Therefore, results from this study will assist in selecting and/or constructing strains of starter cultures which consistently produce Cheddar cheese with enhanced flavor attributes. The results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

## Presentations

"Production of cheese flavor compounds by amino acid metabolism." by Dr. Jim Steele at Dairy Management, Inc's National Cheese Technology Forum. December 1997

*INTERIM REPORT*

# Improvement of Cheddar cheese quality through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese

**Personnel**

Jeff Broadbent, assistant professor, Utah State Univ., Charlotte Brennand, associate professor, Utah State Univ., Marie Strickland, research associate, Utah State Univ. James L. Steele, associate professor, UW-Madison Food Science, Mark E. Johnson, senior scientist, Center for Dairy Research, Yo-Shen Chen, research assistant, UW-Madison Food Science, Jeff Christensen, research assistant, UW-Madison Bacteriology

**Dates**

June 1997 - December 1999

**Funding**

Dairy Management Inc. 133BM91

## Objectives

Define the contribution of starter CEP specificity on peptide pools and bitterness in Cheddar cheese (USU).

Develop a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in cheddar cheese (USU).

Determine the bitter taste threshold for -CN(f193-209) and $_{s1}$-CN(f1-9).

Define the contribution of *Lactobacillus helveticus* CNRZ32 peptidases to the degradation of -CN(f193-209) and $_{s1}$-CN(f1-9) (UW).

Construct *Lactococcus lactis* derivatives with enhanced activity of the peptidases demonstrated to be important in the hydrolysis of -CN(f193-209) and $_{s1}$-CN(f1-9) (UW).

## Summary

The ability of cultures of *Lb. helveticus* CNRZ32 to obtain essential amino acids from - CN(f193-209) and $_{s1}$-CN(f1-9) have been evaluated using a defined media lacking essential amino acids present in the peptide to be examined. The results of these growth studies suggest that CNRZ32 is capable of hydrolyzing both peptides to free amino acids; therefore, as expected, CNRZ32 has the enzymes required to hydrolyze these bitter peptides to non-bitter products. Additionally, the hydrolysis of both putative bitter peptides by CNRZ32 cell-free extracts has been examine by HPLC. These results confirmed that CNRZ32 has the enzymes required to hydrolyze these bitter peptides. Currently, we are developing techniques to enhance the separation of the products of hydrolysis by HPLC to allow us to determine their identity.

Bitterness, which is the result of the accumulation of relatively hydrophobic peptides, is one of the most common defects present in Cheddar cheese. Bitter peptides in Cheddar cheese are generated either directly by chymosin (i.e. -CN(f193-209)) or by the joint action of chymosin and the lactococcal proteinase (i.e.$_{s1}$-CN (f1-9)). The specificity of the lactococcal proteinase is the primary determinant in whether or not a starter culture produces bitter peptides. The hydrolysis of bitter peptides to nonbitter products is widely believed to occur via the action of intracellular peptidases of lactic acid bacteria. Therefore, by selecting or constructing lactic acid bacteria which do not form bitter peptides and which can efficiently hydrolyze bitter peptides to non-bitter products, it should be possible to control bitterness in cheese. The results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

*INTERIM REPORT*

# The improvement of low fat Cheddar cheese through identification and characterization of microbial enzymes responsible for the conversion of aromatic amino acids into off flavor compounds

**Personnel**

Jeff Broadbent, assistant professor, Utah State Univ., Bart Weimer, associate professor, Utah State Univ., James L. Steele, associate professor, Dept. of Food Science, UW-Madison, Mark E. Johnson, senior scientist, Center for Dairy Research, Scott Rankin, assistant Professor, University of Maryland

**Funding**

Dairy Management Inc. UW 133-BM90

**Dates**

June 1997 - December 1999 (Steele component July 1, 1997 to December 31, 1997)

## Objectives

Investigate the role of metabolic cross-feeding between starter, adjunct, and nonstarter cheese bacteria in the production or removal of aromatic off flavor compounds (Broadbent, Weimer, and Steele).

Characterize the contribution of key enzymes to the conversion of aromatic amino acids into off flavor compounds (Broadbent)

Confirm the action of key enzymes in cheese slurries and in low fat Cheddar cheese (Johnson and Rankin).

## Summary

In a previous study we were able to demonstrate that the aromatic aminotransferase from the lactococcal starter, the enzyme which initiates amino acid catabolism, remains active under conditions found in ripening Cheddar cheese. Additionally, it was demonstrated that the product formed by the aminotransferase from phenylalanine, phenylpyruvate, spontaneously degrades to phenethanol a compund with a floral character. Similarly, the product formed by the aminotransferase from tyrosine, *p*-OH-phenyl pyruvate, spontaneously degrades to *p*-cresol a compound with a utensil-like flavor. In this study it was determined that *Lactobacillus casei*, a typical component of the nonstarter lactic acid bacteria flora of ripening Cheddar cheese, is capable of converting these unstable compounds to stable compounds which do not give rise to off-flavors. Therefore, using this information it should be possible to develop starter systems which control the development of off flavor compounds produced via this mechanism.

Also in the previous study we determined that some strains of lactococcal starters produced indole acetate from tryptophan. In the literature it has been reported that some strains of lactobacilli are able to metabolize indole acetate to skatole, an unclean flavor compound. In this study we screened strains of lactobacilli isolated from cheese for the ability to convert indole acetate to skatole. No lactobacilli strains capable of this conversion were identified. Therefore, the production of indole acetate by the starter does not appear to be a good indicator of whether or not off flavors will develop.

By identifying or developing starter systems which avoid the production of off flavor compounds or their precursors, it should be possible to control off flavor development in Cheddar cheese. The results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

*INTERIM REPORT*

# Succinate production by *Lactobacillus casei*: pathways responsible and development of strategies to control its accumulation

**Personnel**

James L. Steele, associate professor, UW-Madison Food Science, Ed Dudley, research assistant, UW-Madison Bacteriology

**Funding**

Dairy Management Inc. 133BM89

**Dates**

July 1997 to December 1999

## Objectives

Screening strains of *Lactobacillus casei* for the ability to metabolize citrate and produce succinate.

Construction and characterization of *Lb. casei* mutants defective in lactate dehydrogenase and oxaloacetate decarboxylase.

Evaluation of the effect of the lactate dehydrogenase and oxaloacetate decarboxylase mutations on the ability of *Lb. casei* to produce succinate in a model cheese ripening system.

## Summary

Efforts to date on this project have been limited to development of methods for the quantification of succinate and a "cheese-like" model system to be used in the screening process.

We will be evaluating the pathways by which *Lactobacillus casei*, the primary nonstarter lactic acid bacteria present in ripening Cheddar cheese, produces the flavor compound succinate. Results from this study will assist in selecting and/or constructing strains of *Lb. casei* which result in cheeses having a consistent controlled level of succinate. By controlling the level of succinate in the cheese matrix, it should be possible to not only have greater control of the flavor of Cheddar cheese consumed directly, but also to control the functionality of Cheddar cheese when used as a food ingredient. Thus, results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

*INTERIM REPORT*

# Construction of exopolysaccharide producing strains of *Streptococcus thermophilus* with increased bacteriophage resistance

**Personnel**

James L. Steele, associate professor, UW-Madison Food Science, Joe Sturino, research assistant, UW-Madison Bacteriology

**Funding**

Wisconsin Milk Marketing Board UW9603

**Dates**

June 1996 - June 1998

## Objectives

Evaluate the effectiveness of bacteriophage-resistance genes derived from *Lactococcus lactis* in strains of *Streptococcus thermophilus*.

Screen industrial strains of *S. thermophilus* for bacteriophage-resistance mechanisms.

Clone and characterize genes encoding bacteriophage defense mechanisms from *S. thermophilus*.

## Summary

The evaluation of the effectiveness of lactococcal bacteriophage-resistance determinants required that they could be cloned into vectors capable of replicating in *S. thermophilus*. The vectors capable of replicating in *S. thermophilus* was evaluated by conducting transformations with cloning vectors based on five distinct replicons. The only replicon found to replicate in *S. thermophilus* was pWV01. However, all attempts to clone lactococcal genes encoding abortive infection mechanisms (*abi*A and *abi*C) were unsuccessful. Transformants containing truncated inserts were observed suggesting that neither of these determinates were stable in this vector. Insert instability is frequently observed in vectors based on the pWV01 replicon, this is believed to be due to its rolling-circle mechanism of replication. Therefore, further attempts to evaluate the effectiveness of lactococcal bacteriophage-resistance determinants in *S. thermophilus* were terminated.

An approach which has been successfully employed in the construction of bacteriophage-insensitive derivatives of lactococci has been the inactivation of genes encoding bacteriophage receptors. We attempted this approach in *S. thermophilus* utilizing the integration vector pGh9::ISS*1* for insertional mutagenesis. This approach has yielded seventeen bacteriophage insensitive mutant (BIM) derivatives of *S. thermophilus* MTC130. These BIMs exhibit resistance against two bacteriophage which infect *S. thermophilus* MTC130. The degree of resistance varies between the BIMs and is also bacteriophage specific. We are currently examining the mechanism(s) of resistance expressed by these BIMs and hope to determine the gene(s) inactivated in the BIM(s) having the highest degree of resistance.

Exopolysaccharide producing cultures of *Streptococcus thermophilus* are an important tool in the manufacture of Italian cheeses with unique functional properties. The produced exopolysaccharide function in the cheese to bind water, increase viscosity, and affect mouth feel. With an increased emphasis on producing low-fat cheese varieties, these functional attributes, especially water binding, are increasing in importance. The difficulty with these cultures is that they are closely related to each other and bacteriophage problems are

increasing. Traditional methods of developing/isolating bacteriophage unrelated strains have not been successful; therefore this project will take advantage of what is known of bacteriophage defense mechanisms in *Lactococcus lactis* and use this knowledge to construct exopolysaccharide producing strains of *S. thermophilus* with enhanced bacteriophage resistance.

*FINAL REPORT*

# Development of process technology to reduce pink discoloration in annatto-colored pasteurized process cheese

**Personnel**

W.L. Wendorff, associate professor, Dept. of Food Science; Ellen Shumaker, graduate research assistant, Dept. of Food Science

**Funding**

Dairy Management Inc. WND97

**Dates**

July 1996 – June 1997

## Objectives

To determine factors affecting the pink discoloration of annatto-colored pasteurized process cheese products.

To determine potential processing parameters or agents that may be used to reduce or inhibit the pinking defect in process cheese products.

## Summary

The process cheese industry has reported problems with pink discoloration of their annatto-colored process cheeses. This discoloration is not a surface discoloration, like the light-induced defect experienced in natural cheeses (CDR Ann. Report 1994), but it is observed throughout the entire cheese loaf. Color is used to judge the quality and acceptability of food products. Discoloration of process cheese may reduce consumer confidence in the overall quality of the product.

Initially, 20 commercial colorants used for production of processed cheese were evaluated for color stability in heated cheese pastes. Annatto colorants were added to the cheese paste at 0.3% (w/v) and heated at 70˚C for up to 76 hours. Samples were taken at 2, 4, 6, 8, 25, 50, 76 hours and analyzed for color with a Hunter colorimeter. The type of annatto colorant was related to some differences in color stability during heating. Annatto emulsions showed the greatest tendency for pink discoloration while annatto suspensions were the most stable to heat processing. Propylene glycol, which is one of the additives used in annatto emulsions, did not contribute toward the pinking problem when added to annatto suspensions at 2-5 % (w/w). Apo-carotenal exhibited the largest change in color properties with only 2 hours of continuous heat.

The effect of process temperature, emulsifying salts, utilization of colored cheese, age of cheese, and pH of cheese were evaluated in pasteurized process cheese. Pasteurized process cheese was manufactured with a 5-L direct steam injected cheese processor. Process cheese was prepared with 6-week old uncolored Cheddar cheese and 2.5% (w/w) emulsifying salt in 1.0-kg lots. Process cheese lots were made by blending cheese ingredients, emulsifying salts and 0.1% (v/v) annatto emulsion colorant with steam injection for 5 minutes at 74˚C, except in the process temperature studies. Process cheese was poured into 500-g molds and cooled immediately to 8˚C. Color of each sample was evaluated by Hunter colorimeter.

When process cheese was cooked at various process temperatures from 68˚C to 80˚C, samples at higher process temperatures had higher a values (redness) and lower hue angles (increased pinking). Samples cooked at 68˚C and then held at 65˚C for 8 hours (to simulate retention in the

cooker or holdup in the distributors to chill rolls) exhibited increased pinking. Process cheeses ranging in pH from 5.4 to 5.8 did not show any significant differences in color attributes.

Process cheeses made with higher concentrations of sodium citrate had lower hue angles than cheeses made with disodium phosphate in the emulsifying salt blends. As the percentage of colored Cheddar cheese was increased in the process cheese blend, the potential for pinking was significantly increased. This is primarily due to the heat sensitivity of norbixin which is the primary annatto component present in natural cheese colorants. The use of more aged cheese in the process cheese blend resulted in lower L values (brightness), but greater a (redness) and b (yellowness) values. However, the b values increased at a greater rate than the a values so there was no significant reduction in hue angles.

Process cheese food was produced with various added whey solids to determine potential impact of those ingredients on pinking. Acid whey exhibited the greatest tendency for pinking while sweet whey resulted in the highest hue angles. Whey protein concentrate showed a greater tendency for browning, especially with delayed cooling of the process cheese food.

In conclusion, cheese processors can reduce the potential for pinking in process cheese by using uncolored cheeses, annatto suspension as the colorant, and limited use of citrates as emulsifying salts. Pasteurizing temperatures for producing process cheese should be kept close to the minimal pasteurization temperatures and product should be properly chilled as soon as possible after heat processing. Some additional studies need to be conducted on the composition of annatto suspensions in relation to annatto emulsions to determine why the emulsions tend to be more heat sensitive and susceptible to pinking.

Acknowledgements

Additional funding for this project was provided by Rhone Poulenc of Madison, WI and Dinesen Trading Co. of Grafton, WI.

## Publications/Presentations

Shumaker, E.K. 1997. Development of process technology to reduce the potential for pink discoloration in annatto-colored pasteurized process cheese. M.S. Thesis, UW-Madison.

Shumaker, E.K., and W.L. Wendorff. 1998. Factors affecting pink discoloration in annatto-colored pasteurized process cheese. (submitted to J. Food Sci.)

*INTERIM REPORT*

# Characterization of interactions between ingredients and cheese constituents for improved functionality of fat-free processed cheese

**Personnel**

William L. Wendorff, associate professor, Dept. of Food Science, Brad Swenson, graduate research assistant.

**Dates**

July 1997 - June 1999

**Funding**

Wisconsin Milk Marketing Board UW9702

## Objectives

Determine the interactive effect of stabilizers, emulsifying salts and other dairy ingredients on the functionality of no-fat pasteurized processed cheese spreads.

Evaluate effect of stabilizers and other dairy ingredients on skin formation during heating of no-fat processed cheese products.

Evaluate water retention in the protein matrix when fat is eliminated in processed cheese products as versus full-fat processed cheese.

## Summary

In the first 4 months of the project, we have established the analytical procedures we will use to evaluate no-fat processed cheese spreads and we have applied those to full-fat and reduced-fat processed cheese spreads from the retail market. This gives us an informational base for comparison with our no-fat spreads. We had some delays in getting an attachment for the CDR Texture Analyzer for assessment of the spreads. We also had some delay in getting a drum of skimmilk Cheddar cheese into Babcock Hall for our base cheese for spreads. The current production of skim cheese is concentrated in Minnesota plants and we had some difficulty in arranging transportation for a single drum of cheese. Emulsifying salts and stabilizers have been obtained and preliminary runs have now been made on the Kustner system.

Additional trials will continue toward the accomplishment of the first objective of determining the interactive effect of the various ingredients in no-fat processed cheese spread. Effects of emulsifying salts alone are currently being completed and the stabilizer trials should proceed through the second semester. As that series of trials continues, information will be recorded to then address Objectives 2 and 3.

VISITING SCIENTIST REPORT

# Objective method of measuring cheese melt/flow characteristics

**Personnel**

Muhammet Mehmet Ak, Istanbul Technical University, Chemical-Metallurgical Engineering Faculty, Food Engineering Department, Sundaram Gunasekaran, Dept of Food Science

**Dates**

July – August, 1995

## Objectives

1. To develop a simple apparatus (based on fundamental rheological principals) for objectively evaluating melt/flow characteristics of cheese.

2. To verify the validity of the new method.

## Summary

The apparatus developed for measuring melt/flow behavior of cheese is referred to as a melt-meter. It can be custom made with a budget around $1,000 (excluding the computer). It is available in the Food Engineering Laboratory in the Agricultural Engineering Department. It consists of the following components:

1. Melt-meter body (made in Agricultural Engineering machine shop) with a doughnut shape heater inside (~$50.00),

2. A micro controller unit (CN4400, Omega Engineering, Inc. Stanford, CT, ~$200.00) to operate the heater,

3. Linear variable differential transformer (LVDT, Schaevitz Engineering, Pennsauken, NJ, ~$450.00) to monitor the flow of cheese,

4. Data acquisition board (DAS 16G High Speed Analog I/O Board, Metrabyte Corp., Taunton, MA) and software (Easyest IX Software, Asyst Software Technologies, Inc., Rochester, NY), [If a computer is not available, the voltage output of LVDT can be registered using a chart-recorder.]

5. Personal computer for data collection and analysis (Gateway-2000, North Sioux City, SD).

In rheological terms, this is a constant force (creep) apparatus. The operation of the melt-meter imitates the spread of melting cheese on a pizza dough. The strain rates (i.e., the rate of spread of melted cheese) attained with the melt-meter are small and can be varied by using different weights (force). Presumably, the strain rate of melting cheese on pizza is also low. Therefore, results obtained with the melt-meter are expected to relate to real applications.

The melt-meter can also be operated in constant speed mode. This requires an additional unit such as Instron Testing Machine to provide the constant deformation rate. An Instron is available in the Food Engineering Laboratory. We have performed both types of experiments but so far only analyzed the data from constant weight experiments.

Output of the melt-meter is voltage vs. time. This was converted into biaxial elongational viscosity (BEV) and radial strain rate using the formulas given in

the publications listed below. BEV represents the resistance of melted cheese to radial flow. Hence, a large value of BEV would mean a less spread of cheese during melting on a pizza dough. Results from constant weight experiments and detailed information regarding the design of the melt-meter will be presented in the publications listed below.

Melt/flow characteristics of various cheeses can be determined with the melt-meter. Such a study is going on in Prof. Gunasekaran's lab. This will establish the utility of the apparatus.

A user-friendly computer program can be developed to process the data, and to report the results in graphical or tabular form. This can facilitate the use of melt-meter in the dairy and related industries as well as in dairy research laboratories.

Dairy industry and food processors have long used empirical tests to determine what is called "meltability" of cheese. Unfortunately, the methods used provide information that can only compared within a particular study. This main reason for this limited utility of the empirical methods is that the results are generally expressed in terms of arbitrarily selected parameters, rather than well defined quantities. This makes comparing findings from different laboratories, or using other researchers' data, very difficult.

We now can offer not only a simple system (i.e., melt-meter), but also a system that is based on fundamental engineering and rheological principles. It can provide data on material properties at high temperature. Such information is scarce in cheese literature. We can express the results in terms of well defined rheological parameters such as elongational viscosity. This allows us to compare data from different sources as well as to form a database on the effects of various factors on melt/flow behavior of cheeses. Such a database would eventually enable us to improve and control the quality of cheese (regular or reduced fat) for ingredient applications.

## Publications

Presentation: Ya-Chun Wang, M. M. Ak, K. Muthukumarappan and S. Gunasekaran, "Melting characteristics of Mozzarella and Cheddar cheeses", poster to be presented at the Midwest Food Processing Conference, October 2-3, 1995, LaCrosse, WI.

Ya-Chun, M. M. Ak, K. Muthukumarappan and S. Gunasekaran. "Melting behavior of regular and reduced fat Mozzarella cheese." In preparation for submission to Journal of Texture Studies.

# chapter 2, section 2

# Cheese safety

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*FINAL REPORT*

# Application of biopreservatives as antilisterial agents in Queso Fresco and Cheddar cheese

**Personnel**

John B. Luchansky, associate professor, Food Research Institute, Mark E. Johnson, senior scientist, Center for Dairy Research, Nana Y. Farkye, research scientist, California Polytechnic State University Alan J. Degnan, senior research specialist, Food Research Institute

**Funding**

Dairy Management Inc. LCH 95

**Dates**

July 1994 – June 1996

## Objectives

Identify lactic acid bacteria (LAB), for use in Queso Fresco, that produce bacteriocins effective against *Listeria monocytogenes* without producing significant amounts of organic acid(s). Identify bacteriocinogenic starters or adjuncts for use in Cheddar cheese.

Validate bacteriocinogenic LAB and fermentates of LAB as antilisterial agents in Queso Fresco and Cheddar cheese at different steps during manufacture.

Evaluate the effect of biopreservatives on sensory or biochemical qualities of Queso Fresco andCheddar cheese during storage at refrigeration and abuse temperatures.

## Summary

Queso Fresco (QF) is a Hispanic, fresh-style soft cheese that relies on enzymes rather than acid to coagulate the cheese milk. Thus, the final product is a low acid (pH 6.2-6.4), high moisture (ca. 55%), buttery-tasting cheese, whose popularity is growing rapidly, especially in the southern and western regions of the United States. The absence of an acid "hurdle" due to the relatively high pH provides favorable conditions for bacterial proliferation and, presumably, a greater potential for foodborne hazard. *Listeria monocytogenes* is a foodborne pathogen which persists in a wide range of raw and processed foods. The proximate composition and manufacture/storage conditions for QF provide a suitable growth environment for this acid and salt tolerant, cold-loving pathogen. Therefore, we investigated the efficacy of biopreservatives delivered during production of QF for the control of *L. monocytogenes.*

In preliminary experiments, bacteriocinogenic lactic acid bacteria (LAB) from our culture repository with antilisterial activity (Table 1) were evaluated to identify strains which grew adequately and produced sufficient bacteriocin but minimal lactic acid during growth in milk compared to synthetic media. Each

Table 1. Bacteriocinogenic lactic acid bacteria with antilisterial activity.

| Strain | Bacteriocin |
|---|---|
| *Lactococcus lactis* subsp. *lactis* ATCC 11454 | nisin |
| *Pediococcus pentosaceus* FBB611 | pediocin |
| *Lactobacillus sake* LB706 | sakacin A |
| *Enterococcus faecium* 1083 | enterocin 1083 |
| *Pediococcus acidilactici* JBL1095 | pediocin AcH |

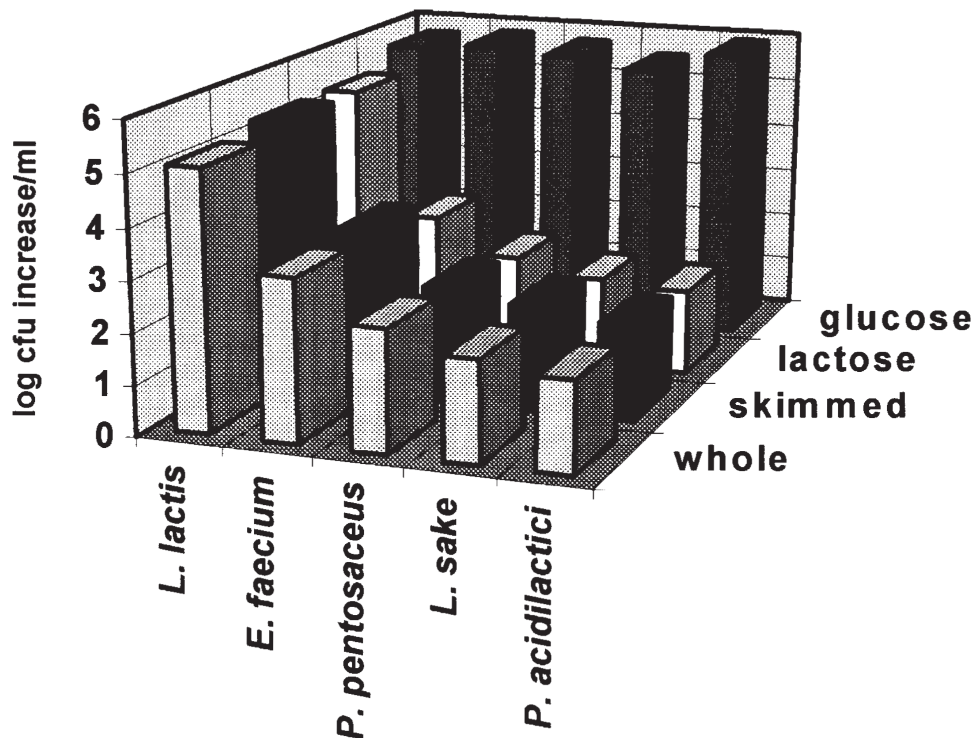



Figure 1a.
Population increases ($log_{10}$ cfu/ml) of bacteriocinogenic lactic acid bacteria in milk and lactose- or glucose-based synthetic media.

strain was separately grown at 30°C for 20 hours in whole or skim milk, as well as a lactose-based or glucose-based synthetic medium. Strains that did not readily utilize lactose showed low growth rates (Figure 1a) and little or no bacteriocin production (Figure 1b) in either milk or lactose-based media compared to the glucose-containing synthetic medium. Only *Lactococcus lactis* subsp. *lactis* ATCC 11454 produced sufficient antilisterial activity [ca. 25,000 Arbitrary Units (AU) per ml of skim milk] for use in cheese-making trials. As a disadvantage, *L. lactis* also produced more acid in milk (pH 5.7) than was considered ideal (data not shown). However, it was assumed that adding this culture, or milk prefermented with this culture, to a relatively large volume of cheese milk would have little effect on the pH of QF.

Queso Fresco was prepared using a traditional process that resulted in a final product with about 52% moisture, 38% fat, 1.9% NaCl, and pH 6.4. To inoculate QF with *L. monocytogenes*, pasteurized, whole milk was challenged with a cocktail containing strains V7, Ohio, and Scott A at about $10^4$ cfu/ml. The final product was stored at 4 or 12°C and tested for viable *L. monocytogenes* at 0, 1, 3, 7, 14, and 21 days. For experimental treatments, the bacteriocin, nisin was added to QF by 3 different strategies. First, a prefermented milk (PFM) was prepared by overnight growth of *L. lactis* ATCC 11454 in 2.5 L of whole milk at 30°C and the resulting nisin-containing PFM (ca. 25,000 AU/ml; pH 5.5) was added to the cheese milk. The PFM was mixed at a level of 5% with the cheese milk containing the *L. monocytogenes* cocktail just before adding the starter culture, delivering about 500 AU of nisin per ml of cheese milk. Second, nisin activity (1,000 AU/ml) was added directly to cheese milk by blending 50 g of Nisaplin™, a commercial preparation of nisin, with 50 kg milk. Third, nisin activity was delivered by "salting" 50 g Nisaplin simultaneously with cheese salt directly onto 5 kg of curd (produced from 50 kg of milk). The salt/nisin combination was thoroughly distributed throughout the cheese curd by hand mixing just prior to hooping and pressing.

In control batches (nisin-free) of QF stored at 12°C, counts of *L. monocytogenes* increased over 3 $log_{10}$ cfu/g cheese within 3 days and remained at maximum levels over the 21 day sampling period (Figure 2). At 4°C, pathogen counts in the control batches increased more slowly, reaching about 1 $log_{10}$ cfu/g over initial inoculum levels over 21 days (Figure 2). For QF prepared with PFM and stored at 12°C, populations of *L.*

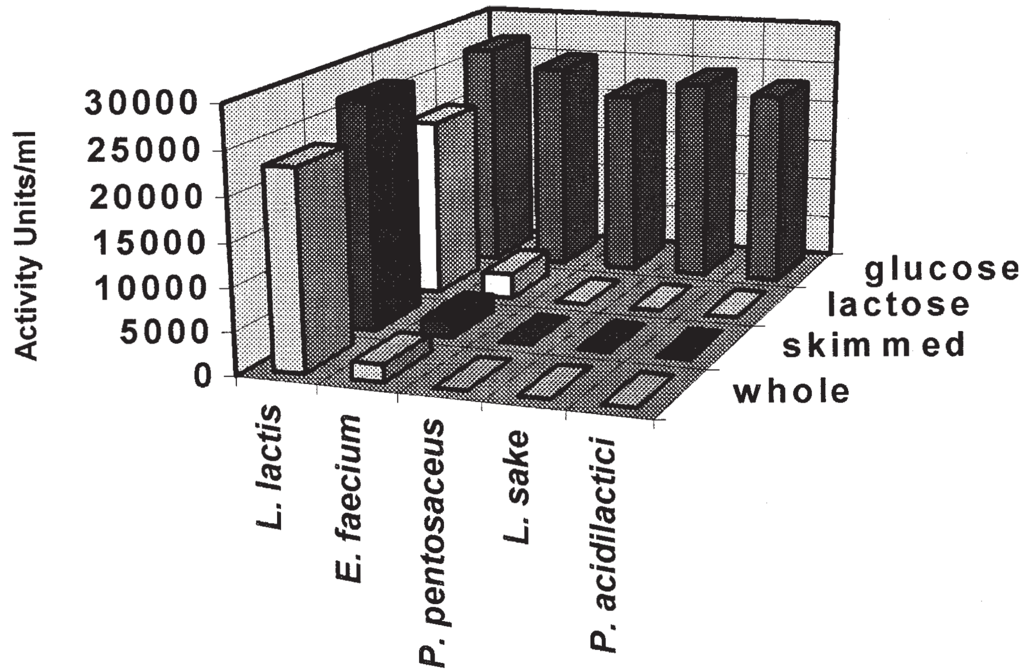
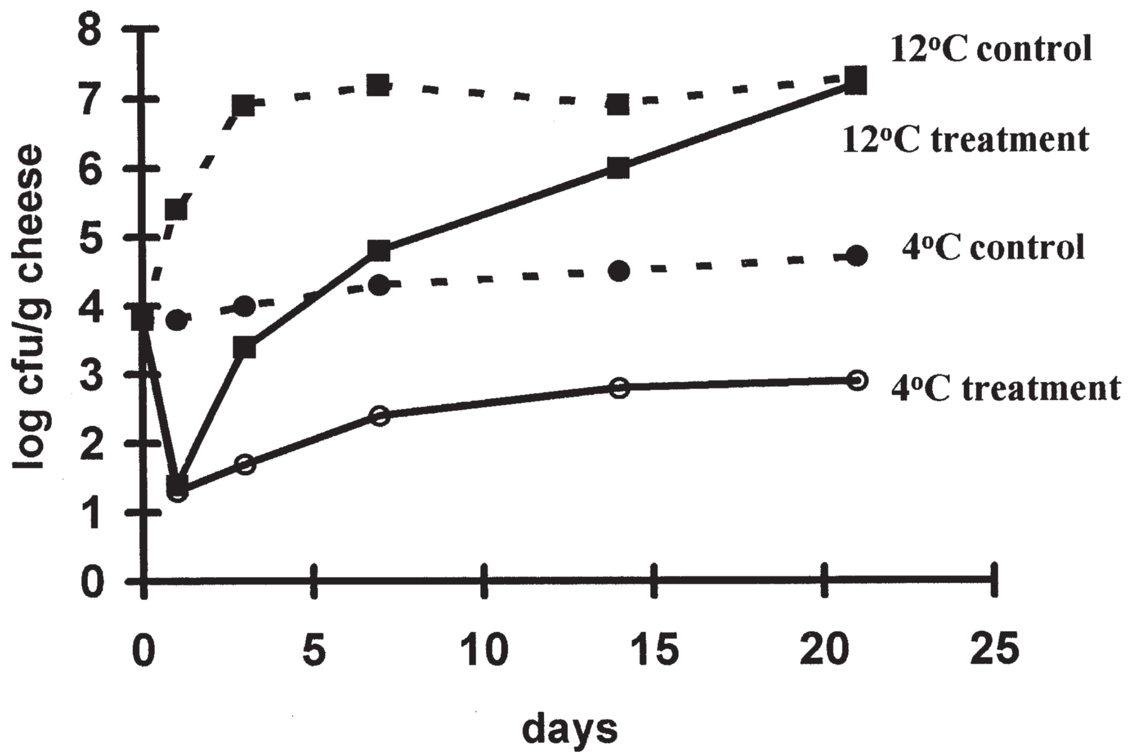



Figure 1b.
Antilisterial activity (AU/ml) produced by bacteriocinogenic lactic acid bacteria in milk and lactose- or glucose-based synthetic media.

Figure 2.
Fate of *L. monocytogenes* in Queso Fresco prepared with nisin (500 AU/ml) added via prefermented milk (PFM; 5%).

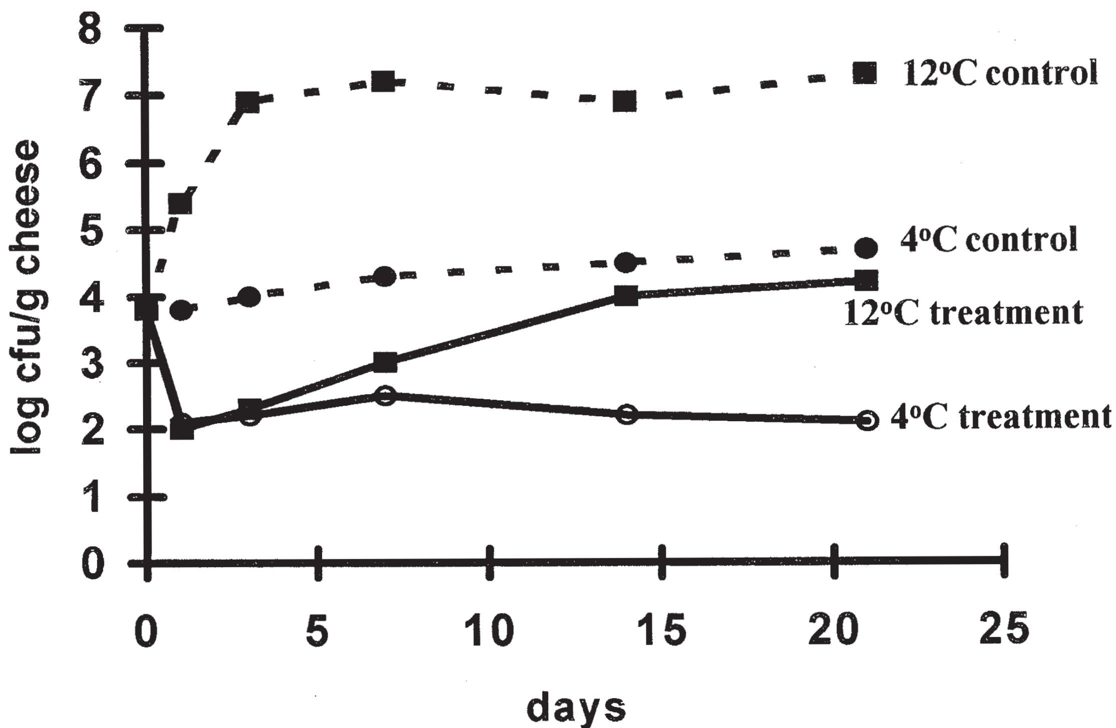


*monocytogenes* decreased about 2 $log_{10}$ units within 1 day. However, counts recovered similar to those in control batches within 21 days. When stored at 4°C, counts of the pathogen also declined about 2 $log_{10}$ units within 1 day, and counts remained about 2 $log_{10}$ units below counts of the control over the 21 day sampling period. Compared to storage at 12°C, after the initial 2 $log_{10}$ unit decrease the additional hurdle of refrigeration substantially suppressed the pathogen during storage at 4°C. The initial decrease in pathogen numbers observed in QF prepared with PFM was attributed to the presence of nisin and perhaps the slight acidity introduced via the PFM.

In QF prepared with Nisaplin added to the cheese milk, and then stored at 12°C, counts of *L. monocytogenes* decreased 1-2 $log_{10}$ units within 1 day and remained 2-3 $log_{10}$ units below those in control batches over the 21 day storage period (Figure 3). Similarly, in batches of QF stored at 4°C, populations of *L. monocytogenes* decreased about 1-2 $log_{10}$ units within 1 day and remained static thereafter at 2-3 $log_{10}$ units below control populations over 21 days at 4°C. However, the combination of Nisaplin and refrigeration displayed greater antilisterial activity than using Nisaplin and storing at 12°C. The greater reduction of *L. monocytogenes* achieved by adding Nisaplin to the cheese milk rather than adding nisin to the cheese milk via PFM was attributed to the higher levels of nisin activity (1,000 vs 500 AU/ml) in the former. Also, the use of a nisin preparation compared to a nisin-producing strain in PFM enabled precise control over the degree of antilisterial activity. Sufficient antilisterial activity was not produced by *L. lactis* ATCC 11454 for efficacious use of PFM at a level of 5%.

In QF prepared with Nisaplin "salted" into cheese curd with subsequent storage at 12°C, counts of *L. monocytogenes* initially dropped 3 $log_{10}$ units within day 1 (Figure 4). Despite a 2-3 $log_{10}$ resurgence in pathogen levels, counts of *L. monocytogenes* remained 2-3 $log_{10}$ units below levels observed in control batches. Similarly, for QF stored at 4°C, pathogen numbers dropped below detection (5 cfu/g cheese) and remained static for up to 14 days, after which low levels of *L. monocytogenes* were detected. The difference between populations of *L. monocytogenes* in control (nisin-free) cheese compared to cheese prepared with Nisaplin added at the salting stage at 4°C was about 4 $log_{10}$ units.

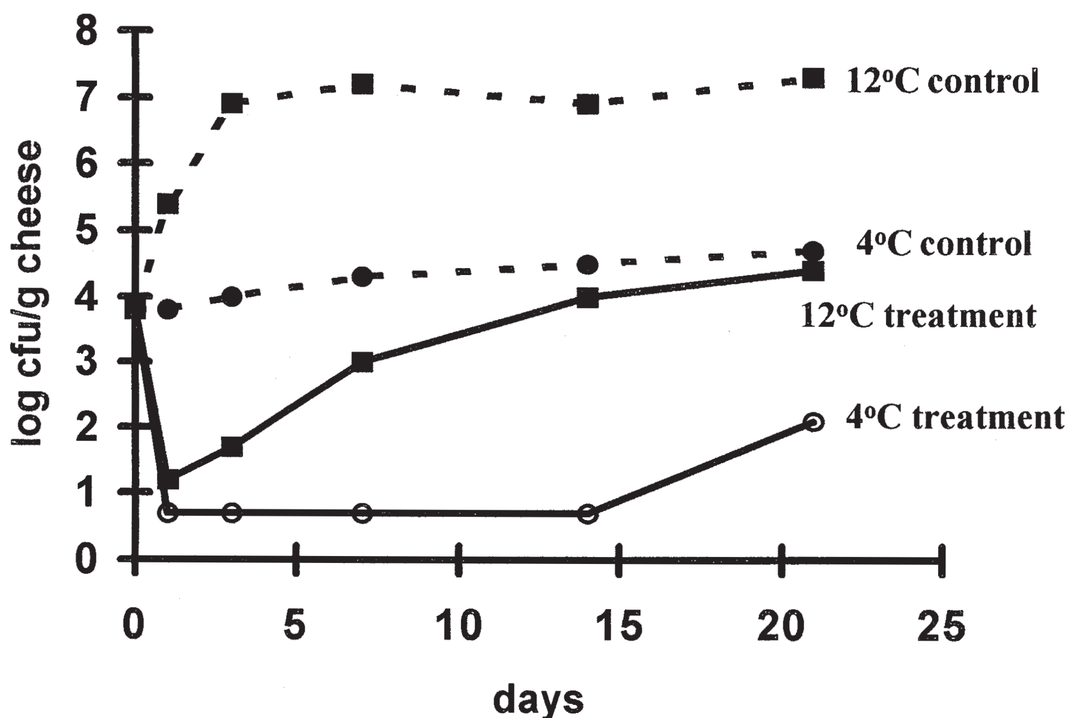
Figure 3.
Fate of *L. monocytogenes* in Queso Fresco prepared with 50 g Nisaplin added to 50 kg cheese milk.



Batches of QF treated with Nisaplin received equivalent amounts, whether added to the cheese milk (50 g Nisaplin/50 kg milk), or "salted" onto the curd (50 g Nisaplin/5 kg curd). However, activity levels were increased by adding nisin activity to 5 kg curd (10,000 AU/g) compared to 50 kg milk (1,000 AU/g). This may explain, in part, why populations of *L. monocytogenes* were lower in QF prepared by adding Nisaplin to the curd rather than the cheese milk. Also, delivering nisin activity to the curd precludes the loss of activity that presumably occurs when the whey is drained. Thus, the "salting" method of delivering antilisterial activity to QF at the curd phase was more efficient than delivering an equivalent amount to the cheese milk. Regardless of when or how antilisterial activity was delivered to QF, the presence of the bacteriocin was sufficient to reduce pathogen numbers appreciably for up to 5 days, which is the approximate timeframe for consumption of QF. Lastly, the addition of nisin-containing PFM or Nisaplin to cheese milk or curd did not appreciably alter pH. Generally, pH values in all treatments discussed in this report were not significantly different, with batches stored at 12°C ranging from an initial pH of pH 6.4 to a final pH of pH 5.7 at day 21. The pH of the cheese stored at 4°C ranged from an initial pH of pH 6.4 to a final pH of pH 6.2 at day 21.

Based on the experiments conducted using Queso Fresco, Cheddar cheese curds were inoculated with a 2-strain inoculum of *L. monocytogenes* (about $10^7$ cfu per gram of cheese) and different levels and combinations of bacteriocins. As shown in Figure 5, in curds containing low levels (about 10 AU per gram total) of a bacteriocin cocktail (crudely purified pediocin, sakacin, and enterocin plus highly-purified nisin) pathogen numbers decreased to <10 cfu per gram within 3 weeks of storage at 6˚C. Low levels of pediocin, enterocin, or sakacin used alone did not result in an appreciable decrease of *L. monocytogenes* compared with control samples that did not contain bacteriocins. In Cheddar cheese containing low levels of nisin, pathogen numbers were reduced to <10 cfu per gram within 5 weeks during storage at 6˚C. As shown in Figure 6, in curds containing higher levels (about 60 AU per gram total) of pediocin, sakacin, or nisin alone, as well as in curds containing the bacteriocin cocktail, pathogen numbers decreased to <10 cfu per gram in 5, 5, 4, and 2 weeks, respectively, during storage at 6˚C. The antilisterial effect of higher levels of enterocin was marginal, as counts of the pathogen were about $10^3$ cfu per gram after 5 weeks of storage at 6˚C. Previous studies revealed pathogen numbers decreased as the storage temperature increased.

Figure 4.
Fate of *L. monocytogenes* in Queso Fresco prepared with 50 g Nisaplin "salted" onto 5 kg curd prepared from 50 Kg cheese milk.



Queso fresco (QF) is a high pH, high moisture soft cheese prepared with minimal starter activity, considerable hand manipulations, and is stored at elevated temperatures. This, QF is particularly prone to microbial hazard, notably from the foodborne pathogen *L. monocytogenes*. Although good manufacturing practices and proper sanitation can reduce the likelihood of hazard, additional strategies are needed to manage *L. monocytogenes* in Hispanic-style cheeses. Our results demonstrate that biopreservatives can appreciably reduce numbers of *L. monocytogenes* in QF, particularly when added directly to the cheese milk or curd. As such, bacteriocins offer great potential for improving the safety of QF and dairy products in general.

The results of this study also revealed that low levels (10 AU per gram) of a bacteriocin cocktail or higher levels (60 AU per gram) of pediocin or sakacin displayed the greatest potential for use as biopreservatives to control *L. monocytogenes* in Cheddar cheese.

## Publications

Glass, K. A., B. Prasad, J. H. Schlyter, H. E. Uljas, N. Y. Farkye, and J. B. Luchansky. 1995. Effects of acid type and ALTA™2341 on *Listeria monocytogenes* in a Queso Blanco cheese. J. Food Prot. 58:737-741.

Luchansky, J. B., and N. Y. Farkye. 1996. Controlling pathogenic and spoilage bacteria in cheese with biopreservatives. California Dairy Beat 3(3):6.

Degnan, A. J., A. Piva, and J. B. Luchansky. 1996. Preservation of food using lactic acid bacteria and bacteriocins. Food Testing & Analysis 2(2):17-19.

## Abstracts

Degnan, A. J., N. Y. Farkye, M. E. Johnson, and J. B. Luchansky. 1996. Use of nisin to control *Listeria monocytogenes* in a Queso Fresco cheese. Abstracts of the Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians (#154), p70.

## Presentations

"Bacteriocin use in foods." Invited speaker at the FDA Science Forum on Regulatory Sciences. Washington, DC, September 30, 1994. (J.B. Luchansky)

"Managing pathogens in processed food." Presentation at the Vision for Food Safety Symposium. Madison, WI, January 18, 1995. (J.B. Luchansky)

"Update on biocontrol and subtyping of foodborne pathogens." Scientific Lectureship, Institute of Food

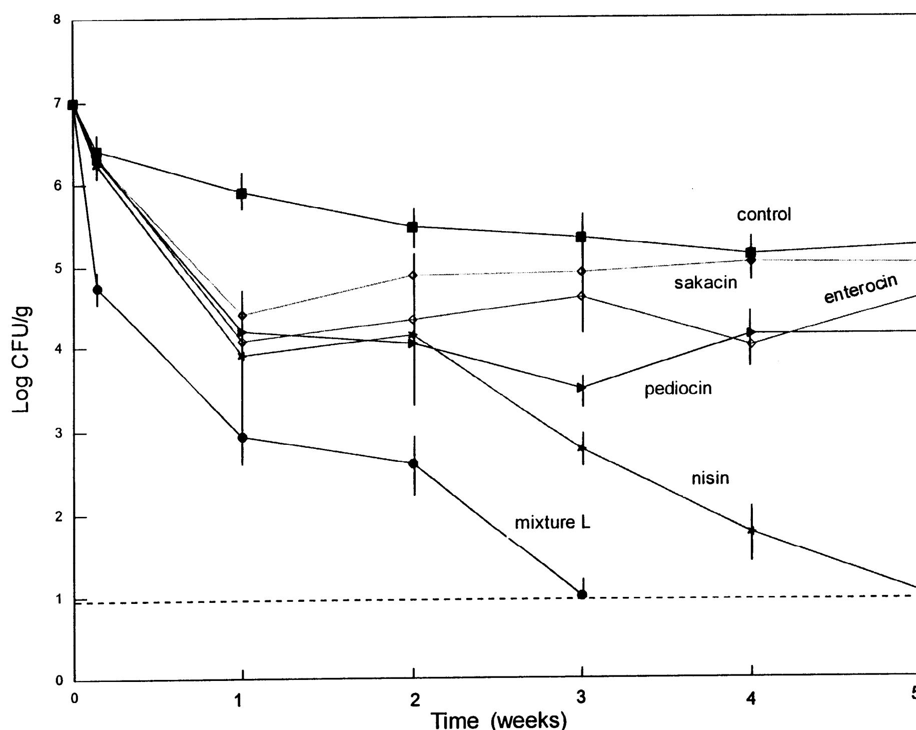



Figure 5

Effect of low level of pediocin, sakacin, enterocin, nisin or mixture L on the survival of two-strain cocktail of *L. monocytogenes* in Cheddar cheese curds stored at 6˚C for 5 weeks. Data represents the average of 3 trials. ■ = control, ♦ = contained sakacin, =➧ containing pediocin, ☆ = containing enterocin, * = containing nisin, and ● = containing mixture L. Dotted line and vertical bars indicate the limit to detection and calculated standard errors respectively.

Technologists. Honolulu, HI, January 25, 1995. (J.B. Luchansky)

"Applications of bacteriocins and bacteriocin-producing lactic acid bacteria in foods." Presentation at the International Dairy Lactic Acid Bacteria Conference. Palmerston North, New Zealand, February 20, 1995. (J.B. Luchansky)

"Applications of pulsed-field gel electrophoresis and lactic acid bacteria for subtyping and biocontrol of foodborne pathogens." Distinguished Science in Microbiology and Cell Science Lecturer, University of Florida. Gainesville, FL, March 24, 1995. (J.B. Luchansky)

"Microbial safety of reduced fat Cheddar cheese." Invited speaker at the International Business Communications Conference of Fat and Cholesterol-reduced Foods. New Orleans, LA, March 30, 1995. (J.B. Luchansky)

"Applications of lactic acid bacteria in food preservation." Keynote speaker at the Advanced Workshop on Bacteriocins of Lactic Acid Bacteria. Banff, Alberta, Canada, April 18, 1995. (J.B. Luchansky)

"Applications of genomic fingerprinting and bacteriocins for food microbiology." Annual Meeting of the Food Research Institute. Madison, WI, May 16, 1995. (J.B. Luchansky)

"Molecular typing and biocontrol of *L. monocytogenes* in foods." Distinguished Lectureship, Czechoslovakia Society of Microbiology. Brno, Czech Republic, October 25, 1995. (J.B. Luchansky)

"Dairy applications for biopreservatives and molecular subtyping." The 63rd Annual Dairy and Food Industry Conference, The Ohio State University. Columbus, OH, February 14, 1996. (J.B. Luchansky)

"Applications of biopreservatives and pulsed-field fingerprinting for the dairy and foods industries." Invited speaker at the California Polytechnic State University, Dairy Products Technology Center. San Luis Obispo, CA, March 11, 1996. (J.B. Luchansky)

"Use of biopreservatives to control foodborne pathogens." Invited speaker at the 15th Brazilian Food Science and Technology Congress. Pocos De Cladas, Minas Gerais, Brazil. August 6, 1996.

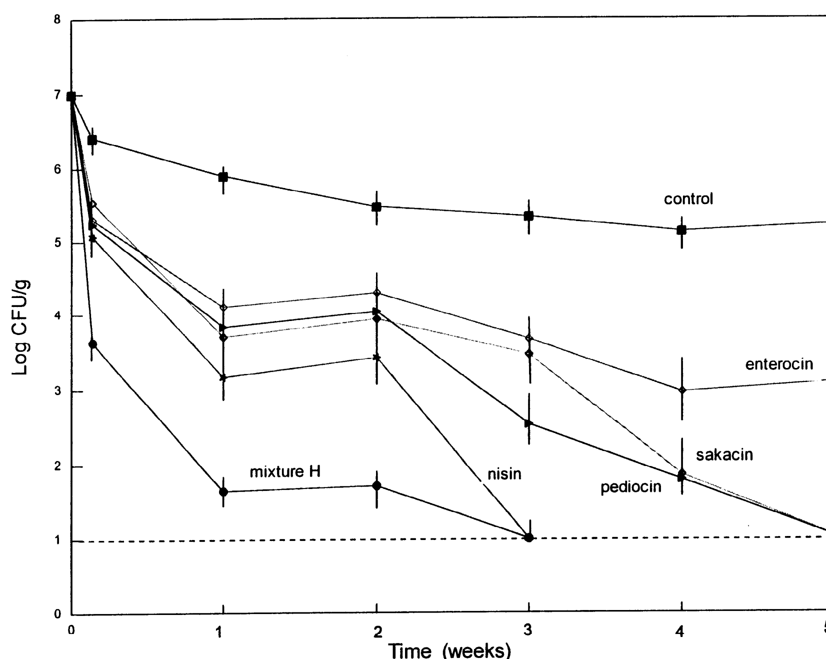



Figure 6

Effectof high levels of pediocin, sakacin, enterocin, nisin or mixture H on the survival of a two-strain cocktail of *L. monocytogens* in Cheddar cheese curdes stored at 6° C for 5 weeks. Data represents the average of 3 trials. ■ = control, ♦ = containing sakacin, ◗= containing pediocin, ☆ = containing enterocin, * = containing nisin, and ● = containing mixture H. Doted line and vertical bars indicate the limit of detection and calculated standard errors, respectively.

*FINAL REPORT*

# Potential uses of microbiological testing in cheese plant HACCP and quality assurance systems

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## Objectives

Develop microbiological verification/sampling procedures for use by cheese plants in HACCP and quality assurance programs.

Assess potential uses of microbiological testing in cheese plants for HACCP and Quality Assurance Systems

## Summary

It is widely accepted that microbiological testing is an important verification activity within a Hazard Analysis Critical Control Point (HACCP) food safety system. The goal of verification activities is to confirm that the HACCP system effectively ensures food safety and, if it does not, to alert HACCP team members to revise the HACCP plan or take other corrective actions. Because verification activities are done periodically and may be done for product that has already been shipped, the time required for microbiological testing is not a deterrent to its use. Monitoring of Critical Control Points, on the other hand, must indicate compliance or the product involved will be held pending corrective action. An ideal monitoring method would be one that provides instantaneous results and 100% product coverage. Therefore, microbiological testing is generally regarded as an inappropriate monitoring activity within a HACCP system because of excessive time required to perform the analyses.

In addition to its function for HACCP verification, microbiological testing can also be useful in verifying that prerequisite programs such worker hygiene, whey handling, cleaning, and sanitizing are effective. Therefore, microbiological analyses done in a cheese plant may include 1) testing for pathogenic bacteria that were identified as significant hazards during a hazard analysis, 2) testing for indicator microorganisms whose presence or numbers may be correlated with the presence of pathogens, and 3) testing for indicator microorganisms whose presence or numbers reflect poor control of a plant's prerequisite sanitation programs.

A wide variety of cheeses are made commercially in the USA. Even for a particular cheese variety, plants may differ markedly in the processing steps utilized. In the present study, comprehensive microbiological testing was done in three very different cheese processing plants. The goal of the project was to identify sampling sites and microbiological analyses that would be useful to cheesemakers for verification of HACCP, HACCP prerequisite (worker hygiene, sanitation) and quality assurance systems performance.

Each of three cheese plants was visited approximately twice quarterly over an 18 month period. The three plants involved in the project represented "large," "medium," and "small" sized plants in the United States. The types of cheeses made in the three plants included Cheddar and

related cheeses, Mozzarella, Muenster, and various fresh cheeses such as Queso Blanco. At each sampling visit, samples were taken from multiple lots of cheese. Samples were collected at steps from raw milk through one-month old finished cheeses. For milk, starter, combined curds and whey, whey, and whey cream the sample size was approximately 100 g. For curds, fines, and finished products the sample size was at least 10 g. Samples were obtained aseptically. Sampling sites for each plant are listed in Table 1.

All samples were tested for microorganisms whose presence or numbers might provide indications of safety or sanitary condition. Analyses were done in two cooperating laboratories at the University of Wisconsin-Madison. Analyses for coliforms, enterococci, presumptive lactobacilli, and yeasts and molds were done in one laboratory and analyses for presumptive *Staphylococcus aureus*, *Salmonella* spp., *Listeria monocytogenes*, and *Bacillus cereus* were done in the second laboratory.

The raw milk supplied to the three cheese plants was often contaminated with pathogenic bacteria (Table 2). *S. aureus* and *L. monocytogenes* were the most frequently present pathogens. *Salmonella* spp. and *B. cereus* were present much less frequently. When *S. aureus* was present in raw milk, its concentration varied (Table 3). Although raw milk at plant B was contaminated with *S. aureus* in 78.1% of samples, there were no samples from plant B containing > $1.0 \times 10^3$ CFU/ml (Table 2). The concentration of *S. aureus* in raw milk from plants A and C was occasionally > $1.0 \times 10^3$ CFU/ml, indicating either that contamination was greater or that raw milk holding temperatures were more conducive

Table 1. Microbiological sampling sites from cheese plants A, B, and C.

| Plant A | Number of samples collected |
|---|---|
| starter | 29 |
| raw milk | 28 |
| curds + whey from transfer line | 12 |
| whey storage in separator room | 17 |
| cold whey storage | 31 |
| hot whey storage | 22 |
| cold condensed whey storage | 27 |
| fines | 28 |
| whey cream | 31 |
| one-month old cheese | 29 |

| Plant B | Number of samples collected |
|---|---|
| raw milk | 32 |
| curds + whey after cutting | 31 |
| whey | 59 |
| brine | 12 |
| freshly packaged cheese | 8 |
| one-month old cheese | 35 |

| Plant C | Number of samples collected |
|---|---|
| starter | 40 |
| raw milk | 41 |
| whey after cutting | 39 |
| fines | 40 |
| whey in finishing vat | 42 |
| curd from mixer | 21 |
| chill water | 29 |
| brine | 21 |
| curd from blocks or molds | 21 |
| drippings from blocks or molds | 21 |
| one-month old cheese | 39 |

to growth. The four raw milk samples from plant C that contained *B. cereus* had very low concentrations of this pathogen ($1.0 - 9.0 \times 10^1$ CFU/ml) as did the one raw milk sample from plant A that contained *B. cereus* ($7.0 \times 10^1$ CFU/ml). Pasteurization eliminated all pathogens from milk except for *B. cereus* endospores (data not shown). Nearly all raw milk samples contained coliform bacteria at concentrations of $\geq 1.0 \times 10^1$ CFU/ml (Table 4). The percentage of raw milk samples that contained enterococci at concentrations of $\geq 1.0 \times 10^1$ CFU/ml varied, but was always much lower than for coliforms. In addition to being found in feces, coliform bacteria are present in vegetative matter, and thus the high

Table 2. Prevalence of *Salmonella* spp. , *Listeria monocytogenes* (L. mono.), *Staphylococcus aureus* , and *Bacillus cereus* in raw milk supplied to three Wisconsin cheese plants.

| Plant | Number of Samples | Frequency (%) of | | | |
|---|---|---|---|---|---|
| | | Salmonella[1] | L. mono.[1] | S. aureus[2] | B. cereus[2] |
| A | 22 | 18.2 | 77.3 | 68.2 | 4.5 |
| B | 32 | 6.3 | 56.3 | 78.1 | 0 |
| C | 41 | 7.3 | 24.4 | 82.9 | 9.7 |

Table 3. Concentrations of *Staphylococcus aureus* in raw milk supplied to three Wisconsin cheese plants.

| Plant | Number of Samples | Frequency (%) Between Given CFU/ml Values | | |
|---|---|---|---|---|
| | | $0 - 10^2$ | $10^2 - 10^3$ | $> 10^3$ |
| A | 22 | 81.8 | 4.5 | 13.7 |
| B | 32 | 56.3 | 43.7 | 0 |
| C | 41 | 60.9 | 31.7 | 7.3 |

Table 4. Frequency (% of samples) of indicator bacteria present at specified concentrations in raw milk supplied to three Wisconsin cheese plants. Concentrations are in CFU/g.

| Plant | n | Coliforms | Enterococci | Presumptive Lactobacilli | Yeasts & Molds |
|---|---|---|---|---|---|
| | | $\geq 10^{1}$[a] | $\geq 10^1$ | $\geq 10^3$ | $\geq 10^2$ |
| A | 28 | 92.9 | 10.7 | 64.3 | 60.7 |
| B | 32 | 96.9 | 37.5 | 21.9 | 56.3 |
| C | 41 | 97.6 | 9.8 | 36.6 | 36.6 |

percentage of raw milk samples contaminated with coliforms does not necessarily indicate fecal contamination. Enumeration of *Escherichia coli* would perhaps be more useful than coliforms for indicating fecal contamination, but the dairy industry has traditionally used coliform counts as an index of overall sanitary conditions. The genus *Enterococcus* has been studied as an alternative to coliforms for indicating fecal contamination or overall sanitary conditions. The main advantage of using this genus instead of the coliform group is that the enterococci are much more cold-tolerant than coliforms and would be more likely to survive during refrigerated storage of samples to the same extent as any pathogens that are present. Among the enterococci, *Enterococcus faecalis* and *E. faecium* are known to be primarily of fecal origin. *Enterococcus hirae*, *E. durans*, and *E. saccharolyticum* have been associated with cattle. Other *Enterococcus* species have been isolated from the feces of humans, swine, or birds. However, some of the enterococci are also found on vegetation or in soil. Therefore, the presence of enterococci in dairy products does not necessarily indicate that fecal contamination has occurred. In addition, some *Enterococcus* spp. reportedly are used as starter cultures.

Table 5. Percentage of in-process and product samples from Plant A containing pathogenic bacteria and indicator microorganisms. Abbreviations and criteria for each organism are listed below.

| Site | B.c. | L. m. | S.a. | Salm. | Colif. | Ent. | Lb. | YM |
|---|---|---|---|---|---|---|---|---|
| starter | 0* | NT | NT | NT | 3.4 | 0 | 0 | 0 |
| curds + whey from transfer line | 0 | NT | NT | NT | 0 | 33.3 | 0 | 0 |
| whey storage in separator room | 4.3 | 0 | 25.0 | 0 | 86.4 | 0 | 63.6 | 9.1 |
| cold whey storage | 14.3 | 0 | 3.6 | 0 | 85.2 | 0 | 29.6 | 11.1 |
| hot whey storage | 4.5 | 0 | 0 | 0 | 27.3 | 0 | 9.1 | 13.6 |
| cold condensed whey storage | 14.8 | 0 | 9.5 | 0 | 37.0 | 0 | 3.7 | 3.7 |
| fines | 3.6 | NT | NT | NT | 96.4 | 25.0 | 85.7 | 46.4 |
| whey cream | 22.6 | NT | NT | NT | 87.1 | 67.7 | 61.3 | 25.8 |
| one-month old cheese | 0 | NT | NT | NT | 3.4 | 55.2 | 3.4 | 6.9 |

* n= 20
NT = not tested
*B. cereus* (B.c.): % of samples containing $\geq 5.0 \times 10^{0}$ CFU per ml or g.
*L. monocytogenes* (L.m.): % of 25 g samples containing the organism.
*S. aureus* (S.a.): % of samples containing $\geq 5.0 \times 10^{0}$ CFU per ml or g.
*Salmonella* spp. (Salm.): % of 25 g samples containing the organism.
Coliforms (Colif.): % of samples containing $\geq 1.0 \times 10^{1}$ CFU per ml or g.
Enterococci (Ent.): % of samples containing $\geq 1.0 \times 10^{1}$ CFU per ml or g.
Presumptive lactobacilli (Lb.): % of samples containing $\geq 1.0 \times 10^{3}$ CFU per ml or g.
Yeasts and Molds (YM): % of samples containing $\geq 1.0 \times 10^{2}$ CFU per ml or g.

Obviously, concentrations of enterococci could not be used as an indication of sanitation in this situation.

Between 21.9 and 64.3% of raw milk samples contained presumptive lactobacilli at concentrations of $\geq 1.0 \times 10^3$ CFU/ml and between 36.6 and 60.7% of raw milk samples contained yeasts and molds at concentrations of $\geq 1.0 \times 10^2$ CFU/ml. Lactobacilli are normally associated with raw milk and their presence in high numbers is probably of limited value as an indicator of raw milk quality. Yeasts and molds are commonly associated with the dairy farm environment and their presence in raw milk may indicate environmental contamination.

Because each cheese plant varied considerably in size, layout, and types of cheeses produced, the microbiological testing results will be discussed for each plant in turn (Tables 5-7). Analyses for *L. monocytogenes, Salmonella* spp. and presumptive *S. aureus* were performed on whey samples from plant A. None of these samples contained detectable *L. monocytogenes* or *Salmonella* spp. Presumptive *S. aureus* was detected in 25% of whey samples taken from storage in the separator room. Concentrations of *S. aureus* in these positive samples were all $\geq 1.0 \times 10^3$ CFU/ml. Of the remaining whey sampling sites, only three samples contained presumptive *S. aureus* ; none of the three samples had concentrations of $\geq 1.0 \times 10^2$ CFU/ml. *S. aureus* is usually only considered hazardous in foods when present at the high concentrations associated with enterotoxin production. Low numbers of *S. aureus* are only likely to increase to dangerous levels if the product is temperature-abused and if there is little microbial competition. The whey may have been contaminated with *S.* aureus by plant personnel because an estimated 10 to 40% of humans carry this pathogen in the nose. Presence of *S. aureus* in curds or finished cheese may be used to indicate that either poor personnel hygiene practices or sloppy handling of whey occurred. B. *cereus* was detected at low concentrations ($5.0 \times 10^0$ - $6.2 \times 10^2$ CFU/ml) in whey, whey cream, and fines samples in

Table 6. Percentage of in-process and product samples from Plant B containing pathogenic bacteria and indicator microorganisms. Abbreviations and criteria for each organism are listed below.

| Site | B.c. | L. m. | S.a. | Salm. | Colif. | Ent. | Lb. | YM |
|---|---|---|---|---|---|---|---|---|
| curds + whey after cutting | 9.7 | 0 | 3.2 | 0 | 9.7 | 29.0 | 9.7 | 6.4 |
| whey | 10.2 | 0 | 23.7 | 0 | 23.7 | 1.7 | 11.9 | 3.4 |
| brine | 0 | 0 | 8.3 | 0 | 0 | 0 | 16.7 | 0 |
| freshly packaged cheese | 50.0* | 0 | 0 | 0 | 100** | 37.5* | 0 | 0 |
| one-month old cheese | 2.9 | 0 | 25.7 | 0 | 34.3 | 5.7 | 5.7 | 10.5 |

* n = 8

** n = 4

*B. cereus* (B.c.): % of samples containing $\geq 5.0 \times 10^0$ CFU per ml or g.

*L. monocytogenes* (L.m.): % of 25 g samples containing the organism.

*S. aureus* (S.a.): % of samples containing $\geq 5.0 \times 10^0$ CFU per ml or g.

*Salmonella* spp. (Salm.): % of 25 g samples containing the organism.

Coliforms (Colif.): % of samples containing $\geq 1.0 \times 10^1$ CFU per ml or g.

Enterococci (Ent.): % of samples containing $\geq 1.0 \times 10^1$ CFU per ml or g.

Presumptive lactobacilli (Lb.): % of samples containing $\geq 1.0 \times 10^3$ CFU per ml or g.

Yeasts and Molds (YM): % of samples containing $\geq 1.0 \times 10^2$ CFU per ml or g.

plant A. Since *B. cereus* was present in low concentrations in a small proportion of raw milk samples at plant A and pasteurization does not destroy *B. cereus* endospores (1), its presence in whey was not surprising.

Coliforms ($\geq$1.0 x $10^1$ CFU/ml or g) were frequently detected in whey, whey cream, and fines samples from plant A. Enterococci ($\geq$ 1.0 x $10^1$ CFU/ml or g) were not detected in whey samples, but were present in one-fourth of fines samples and over two-thirds of whey cream samples. Yeasts and molds ($\geq$ 1.0 x $10^2$ CFU/ml or g) were present in just over 10% of whey samples, in over 40% of fines samples, and in 25.8% of the whey cream samples. Presumptive lactobacilli were present in concentrations $\geq$ 1.0 x $10^3$ CFU/ml or g in the vast majority of fines and whey cream samples. Although not indicators per se, these organisms may have an effect on cheese quality. The prevalence of coliform bacteria, yeasts and molds, and presumptive lactobacilli in whey, fines, and whey cream samples strongly suggests that these byproducts can serve as sources of microbial contaminants in cheesemaking. However, the

Table 7. Percentage of in-process and product samples from Plant C containing pathogenic bacteria and indicator microorganisms. Abbreviations and criteria for each organism are listed below.

| Site | B.c. | L. m. | S.a. | Salm. | Colif. | Ent. | Lb. | YM |
|---|---|---|---|---|---|---|---|---|
| starter | 2.5 | 0 | 7.5 | 0 | 0 | 0 | 0 | 0 |
| whey after cutting | 2.4 | 0 | 9.5 | 0 | 0 | 7.1 | 0* | 0 |
| fines | 0 | 0 | 12.8 | 0 | 23.1 | 5.1 | 0* | 0 |
| whey from finishing vat | 2.4 | 0 | 4.8 | 0 | 4.8 | 2.4 | 0* | 0 |
| curd from mixer | 4.8 | 0 | 14.3 | 0 | 0 | 28.6 | 66.7 | 0 |
| chill water | 26.7 | 0 | 30.0 | 0 | 26.7 | 0 | 30.0 | 13.3 |
| brine | 28.6 | 0 | 9.5 | 0 | 0 | 0 | 0 | 14.3 |
| curds from molds or blocks* | 0 | 0 | 9.5 | 0 | 9.5 | 23.8 | 14.3 | 0 |
| drippings from molds or blocks* | 0 | 0 | 28.6 | 0 | 61.9 | 0 | 38.1 | 28.6 |
| one-month old cheese | 0 | NT | NT | NT | 7.1 | 33.3 | 0* | 0 |

* Only cheeses made without added lactobacilli
NT = Not Tested
*B. cereus* (B.c.): % of samples containing $\geq$ 5.0 x $10^0$ CFU per ml or g.
*L. monocytogenes* (L.m.): % of 25 g samples containing the organism.
*S. aureus* (S.a.): % of samples containing $\geq$ 5.0 x $10^0$ CFU per ml or g.
*Salmonella* spp. (Salm.): % of 25 g samples containing the organism.
Coliforms (Colif.): % of samples containing $\geq$ 1.0 x $10^1$ CFU per ml or g.
Enterococci (Ent.): % of samples containing $\geq$ 1.0 x $10^1$ CFU per ml or g.
Presumptive lactobacilli (Lb.): % of samples containing $\geq$ 1.0 x $10^3$ CFU per ml or g.
Yeasts and Molds (YM): % of samples containing $\geq$ 1.0 x $10^2$ CFU per ml or g.

only indicator organisms found in more than 10% of the one-month old cheese samples were enterococci. This finding probably reflects the fact that the enterococci are more cold-tolerant than coliforms. The microbiological testing results from plant A suggest the following: a) whey cream and fines, if used in cheesemaking, can serve as a contamination route for indicator organisms and *B. cereus* endospores, b) testing curd or cheese samples for *S. aureus* may be useful for verifying proper whey handling and worker hygiene practices during cheesemaking, c) enterococci appear to be the indicator organisms most likely to survive in the one month old cheese and thus may be the best indication of in-process sanitation.

Production of cheese in plant B (Table 6) was less automated than in plants A and C, and thus contamination of cheese with *S. aureus* via worker handling would be expected to occur more frequently than in plants A and C. Whey from plant B was more frequently contaminated with this pathogen than wheys from plants A and C. Although no freshly packaged cheeses contained detectable levels of *S. aureus*, 25.7% of the one-month old cheeses contained low ($1.0 \times 10^1$ - $1.6 \times 10^2$ CFU/g) concentrations of *S. aureus.* One sample of curds and whey sampled after cutting also contained *S. aureus* at a low concentration ($1.0 \times 10^1$ CFU/g). Plant B samples were predominantly from batches of fresh-type cheese, such as Queso Blanco. Plants A and C did not make fresh-type cheeses. The amount of manual labor involved in cheesemaking, along with the likely absence of competing starter culture bacteria (starter cultures were not used in 80% of the batches sampled), demonstrates the importance of verifying that proper sanitary practices are followed at plant B. *Bacillus cereus* was infrequently ($\leq$ 10%) present in samples, with the exception of the freshly packaged cheeses. Of the eight freshly packaged cheeses tested, half contained low concentrations of this pathogen. However, only 2.9% of one-month old cheeses from plant B contained *B. cereus* . No in-process or cheese samples from plant B contained detectable levels of *L. monocytogenes* or *Salmonella* spp.

Indicator organisms were relatively infrequent in curd and whey samples taken after cutting in plant B. As in plant A, results suggested that whey was a potential source of contamination with indicator organisms, although the percentages of samples containing $\geq 1.0 \times 10^1$ CFU/ml of coliforms, $\geq 1.0 \times 10^2$ yeasts and molds, or $\geq 1.0 \times 10^3$ CFU/ml presumptive lactobacilli were lower than for whey samples from plant A. The brine samples tested did not contain coliforms or enterococci, but one sample did contain a low concentration ($1.5 \times 10^1$ CFU/ml) of *S. aureus*. Presumptive lactobacilli at concentrations of $\geq 1.0 \times 10^3$ CFU/g were found in one-sixth of the brine samples. The relative scarcity of presumptive lactobacilli probably occurred because starter cultures containing *Lactobacillus* were not used in the plant. Coliforms were the indicator organisms most likely to be present in one month old cheese samples. Yeasts and molds at concentrations of $\geq 1.0 \times 10^2$ CFU/g or ml were only detected in 3.4 and 10.5 % of whey and one month old cheese samples, respectively. These results show that a rigorous sanitation and hygiene training program instituted by plant B were effective in reducing microbial indices to levels comparable to those at plants A and C. The results also suggest that a) testing of whey, brine, and finished product for *S. aureus* may be useful to plant B for verifying the maintenance of proper whey handling and worker hygiene during cheesemaking, and b) coliform testing of finished product may provide verification of proper whey handling and plant sanitation practices.

*L. monocytogenes* and *Salmonella* spp. were not detected in any in-process sample at plant C (Table 7). However, *S. aureus* , usually at concentrations of $\leq 1.0 \times 10^2$ CFU/ml or g, was found in 4.8 - 30% of samples taken from in-process sites. This pathogen was most commonly found in chill water, drippings from molds or blocks of cheese, curd taken from the mixer, and fines. *B. cereus* was present in approximately one-quarter of the brine and chill water samples, at concentrations of $5.0 \times 10^0$ - $1.7 \times 10^3$ CFU/ml. Other samples containing *B. cereus* were the starter, whey obtained after curd cutting, whey from the finishing vat, and curd from the mixer.

Coliforms ($\geq 1.0 \times 10^1$ CFU/g or ml) were detected in 23.1% of fines samples, 26.7% of chill water samples, and 61.9% of sampled drippings from molds or blocks. However, only 7.1% of one-month old cheeses contained coliforms at concentrations $\geq 1.0 \times 10^1$ CFU/g. Enterococci at concentrations of $\geq 1.0 \times 10^1$ CFU/g or ml were not detected in the chill water but were detected in over one-fourth of curd samples taken from the mixer. The latter samples did not contain coliforms at concentrations of $\geq 1.0 \times 10^1$ CFU/g. This difference may reflect the fact that Gram-positive bacteria, including the enterococci, are generally more heat-resistant than Gram-negative bacteria and could presumably survive heating during mixing of the curds. Enterococci at concentrations of $\geq 1.0 \times 10^1$ CFU/g were detected in 33.3% of the one-month old cheeses, but coliforms at

these concentrations were only present in 7.1% of these samples. Presumptive *Lactobacillus* spp. should not be used as an indicator group for sanitation when *Lactobacillus* spp. are used in cheese manufacture. Presumptive lactobacilli at concentrations of $\geq 1.0 \times 10^3$ CFU/g or ml were found in some curd and drippings samples from molds or blocks, but were not found in one-month old samples of cheese made without added lactobacilli. Yeasts and molds at concentrations of $\geq 1.0 \times 10^2$ CFU/g or ml were only detected in chill water and brine, and in drippings from molds or blocks. The results of testing in plant C suggest that a) analysis for presumptive *S. aureus* would be useful for verifying worker hygienic practices, b) chill water and brine are potential sources of bacterial contamination, and their microbiological quality and safety should be verified by testing for presumptive *S. aureus* (chill water and brine) and coliforms (chill water only), c) enterococci are probably the most useful indicator organisms for verification of stored cheese quality.

Overall, the results of this project show that a) raw milk received at cheese plants is often contaminated with pathogenic bacteria and should be heated to kill these organisms, b) *S. aureus* is a frequent in-process pathogenic contaminant and testing for this pathogen is an important way of verifying whether prerequisite whey handling and worker hygiene programs are successful, c) whey, fines, whey cream, chill water, and brine are potentially important sources of bacterial contamination, d) enterococci are more likely to be present in one-month old Cheddar-type and Mozzarella cheeses than coliforms and may be a more useful indicator organism for use in finished product testing, and e) coliforms are more likely than enterococci to be present in one-month old fresh-type cheeses and may be an appropriate indicator organism for use with these cheeses. The results of this study emphasize the role that microbiological testing can play in verifying the effectiveness of HACCP and prerequisite programs.

*FINAL REPORT*

# Microbiology of reduced fat and fat free cheese products


### Personnel

Eric A. Johnson, professor; Ann E. Larson, senior research specialist; Eric Manthei, student worker; Jeff Diercks, student worker, Food Microbiology and Toxicology



### Funding

Dairy Management Inc. JHN97


### Dates

July 1996- June 1997

## Objectives

Determine survival and growth of *Salmonella* and *Listeria monocytogenes*, and toxin production by *Clostridium botulinum* in full fat, reduced-fat, and nonfat Cheddar cheese, and in reduced-fat Cheddar containing various fat replacers.


## Summary

Full fat, reduced fat (target levels 25% and 50% reduced fat calculated on a dry basis), and nonfat white Cheddar cheese were prepared from HTST pasteurized non-homogenized whole, 2%, 1%, or skim milk using a standard Cheddar cheese making protocol. Additional batches of 50% reduced-fat Cheddar cheese were prepared using commercially available carbohydrate based (modified corn starch), cellulose based, or whey protein based fat replacers. Spores of *Clostridium botulinum* (mixture of 7 proteolytic A /B and 3 non-proteolytic B/E strains; target $10^4$ spores/g cheese), or vegetative cells of *L. monocytogenes* (mixture of 4 strains; target $10^4$-$10^5$ CFU/g cheese) or *Salmonella* (mixture of 3 strains; target $10^4$-$10^5$ CFU/g cheese) were added to milk before addition of starter culture or rennet. Full fat cheese inoculated with *C. botulinum* was also prepared with washed curd treatment (to increase moisture). After pressing for 16 h, cheeses were cut into 50-100 g blocks, vacuum packaged in gas impermeable polyethylene bags, and incubated at 4°C, 12°C, or 30°C. Duplicate vats of cheese were prepared for each combination of pathogen/fat level/fat replacer.

Triplicate samples from each vat were tested at day 1 (day of packaging) for *C. botulinum* spore inoculum level (5-tube MPN in TPGY-cooked meat broth) or numbers of *L. monocytogenes* (plating on Modified Oxford agar) or *Salmonella* (plating on XLD agar). Additionally, samples were tested for pH, % moisture, toxicity (mouse assay; samples with *C. botulinum*)water activity, % fat (dry basis), % NaCl, and populations of lactic acid bacteria. Table A lists the initial parameters of cheese batches.

After 1, 2, and 3 weeks, and 1, 2, 3, 4, 5, and 6 months of incubation, triplicate samples from each vat at each incubation temperature were examined for odor and appearance (including gas production), and tested for pH, moisture, and toxin production (*C. botulinum* samples; mouse bioassay) or numbers of *L. monocytogenes* or *Salmonella*. Presence of botulinal toxin in samples causing mouse deaths was confirmed using type A, B, and E botulinal antitoxin. In most cases, testing of samples from a vat at an incubation temperature was discontinued either after gross spoilage occurred, or if two or more samples were toxic at two consecutive sampling intervals. Cheese was considered to be grossly spoiled if it had a bad off odor and severe gassing, and was definitely inedible.

No cheese samples containing *C. botulinum* became toxic during incubation at 4°C or 12°C. Table B shows results of toxicity testing of samples incubated at 30°C. No toxin was detected in standard full fat or 25% reduced-fat cheese, but toxin was detected at 2 months in both full fat washed curd vats (before gross

**Table A: Initial Parameters of Cheddar Cheese**[a]

| target fat level | % moisture[b] | pH[c] | $a_w$[d] | LAB[e] | % NaCl[f] | % fat (dry)[g] |
|---|---|---|---|---|---|---|
| full fat | 40.44 ± 1.01 | 5.32 ± 0.25 | 0.96 ± 0.01 | 9.16 ± 0.16 | 2.57 ± 0.42 | 55.84 ± 5.42 |
| 25% reduced fat | 43.14 ± 1.06 | 5.41 ± 0.21 | 0.96 ± 0.01 | 9.09 ± 0.29 | 2.59 ± 0.42 | 38.00 ± 4.08 |
| 50% reduced fat | 47.53 ± 2.33 | 5.30 ± 0.15 | 0.96 ± 0.00 | 9.20 ± 0.16 | 2.42 ± 0.23 | 24.71 ± 3.92 |
| 50% reduced fat (Stellar) | 48.32 ± 1.08 | 5.17 ± 0.12 | 0.97 ± 0.00 | 9.18 ± 0.12 | 2.77 ± 0.38 | 22.20 ± 4.44 |
| 50% reduced fat (Dairy Lo) | 48.63 ± 0.76 | 5.25 ± 0.14 | 0.96 ± 0.00 | 9.33 ± 0.07 | 2.43 ± 0.30 | 25.03 ± 4.37 |
| 50% reduced fat (Novagel) | 46.81 ± 1.65 | 5.24 ± 0.08 | 0.96 ± 0.01 | 9.11 ± 0.25 | 2.35 ± 0.38 | 25.75 ± 6.75 |
| nonfat | 51.12 ± 2.63 | 5.36 ± 0.19 | 0.96 ± 0.01 | 9.07 ± 0.27 | 2.36 ± 0.50 | 5.02 ± 2.58 |
| full fat (washed curd)[h] | 42.16 ± 0.45 | 5.03 ± 0.00 | 0.96 ± 0.00 | 9.39 ± 0.01 | 2.63 ± 0.21 | 54.25 ± 1.34 |

[a] tested at day 1 (after cutting); average ± standard deviation
[b] five-hour 100°C vacuum oven method
[c] pH determined for sample diluted 1:1 in $dH_20$ and homogenized
[d] Decagon CX2 water activity meter
[e] Lactic Acid Bacteria; log CFU/g determined by pour plating in MRS agar
[f] Volhard titration
[g] Babcock method
[h] data only from duplicate vats inoculated with *C. botulinum*

spoilage in one vat). Toxin was detected in 50% reduced-fat cheese at 4 weeks-2 months. Carbohydrate-based fat replacer delayed toxin production to 4 months in one 50% reduced-fat vat, with no toxin produced before gross spoilage in the other vat. The addition of cellulose based or whey protein based fat replacers accelerated toxin production (occurring at 3 weeks at 30°C) compared to 50% reduced-fat cheese without fat replacers. Toxin was detected in nonfat cheese at 30°C at 4 weeks (before gross spoilage in one vat).

At 4°C, *L. monocytogenes* populations remained relatively stable or slightly decreased (≤1 log CFU/g) over 6 months in all cheeses (data not shown). At 12°C, populations decreased 2 log CFU/g in full fat cheese, but remained relatively stable (decrease of ≤1 log CFU/g) in other cheeses. At 30°C, numbers of *L. monocytogenes* decreased steadily in all cheeses, but fastest in full fat cheese (to undetectable numbers after 3 months)

**Table B: Toxicity of Cheddar cheese inoculated with *Clostridium botulinum* at 30°C**

| | | sampling time | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | vat | day 0 | 1 wk | 2 wks | 3 wks | 4 wks | 2 mos | 3 mos | 4 mos | 5 mos | 6 mos |
| ***C. botulinum*** | | | | | | | | | | | |
| full fat | A[a] | 0[b] | 0 | 0 | 0 | 0 | 0[c] | NT[d] | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 0[c] | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 3[c] | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 2 | 3 | 2 | NT | NT | NT |
| 50% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 0[c] | NT | NT | NT | NT |
| carbohydrate based FR | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 |
| 50% reduced fat A | 0 | 0 | 0 | 2[c] | NT | NT | NT | NT | NT | NT | |
| whey protein based FR | B | 0 | 0 | 0 | 2[c] | NT | NT | NT | NT | NT | NT |
| 50% reduced fat A | 0 | 0 | 0 | 2[c] | NT | NT | NT | NT | NT | NT | |
| cellulose based FR | B | 0 | 0 | 0 | 1[c] | NT | NT | NT | NT | NT | NT |
| nonfat | A | 0 | 0 | 0 | 0 | 3[c] | NT | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 1 | 3[c] | NT | NT | NT | NT |
| full fat | A | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| (washed curd) | B | 0 | 0 | 0 | 0 | 0 | 3[c] | NT | NT | NT | NT |

[a] vats A and B are duplicate vats
[b] # toxic samples (out of 3)
[c] grossly spoiled (definitely inedible)
[d] NT=not tested

*Salmonella* populations decreased steadily in all cheese at 4°C (ca. 2-3 log CFU/g over 6 months). At 12°C, *Salmonella* was not detected in full fat or 25% reduced-fat vats after 3-5 months. In nonfat cheese and 50% reduced fat cheese (with and without fat replacers), *Salmonella* numbers decreased ca. 5-6 log CFU/g over 6 months at 12°C. At 30°C, *Salmonella* numbers rapidly decreased to undetectable levels in 2 weeks-3 months (fastest in 50% reduced-fat cheese with cellulose based fat replacer).

Due to the higher moisture content and other properties of reduced fat cheeses, concerns have been raised regarding the possibility of growth of pathogens such as *C. botulinum*, *L. monocytogene*s and *Samonella*. Results of this study indicate the potential of toxin production by *C. botulinum* in reduced fat Cheddar cheese upon severe temperature abuse. However, *L. monocytogenes* or *Salmonella* populations remained stable or decreased in all cheeses tested.

*FINAL REPORT*

# Control of *Clostridium botulinum* and related sporeformers in full fat and reduced fat Cheddar cheese

**Personnel**

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**Funding**

Dairy Management Inc. JHN95

**Dates**

July 1994-September 1997

## Objectives

Isolate clostridia responsible for gaseous spoilage of commercial Cheddar cheese obtained from manufacturers in Wisconsin.

Evaluate the ability of *Clostridium botulinum*, *C. sporogenes*, and toxigenic *C. butyricum* to grow and produce toxin or cause spoilage in full fat versus reduced fat Cheddar cheese.

Examine the conditions contributing to growth and toxin production by these *Clostridia* in full fat and reduced fat Cheddar cheese.

Prepare cationic peptides and lipid fractions from full fat and lowfat Cheddar cheese and test these for inhibitory activity against pathogens.

## Summary

Efforts to isolate anaerobic sporeformers from samples of gaseous cheese obtained from the cheese industry were unsuccessful. However, we continue our efforts to obtain natural isolates of clostridia species from spoiled cheeses.

To determine the ability of added nutrients to stimulate *C. botulinum* toxin production in process cheese, one percent (w/v) yeast extract, glucose, and enzymatic casein hydrolysate were added alone or in combination to commercial nacho sauce implicated in a botulism outbreak. Two strains (one each type A and B) of *C. botulinum* were inoculated ($10^4$ spores/g) into the sauce, samples incubated at 20°C or 32°C, and toxin production determined by mouse assay. At both 20°C and 32°C, toxin production was accelerated by all three additives (espectially yeast extract) compared to control cheese.

Lipids were extracted from commercial full fat and reduced fat Cheddar cheese samples with a mixture of ethanol and acetone, concentrated, and dried over sodium sulfate. Extracts (up to 1% v/v) were tested for inhibition of pathogens and spoilage organisms in broth media. Lipid extracts did not inhibit growth of *L. monocytogenes*, *Salmonella hiedelberg*, *C. botulinum* (56A and 17B), *E. coli* O157:H7, *Staphylococcus aureus*, cheddar cheese starter culture, *Clostridium difficile*, *Helicobacter pylori*, or *Zygosaccharomyces bailii*.

Cationic peptide fractions were prepared from whey permeate by TCA precipitation of proteins, centrifugation, suspension in 20 mM sodium phosphate buffer (pH 7.0), and gel filtration using a Sephadex C-50 column. Peptides were eluted with aqueous 1.0 M NaCl, and fractions tested in broth media for inhibition of growth of *C. botulinum* (56A and 17B), *L. monocytogenes*, *Salmonella*, *E. coli* O157:H7, and *S. aureus*. No inhibition of growth was observed.

**Table 1: Initial Parameters of Cheddar Cheese[a]**

| target fat level | % moisture[b] | pH[c] | $a_w$[d] | LAB[e] | % NaCl[f] | % fat (dry)[g] |
|---|---|---|---|---|---|---|
| full fat | 40.16 ± 0.75 | 5.48 ± 0.17 | 0.960 ± 0.01 | 9.18 ± 0.19 | 2.49 ± 0.24 | 52.48 ± 3.16 |
| 25% reduced fat | 43.25 ± 0.87 | 5.45 ± 0.19 | 0.959 ± 0.01 | 9.09 ± 0.27 | 2.39 ± 0.70 | 37.10 ± 2.83 |
| 50% reduced fat | 45.73 ± 0.51 | 5.40 ± 0.12 | 0.960 ± 0.00 | 9.27 ± 0.05 | 2.11 ± 0.25 | 25.56 ± 3.51 |
| 50% reduced fat (fat replacer) | 48.95 ± 0.72 | 5.25 ± 0.07 | 0.970 ± 0.017 | 9.25 ± 0.17 | 2.53 ± 0.46 | 25.48 ± 2.58 |

[a] tested at day 1 (after cutting); average ± standard deviation
[b] five-hour 100°C vacuum oven method
[c] pH determined for sample diluted 1:1 in $dH_20$ and homogenized
[d] Decagon CX2 water activity meter
[e] Lactic Acid Bacteria; log CFU/g determined by pour plating in MRS agar
[f] Volhard titration
[g] Babcock method

Full fat and reduced fat (target levels 25% and 50% reduced fat calculated on a dry basis) white Cheddar cheese was prepared from HTST pasteurized non-homogenized whole, 2%, or 1% milk using a standard Cheddar cheese making protocol. A carbohydrate-based fat replacer (4% w/w) was added to milk during the making of half of the batches of 50% reduced-fat milk. Spores of *Clostridium botulinum* (mixture of 7 proteolytic A / B and 3 non-proteolytic B/E strains), *C. butyricum* (mixture of 3 toxigenic strains that produce type E botulinal toxin), or *C. sporogenes* (mixture of 3 strains) were added to milk before addition of starter culture or rennet. After pressing for 16 h, cheeses were cut into 50-100 g blocks, vacuum packaged in gas impermeable polyethylene bags, and incubated at 4°C, 12°C, or 30°C. Duplicate vats of cheese were prepared for each combination of pathogen/fat level.

Triplicate samples from each vat were tested at day 1 (day of packaging) for spore inoculum level [5-tube MPN in TPGY-cooked meat broth for *C. botulinum* and *C. butyricum*; plating on *Clostridium botulinum* isolation (CBI) agar for *C. sporogenes*], pH, % moisture, toxicity (mouse assay; samples with *C. botulinum* and *C. butyricum* only), water activity, % fat (dry basis), % NaCl, and populations of lactic acid bacteria. Table 1 lists the initial parameters of cheese batches.

After 1, 2, and 3 weeks, and 1, 2, 3, 4, 5, and 6 months of incubation, triplicate samples from each vat at each incubation temperature were examined for odor and appearance (including gas production). Additionally, samples were tested for pH, moisture, and toxin production (*C. botulinum* and *C. butyricum* samples; mouse bioassay) or levels of *C. sporogenes* (plating on CBI agar). Presence of botulinal toxin in samples causing mouse deaths was confirmed with type A, B, and E botulinal antitoxin. In most cases, testing of samples from a vat at an incubation temperature was discontinued either after gross spoilage occurred, or if two more samples were toxic at two consecutive sampling intervals. A cheese sample was considered to be grossly spoiled if it had a moderate or severe off odor and severe gassing, and was definitely inedible.

**Table 2: Toxicity of Cheddar cheese inoculated with *Clostridium botulinum* or *C. butyricum* and incubated at 30°C[b]**

| | | sampling time | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | vat[a] | day 0 | 1 week | 2 weeks | 3 weeks | 4 weeks | 2 months | 3 months | 4 months | 5 months | 6 months |
| ***C. botulinum*** | | | | | | | | | | | |
| full fat | A | 0 | 0 | 0 | 0 | 0 | 0[c] | NT[d] | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 0[c] | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 3[c] | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 2 | 3 | 2 | NT | NT | NT |
| 50% reduced fat with fat replacer | A | 0 | 0 | 0 | 0 | 0 | 0[c] | NT | NT | NT | NT |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 |
| ***C. butyricum*** | | | | | | | | | | | |
| full fat | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 50% reduced fat | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 | 0[c] | NT | NT | NT | NT |
| 50% reduced fat with fat replacer | A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | B | 0 | 0 | 0 | 0 | 0 | 0 | 0[c] | 0[c] | 0[c] | 0[c] |

[a] vats A and B are duplicate vats
[b] # toxic samples (out of 3)
[c] grossly spoiled (definitely inedible)
[d] NT=not tested

No cheese samples containing *C. botulinum* or *C. butyricum* became toxic during incubation at 4°C or 12°C. Table 2 shows results of toxicity testing of samples incubated at 30°C. No botulinal toxin was detected in samples containing *C. butyricum* at 30°C, although gross spoilage occurred in one vat each of 50% reduced fat and 50% reduced fat with fat replacer at 2 months and 3 months, respectively.

At 30°C, one vat each of full fat and 25% reduced fat cheese containing *C. botulinum* were grossly spoiled at 2 months, but not toxic. In 50% reduced fat cheese containing *C. botulinum* at 30°C, one vat became toxic at 2 months, concurrent with gross spoilage, but toxin production occurred in the duplicate vat at 4 weeks without gross spoilage. It should be noted that determination of spoilage was very conservative. Cheeses

considered grossly spoiled were considered to be very obviously inedible, with severe off odor and gas production. At 30°C, one vat of 50% reduced fat cheese made with fat replacer and containing *C. botulinum* was grossly spoiled without toxin production at 2 months, and the other was toxic without gross spoilage at 4 months.

Populations of *C. sporogenes* remained relatively stable in all cheeses incubated at 4°C, and in full fat and 25% reduced fat cheese at 12°C, with no gas production noted (data not shown). At 12°C in 50% reduced fat cheese, *C. sporogenes* numbers remained relatively stable, but gas production occurred in 2-3 months and 3 months in duplicate vats without and with added fat replacer, respectively. At 30°C, numbers of *C. sporogenes* remained relatively stable in full fat and 25% reduced fat cheese, but gas production was noted in one vat of each at 5 months and 3 months, respectively. At 30°C, in 50% reduced fat cheese both with and without fat replacer, numbers of *C. sporogenes* rapidly increased, with gas production occurring in all vats at 2 weeks.

Due to the higher moisture content and other properties of reduced fat cheeses, concerns have been raised regarding the possibility of growth of toxigenic and spoilage clostridia. The results of this study show that toxin production by *C. botulinum* occurred in 50% reduced fat cheese with and without carbohydrate fat replacer after severe temperature abuse for a month or longer. Gas production by *C. sporogenes* also occurred faster at 12°C and 30°C in 50% reduced fat cheese with or without fat replacer than in full fat or 25% reduced fat cheese.

*INTERIM REPORT*

# Prevention of germination and growth by gas-forming *Clostridium tyrobutyricum* in high-pH cheeses

**Personnel**

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**Funding**

Wisconsin Milk Marketing Board UW9607

**Dates**

July 1996 - June 1998

## Objectives

Determine typical concentrations of *Clostridium tyrobutyricum* endospores in Wisconsin cheese milks.

Determine the germination and growth rates of *C. tyrobutyricum* in high-pH cheese made using commercial and altered (lower ripening temperature, higher % salt) processing conditions.

Determine typical percentage removal of *C. tyrobutyricum* endospores achieved during centrifugation of milk and evaluate the potential of this technique for preventing late blowing in high-pH cheeses.

## Summary

*Clostridium tyrobutyricum* is believed to be the major cause of the late blowing defect in high-pH cheese such as Gouda and Edam. This defect results from the fermentation of lactate and the resulting production of gas and malodorous butyric acid. There are a variety of published methods for enumerating endospores of *Clostridium tyrobutyricum* in milk. Most of these methods are relatively non-specific, enumerating a variety of *Clostridium* spp. capable of fermenting lactate and producing gas. Alternatively, qualitative methods using DNA probes are available to determine if *C. tyrobutyricum* is present. Using a quantitative Most Probable Number (MPN) method, we surveyed 21 pasteurized milk samples obtained from eight different cheese plants in Wisconsin between October, 1996 and December, 1997. Numbers of endospores of lactate-fermenting, gas-producing *Clostridium* spp. never exceeded a concentration of 10 endospores per ml, but at least one endospore per 50 ml was present in all samples tested. Further evaluation of 14 milk samples showed, however, that most of these endospores were produced by *Clostridium* spp. other than *C. tyrobutyricum*. Further characterization of 33 isolates obtained from the milk samples yielded 5 isolates that were presumptively identified as *C. tyrobutyricum*. Of 24 *Clostridium* spp. isolates tested, all but one produced significant amounts of gas during ripening of Gouda cheese following inoculation of high concentrations of endospores. The most discriminating method for differentiating among the *C. tyrobutyricum* isolates, as well as the other 28 non-tyrobutyricum *Clostridium* spp., was gas chromatographic analysis of cell membrane fatty acids. Pulsed field gel electrophoresis of genomic DNA was also found to be a very discriminating method for differentiating among the *C. tyrobutyricum* isolates. Gas chromatographic analysis of volatile organic acid cell byproducts was somewhat less discriminating method, but gas chromatographic analysis of non-volatile organic cell byproducts and carbohydrate fermentation profiling were considerably less useful. In summary, it appears that low concentrations of endospores produced by

lactate-fermenting, gas-producing *Clostridium* spp. are present in Wisconsin milk used to make cheese. Although the majority of these endospores are not *C. tyrobutyricum*, in high concentrations they may still be capable of producing deleterious amounts of gas during the ripening of high-pH cheeses. Further studies will examine how cheese ripening conditions or centrifugation of milk may be used to prevent germination and growth by these *Clostridium* spp.

*FINAL REPORT*

# Survival of *Mycobacterium paratuberculosis* in cheese

**Personnel**

Michael T. Collins, professor, Nackmoon Sung, graduate research assistant, Animal Health and Biomedical Sciences

**Funding**

Wisconsin Milk Marketing Board UW 9507

**Dates**

July, 1995 - June 1997

## Objectives

Define standard parameters of thermal tolerance for *M. paratuberculosis* isolates of human and animal origin.

Define the length of time *M. paratuberculosis* can survive under pH and salt combinations found in cheese.

Evaluate the ability of heat-stressed *M. paratuberculosis* to survive pH and salt exposure.

Perform pilot studies on survival of *M. paratuberculosis* in naturally and experimentally infected cheese.

## Summary

*Mycobacterium paratuberculosis* causes a chronic intestinal disease of dairy cattle and other ruminants known as Johne's disease. In the latter stages of infection in cows, this bacterium is excreted in raw milk as well as feces. Three studies all confirm that *M. paratuberculosis* can be found in raw milk of infected, but clinically normal cows. This same bacterium has been found in the intestinal tissues of people with Crohn's disease, a chronic, inflammatory bowel disease that resembles Johne's disease in clinical signs and pathology. The cause of Crohn's disease is not known, but some investigators propose that it might by caused by *M. paratuberculosis*. Concerns have been raised about the ability to this organism to survive pasteurization and thereby expose humans to this potential pathogen. The goal of this project was to provide the industry with quantitative data on the ability of *M. paratuberculosis* to resist killing by heat, low pH and salt. The industry could then evaluate the ability of manufacturing practices to inactivate this potential microbial contaminant of milk. Decimal reduction times (D-values), the time for a one log10 reduction in viable counts to occur, was established as the standard measure of resistance to each condition studied.

The average D-value of *M. paratuberculosis* at 71˚C (160˚F) was 12 sec. D-values of all *M. paratuberculosis* strains tested were considerably higher than those published for *Listeria monocytogenes, Salmonella, Coxiella burnetti* and *Mycobacterium bovis*. These findings indicate that *M. paratuberculosis* may survive HTST pasteurization when the initial *M. paratuberculosis* concentration is greater than 10 bacterial cells/ml milk. *M. paratuberculosis* survival rates in 0% to 6% salt did not differ. Lower pH, however, was directly associated with decreased rate of *M. paratuberculosis* survival:. D-values at pH 4, 5, 6, and 7 were 10 days, 19 days, 33 days, and 68 days, respectively. Although eventually killed by low pH conditions, comparison of *M. paratuberculosis* survival rates in the face of low pH to those described for other bacteria indicate that *M. paratuberculosis* is more resistant to low pH than other potential bacterial pathogens that could contaminate milk. When milk spiked with *M. paratuberculosis*

was used to make Queso Fresco, very little decline in viable *M. paratuberculosis* numbers was seen over the 30 day study period. The estimated D-value for *M. paratuberculosis* in Queso Fresco (pH 6; 2% NaCl) stored in a refrigerator (4˚C) was 60 days.

### Strains of *M. paratuberculosis* studied

Two human-origin (Ben and Dominic) and two bovine-origin (BO45 and ATCC 19698) strains were studied. ATCC 19698 is a type strain that has been maintained in laboratory culture for many years. The other strains were low passage clinical isolates. As this bacterium has a strong tendency to form clumps of bacterial cells, and this property can potentially affect resistance to physical or chemical factors, both clumped and single celled preparations of strains were tested.

### Menstruums studied

For thermal tolerance studies *M. paratuberculosis* cells were suspended in lactate buffer (pH 6.8) or raw milk (Holstein cow). For pH and salt studies, acetate buffer was used as a base menstruum with addition of lactic acid to adjust the pH to the desired level. Sterile distilled water was used as a control. A Hispanic-style soft white cheese (Queso Fresco; pH 6.15, 2% NaCl) was used as a laboratory model cheese. The cheese was made from *M. paratuberculosis* spiked retail 2% fat milk and it was stored at 4˚C for 30 days of the trial. Microbial (non-mycobacterial) degradation of the cheese prevented continuing the cheese study for more than 30 days.

### *M. paratuberculosis* counting system, D-value and Z-value estimation

The number of *M. paratuberculosis* surviving at each time period of treatment was determined using a radiometric counting system. This technique converts measurements of 14CO2 release by cultures in medium with 14C-palmitate into counts of *M. paratuberculosis* cells based on a standard curve. D-values were calculated as the slope of the best-fit linear regression line for log10 counts of surviving *M. paratuberculosis* cells versus time using Minitab software. Z-values were determined by linear regression of the log10 D-values versus the reaction temperature.

### Heat, pH and salt treatments

Lactate buffer and milk suspensions of *M. paratuberculosis* were heated at 62˚C, 65˚C, 68˚C, and 71˚C. At set time intervals, surviving *M. paratuberculosis* numbers were counted. As a separate investigation, *M. paratuberculosis* was added to acetate buffer suspensions adjusted to have all 12 combinations of pH (4, 5, and 6) and NaCl (0%, 2%, 4%, and 6%). Surviving organisms were counted up to 200 days.

### Results and conclusions

Killing of *M. paratuberculosis* by heat exposure occurred in a linear fashion with thermal death curves having $R^2$ > 0.90 for all strains at all temperatures tested. The laboratory strain of *M. paratuberculosis* was more heat-resistant than the three clinical strains. Clumping of *M. paratuberculosis* cells had no significant effect on D-values for thermal resistance. Mean D-values for the clinical strains of *M. paratuberculosis* at 62˚C, 65˚C, 68˚C, and 71˚C were 229 sec, 48 sec., 22 sec., and 12 sec., respectively. The Z-value calculated from these data was 7.11˚C. These parameters were used to mathematically estimate the time needed to kill 100% of *M. paratuberculosis* cells when the initial concentration in milk was $10^1$, $10^2$, $10^3$, $10^4$, $10^5$, and $10^6$ *M. paratuberculosis* cells/ml milk. The conclusion from this analysis was that *M. paratuberculosis* is more resistant to killing by heat than the other bacterial pathogens found in milk used to establish pasteurization standards. Based on D values obtained in the lab, HTST methods are sufficient to kill 100% of *M. paratuberculosis* provided the initial concentration in raw milk is less than 10/ml.

Salt concentration did not affect survival rates of *M. paratuberculosis*. Killing curves in acetate buffer with 0%, 2%, 4%, and 6% salt were linear and not significantly different.

Low pH decreased survival of *M. paratuberculosis*. Killing curves at pH 4, pH 5, and pH 6 were linear and gave D-values of 10 days, 19 days, and 33 days (data for all NaCl levels pooled). For comparison, the D-value for *M. paratuberculosis* suspended in water (pH 7.2) was 69 days for a human-origin clinical strain and 92 days for the type strain.

When milk spiked with *M. paratuberculosis* was used to prepare Queso Fresco, linear survival curves indicated a D-value of 60 days (mean of 3 trials). When compared to results of pH and salt trials, these data indicate that some unknown factors in cheese may serve to partially protect *M. paratuberculosis* from the lethal effects of low pH and salt since the D-value in cheese with pH 6.15

and 2% NaCl (60 days) was higher than the D-value in acetate buffer at a comparable pH 6.0 and NaCl concentration.

Heat stressed *M. paratuberculosis* (71˚C for 20 sec) died faster after exposure to pH 5 with 2% NaCl than did non-heat stressed *M. paratuberculosis*. The longer the heat stress the faster the organism died. Thus, the combined effects of multiple treatments (heat, pH and salt) that can potentially accelerate *M. paratuberculosis* killing during the manufacture of dairy products.

Summary evaluation of survival curves for all pH, salt and cheese studies with *M. paratuberculosis* suggests that, although this organism can not multiply in cheese, it is more resistant to the killing effects of low pH and salt than other bacterial pathogens potentially found in cheese. For this reason the levels of contamination of *M. paratuberculosis* in raw milk used for cheese production could potentially be of concern, without further information.

*M. paratuberculosis* infects 20 to 40% of U.S. dairy herds (USDA National Animal Health Monitoring Service 1996 survey). This bacterium is found in raw milk but normal range of *M. paratuberculosis* concentration in raw milk is not known. Some medical research incriminates *M. paratuberculosis* as the cause of, or complicating infection in, Crohn's disease of humans but there are widely disparate opinions on these findings. Dairy products are not the only vehicle for human exposure to *M. paratuberculosis*. Nevertheless, the dairy industry must understand the survival characteristics of this bacterium during food manufacturing processes. The results of this study provide quantitative data to meet this need and identify methods of control.

## Publications

Sung, N., and Collins, M.T. Thermal tolerance of Mycobacterium paratuberculosis. Applied and Environmental Microbiology (in press for March, 1998)

*APPLICATIONS PROGRAM REPORT*

# Safety/Quality applications program

**Personnel**

Marianne Smukowski, safety coordinator, Kristen Houck, analytical microbiologist

**Funding**

Wisconsin Milk Marketing Board
UW A9704

**Dates**

July 1996-June 1997

## Objectives

Survey dairy facilities for potential safety/quality problems

Work with plant management to identify and correct potential quality problems

Participate and assist in UW and industry sponsored courses

Serve as a technical adviser for QC programs and HACCP implementation

Participate with the Master Cheese Maker program

## Summary

The Safety/Quality Applications program was initiated July 1, 1997. This program was established to maintain and improve HACCP-based programs used by Wisconsin manufacturers. A safety/quality program of a dairy plant affects the quality of all the dairy products manufactured in the plant. The need for a proper sanitation program is also key to the development of a functional dairy product.

The Safety/Quality Applications program assisted dairy plants in Wisconsin by helping them design and upgrade their HACCP plans, thus improving the quality of their dairy products. This was done by visiting plants, presentations given at conferences, and by networking. A total of 20 plant visits were made this past year, and over 50 contacts were made concerning HACCP implementation. During plant visits a walk through of the plant was performed and samples and swabs were taken throughout the processing/manufacturing system. Recommendations were left with plant management on how to remedy/correct their situation and followup phone calls or visit s were made to insure the recommendations were followed.

In addition, the applications program assists the Master Cheese Maker and Cheese program with safety issues by visiting cheese plants and making recommendations to correct any deficiencies noted. As a member of the UW/CDR dairy plant committee, I have written CDR dairy plant guidelines for the facilities used in Babcock Hall.

In conclusion, safe, high quality dairy products are the goals of manufacturing plants. While recent advances in technology allows for more sophistication and automation, safety is still the primary issue. With this program, I am able to assist the dairy plants in improving GMP's and HACCP plans.

## Presentations

Wisconsin CIP Workshop Madison, WI March 25,1997, Plant Sanitation Audits

WI Cheese Grading Short Course—Italian Cheese Evaluation

WDPA Cheese and Butter Evaluation Clinic—Overview of Butter Grading

Lead Butter Judge for the Intercollegiate Dairy Products Evaluation Contest

Producing Safe Dairy Foods Workshop—Control of Foodborne Pathogens, Including HACCP

WAMFES Dairy Food and Environmental Health Symposium—Extended Production Runs, on the Farm and in the plant

WI Cheese Industry Conference Green Bay, WI April 2, 1997, CDR Safety/Quality Applications Program

# chapter 2, section3

# Cheese whey

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*FINAL REPORT*

# Fractionation of whey proteins using ion exchange membranes

**Personnel**

Mark. R. Etzel, associate professor, Dept. of Food Science; Clovis Ka Kui Chiu, graduate student, Dept. of Food Science; Ida A. Adisaputro, student, Dept. of Chemical Engineering

**Funding**

Wisconsin Milk Marketing Board 93-9

**Dates**

November 1993 - November 1997

## Objectives

The overall objective of this research is to develop an economical large-scale technology for producing pure individual whey proteins so that existing whey protein concentrate manufacturers can convert over without having to invest in a new plant. This new technology is needed to exploit the unique nutritional and functional properties of dairy proteins not found with other proteins derived from soy beans and eggs. The specific objectives are to:

Show that glycomacropeptide, lactoferrin and lactoperoxidase can be fractionated from whey using ion-exchange membranes.

Show that beta-lactoglobulin, alpha-lactalbumin and immunoglobulin G can be fractionated into pure products using multicomponent adsorption behavior of the ion-exchange membrane.

Optimize the fractional properties by modifying processing conditions such as whey pH and loading volume, and eluant pH.

Scale-up the process by collecting pilot-plant data needed to design, build and operate a successful commercial-scale process.

## Summary

Lactoferrin and lactoperoxidase were fractionated from whey using a bench-top scale sulfopropyl ion exchange membrane cartridge (model S-100, Sartorius Corp., Edgewood, NY) having a membrane volume of 2 ml. Using filtered whey (0.7 µm filter paper, Micro Filtration Systems, Dublin, CA), flow rates of 10 and 50 ml/min were used without significant pressure drop or loss of performance. Up to 250 ml of whey was loaded before breakthrough of lactoperoxidase or lactoferrin (Fig. 1). Loading more whey caused the lactoperoxidase activity of the effluent to exceed the lactoperoxidase activity of the inlet whey because lactoferrin, which binds more tightly than lactoperoxidase, displaced lactoperoxidase from the membrane.

Using a 250 ml loading volume of whey, essentially all the lactoferrin and lactoperoxidase were recovered from the whey. Two elution peaks were collected. In the first peak, using 0.3 M NaCl, pure lactoperoxidase was obtained. In the second peak, using 0.9 M NaCl, pure lactoferrin was obtained. Repeated loading and elution cycles were performed in sequence without cleaning between cycles, and performance was stable (Table 1).

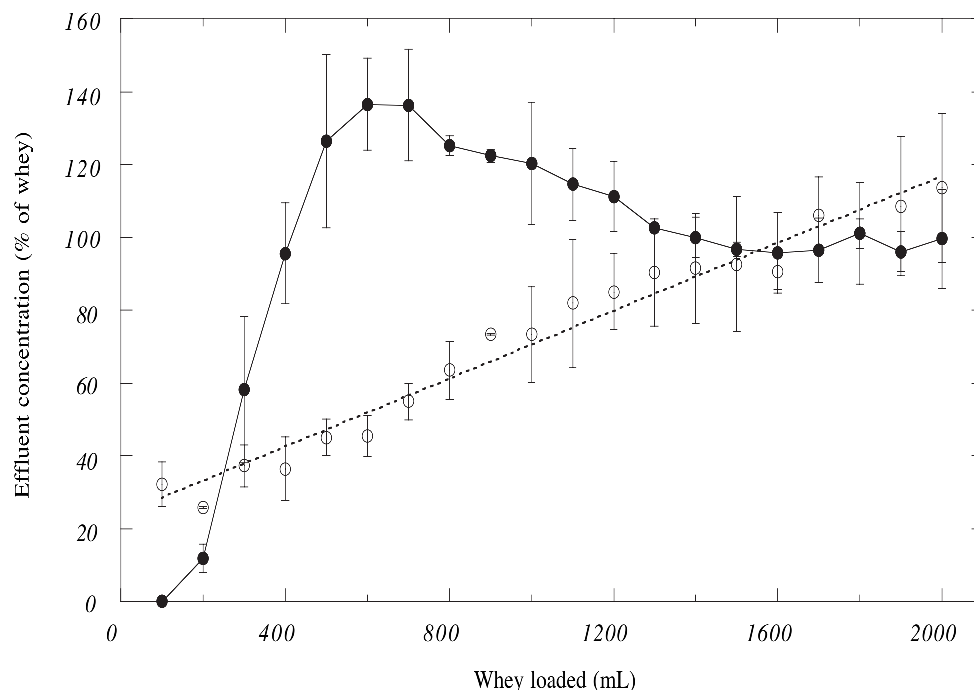


**Figure 1**. Breakthrough curves of lactoperoxidase (solid circles) and lactoferrin (hollow circles) for loading whey into the S-100 membrane. Each point is the average of two trials, and error bars span ± one standard deviation.

Based on these successful laboratory-scale experiments, the process was scaled-up 8-fold by using a pilot-plant-scale membrane having a volume of 16 mL. The cycle time was 9 min, the flow rate was 400 ml/min, and the loading volume was 2000 ml. Matching performance was observed. Thus, the membrane system was more rapid, smaller in size, and used a higher flow rate than traditional bead-based systems.

Next, attention was centered on fractionating kappa-casein glycomacropeptide (GMP) from whey. GMP is the moiety cleaved from kappa-casein by chymosin during cheese making. It occurs at a concentration of 1.2 to 1.5 g/L in sweet whey, comprising 15 to 20% of the total protein. Glycomacropeptide is an example of a purified whey protein that has unique medical or health benefits e.g. control of satiety and phenylketonuria, properties not found with other proteins. Developing a technology for fractionation of GMP from whey will allow production of a high-value nutraceutical product from whey.

We found that GMP could be fractionated from whey using a quaternary methyl amine ion exchange membrane cartridge (model PSC10-QM, BPS Separations Ltd., Spennymoor, County Durham, U.K.) having a 10-mL membrane volume and a large pore size. Optimal conditions were: whey pH of 5.0-6.0, loading volume of 100-150 ml, flow rate greater than 15 ml/min, and 0.3 M NaCl for elution. The process was scaled-up by a factor of four using the optimal conditions. Ten cycles were successfully conducted, yielding 3.2 glycomacropeptide from 4.8 L of whey, or ~ 50% recovery.

Further research was aimed at increasing the purity of the GMP using a second adsorption step and scaling up the process. Both goals were met. This work was the subject of a patent application (P97071US) and an ongoing partnership with a company for commercialization of the technology.

Lastly, the fractionation of alpha-lactalbumin (ALA) and beta-lactoglobulin (BLG) from whey using ion exchange was investigated. We discovered a means to produce both products in a highly pure and undenatured form. This has not before been possible. Pure undenatured ALA is of keen interest to the infant formula industry because bovine milk and whey are deficient in ALA compared to human milk. Supplementing infant formula with pure undenatured ALA would "humanize" infant formula and promote infant health through better nutrition. BLG is a potent gelling agent for use in foods e.g. to replace egg white. Our process uses one unit operation to produce both products and has been disclosed to WARF.

**Table 1**. Lactoperoxidase (LP) activity and lactoferrin (LF) content of the effluent, LP peak, and LF peak (% of total loaded) for repeated cycles using the S-100 module without cleaning between cycles.

| Cycle | Effluent | | 0.3 M NaCl | | 0.9 M NaCl | | Total | |
|---|---|---|---|---|---|---|---|---|
| number | LP | LF | LP | LF | LP | LF | LP | LF |
| 1 | 5 | 50 | 60 | 1.3 | 1.0 | 35 | 66 | 86 |
| 2 | 6 | 45 | 71 | 2.3 | 1.5 | 41 | 78 | 89 |
| 3 | 7 | 47 | 78 | 2.0 | 1.4 | 40 | 86 | 89 |
| 4 | 8 | 43 | 65 | 3.0 | 0.8 | 44 | 73 | 90 |
| 5 | 7 | 43 | 65 | 2.3 | 0.9 | 41 | 73 | 86 |
| 6 | 8 | 41 | 86 | 2.0 | 1.0 | 40 | 95 | 83 |
| 7 | 8 | 41 | 73 | 1.8 | 1.0 | 39 | 83 | 82 |
| 8 | 9 | 41 | 71 | 1.3 | 1.1 | 41 | 81 | 84 |
| 9 | 9 | 41 | 82 | 1.7 | 0.7 | 42 | 92 | 84 |
| 10 | 9 | 35 | 63 | 1.4 | 0.7 | 39 | 73 | 75 |
| 11 | 10 | 39 | 70 | 1.5 | 0.7 | 39 | 81 | 79 |
| 12 | 10 | 42 | 77 | 2.0 | 0.5 | 38 | 87 | 82 |
| Average | 8 | 42 | 72 | 1.9 | 0.9 | 40 | 81 | 84 |
| Std. Dev. | 2 | 4 | 8 | 0.5 | 0.3 | 2 | 9 | 4 |

Although the U.S. market for high value, purified whey proteins is small at present, several factors indicate it will grow. Other major dairying countries are developing markets for these purified individual whey protein products. In the future, the food industry will demand proteins with higher nutritional and functional properties because of the trend towards foods with enhanced health benefits, lower fat content and lower lactose content.

The proposed ion exchange membrane process offers numerous advantages. Evaluating and optimizing the process should provide the stimulus for the U.S. dairy industry to expand into the new domestic and international markets for whey protein products with enhanced nutritional and functional properties.

## Publications/Presentations

Adisaputro, I.A., Wu, Y.-J., and Etzel, M. R. Strong cation and anion exchange membranes and beads for protein isolation from whey. *J. Liq. Chrom. & Rel. Technol.*, 19(9), 1437-1450 (1996).

Chiu, C.K. and Etzel, M.R. Fractionation of lactoperoxidase and lactoferrin from bovine whey using a cation exchange membrane. *J. Food Sci.* **62**(5), 996-1000 (1997).

Chiu, C.K. Fractionation of casein glycomacropeptide, lactoperoxidase and lactoferrin from bovine whey using ion exchange membranes or beads. M.S. thesis, Univ. of Wisconsin, Madison (1996).

Etzel, M.R. Production of k-casein macropeptide for nutraceutical uses. Pat. Appl. 97071US (1997).

*INTERIM REPORT*

# Fractionation of κ-casein glycomacropeptide from whey for nutraceutical uses: scale up of the ion exchange membrane technology

**Personnel**

Mark. R. Etzel, associate professor, Shinta Dermawan, graduate student, Dept. of Food Science, Laura T. Marschoun, graduate student, Dept. of Biological Systems Engineering

**Funding**

Dairy Management Inc. CW47

**Dates**

July 1997 - July 1999

## Objectives

Increase the use of whey proteins for health and nutrition applications by developing processing, fractionation, and modification technologies, specifically:

Scale up the anion exchange membrane technology for fractionation of k-casein glycomacropeptide from whey.

## Summary

Glycomacropeptide (GMP) is the moiety cleaved from κ-casein at the $Phe^{105}$-$Met^{106}$ position by chymosin during cheesemaking. GMP occurs at a concentration of 1.2 to 1.5 g/L in sweet whey, comprising 15 to 20% of the total protein. Known biological functions of GMP, as consolidated from several nutritional and biological research reports, include the utilization of GMP in dietetic foods and pharmaceuticals. Aromatic amino acids (phenylalanine, tryptophan and tyrosine) are absent from GMP. Thus, GMP may be useful for the treatment of phenylketonuria, a hereditary disorder in which aromatic amino acids cannot be metabolized. Furthermore, consumption of as little as 25 mg of GMP stimulates the release of cholecystokinin, a hormone that regulates food intake by creating the sensation of satiety. Thus, GMP may be useful in the billion-dollar weight loss or diet foods marketplace.

The objectives of our past research were to develop a new and more economical method for the large-scale fractionation of GMP from bovine whey based on ion exchange membranes. The basis of the ion exchange method is that GMP has a negative net charge in whey at acidic pH, while other whey proteins are charged positive. Thus, when whey is contacted with anion exchange membranes or beads at acidic pH, GMP binds to the ion exchange media while the other whey proteins remain in the fluid phase. The bound GMP can be eluted from the media using a salt solution. Experiments were conducted to: 1) determine the effect of operating conditions on the purity and recovery of GMP, 2) operate consecutive cycles of loading and elution to simulate an industrial process, and 3) compare the performance of ion exchange membranes to ion exchange beads, the conventional media.

In this follow-up project, we have discovered a process to increase the purity, lower the cost of manufacture, and increase the commercial value of GMP. Our process for producing a GMP having nutraceutical properties utilizes whey and two ion exchangers of opposite polarity in series. A hydrolyzed GMP nutraceutical food product having less that about 4% total of the hydrophobic aromatic amino acids phenylalanine, tryptophan

and tyrosine can also be produced using our process. This invention was the subject of a patent application (P97071US) and an ongoing partnership with a company for commercialization of the technology.

The nutraceutical, κ-casein glycomacropeptide, has been shown to have special medical benefits. GMP comprises 15 to 20% of the protein in whey, making it plentiful in supply. However, prior attempts to purify it from whey have resulted in low recovery percentages, low purity and unfavorable economics. Recently, we developed an ion exchange membrane process that overcomes this limitation and that has a higher relative throughput than prior processes. In this research, we will further develop and scale up this new ion exchange process.

## Publications/Presentations

Etzel, M.R. Production of κ-casein macropeptide for nutraceutical uses. Pat. Appl. 97071US (1997).

FINAL REPORT

# Process modification of starter cultures for flavor enhancement in low fat cheese

**Personnel**

Mark. R. Etzel, associate professor, Brian Chi-Shung Brian To, graduate student, Dept. of Food Science

**Dates**

July 1994 - June 1997

**Funding**

Dairy Management Inc. ETZ 95

## Objectives

The overall objective of this research is to develop more flavorful low-fat Cheddar cheese by using process-modified starter culture adjuncts that accelerate the ripening process. Processing conditions are to be developed which reliably modify the culture characteristics to permit flavor enhancement in low-fat cheese. This allows the manufacture of more consistent and flavorful low-fat cheeses, thus increasing the demand for cheese and milk. The specific objectives are to:

Establish methods for small-scale production of cell pastes of candidate cultures.

Freeze, freeze dry and spray dry the cell paste solutions using processing conditions which range from attenuating to preserving of metabolic activity.

Analyze the cell pastes, solutions and cell powders for metabolic activity, specifically cell survival, lactic acid production, and b-galactosidase and aminopeptidase activity.

Select the cultures and processing conditions which are best for flavor enhancement in low-fat cheese, and then produce large amounts of adjunct needed for cheesemaking.

## Summary

The culture *Brevibacterium linens* ATCC 9174 was selected as the first candidate for investigation. *B. linens* is responsible for the ripening of surface-ripening cheeses such as Limburger and Brick. Incorporation of this obligate aerobe into Cheddar cheese, as an adjunct to the normal starter culture, has been shown to enhance the rate and extent of flavor development. Inexpensive methods for producing, preserving, and distributing these adjuncts cultures must be developed before widespread use in the dairy industry is economical. For this reason, the effects of processing (freezing, freeze drying and spray drying) on the characteristics of *B. linens* were measured in this research.

Survival was 100% for *B. linens* after freezing and freeze drying. For spray drying, survival was halved for every 5°C increase in the outlet air temperature. Heat was the sole mechanism decreasing cell survival during spray drying. Thermal resistance was measured vs. moisture content and temperature, resulting in D-values ranging from 120 min at 25.3% solids and 44.0°C to 2.6 min at 46.2% solids and 55°C. This is much more heat sensitive than alkaline phosphatase, an indicator of proper pasteurization of milk. However, thermal inactivation of *B. linens* during spray drying can be eliminated by proper selection of the outlet-

air temperature. By extrapolation of the data, 100% survival would occur at an outlet air temperature of 57°C. The freeze dried and spray dried cultures were stable during prolonged storage at 4°C in the absence of oxygen and moisture.

In the second part of this investigation, five lactic acid bacteria, *Lactococcus lactis* ssp. *cremoris* D11, *Lactobacillus casei* ssp. *pseudoplantarum* UL137, *Lactobacillus delbrueckii* ssp. *bulgaricus* CH3B, *Lactobacillus acidophilus* NCFM and *Streptococcus salivarius* ssp. *thermophilus* CH3TH, were separately frozen, freeze dried or spray dried, and tested for survival and lactic acid production before and after processing. Virtually all the cells survived freezing. Of the survivors, 60 to 70% survived the dehydration step of freeze drying, except for *Lb. bulgaricus*, for which one fifth survived. Survival after spray drying was greatest for *S. thermophilus* and lowest for *Lb. bulgaricus*. In contrast to freezing and freeze drying, spray drying caused a significant delay in lactic acid production and reduction insurvival. After spray drying, survival was greatest for *S. thermophilus* and lowest for *Lc. cremoris*.

The results of this research will help advance the cheese industry. The demand for low fat cheese is growing. This new technology will help to develope low fat cheeses with a flavor and texture comparable to full fat cheeses. These low fat cheeses will be popular for producing spray-dried powders for use in low fat convenience foods such as cheese nachos and frozen dinners, and will appeal to consumers on low-fat diets who may have reduced their consumption of traditional full fat cheeses. This new technology will help to accelerate the already increasing demand for cheese, which will directly result in an increased demand for milk. In addition, the results of this research will help to advance the starter culture industry, most of which is based in Wisconsin.

## Publications/Presentations

To, B.C.S. 1996. Properties of *Brevibacterium linens* and five different lactic acid bacteria attenuated by spray drying, freeze drying, or freezing. M.S. thesis, Univ. of Wisconsin, Madison.

To, B.C.S. and Etzel, M.R. 1996. Spray drying, freeze drying, or freezing of three different lactic acid bacteria species. *J. Food Sci.,* 62(3): 576-578 & 585 (1997).

To, B.C.S. and Etzel, M.R. 1996. Survival of *Brevibacterium linens* (ATCC 9174) after spray drying, freeze drying, or freezing. *J. Food Sci.,* 62(1): 167-170 & 189 (1997).

To, B.C.S. and Etzel, M.R. 1996. Survival of *Brevibacterium linens* ATCC 9174 after spray drying, freeze drying, or freezing. Am. Inst. Chem. Eng. Conf. Food Eng., Nov. 2-3, 1995, poster 8.4.

*APPLICATIONS PROGRAM REPORT*

# Whey applications program

### Personnel

J. Russell Bishop, director, Kimberlee J. Burrington, associate researcher, Tom Szalkucki, assistant director, Center for Dairy Research

### Funding

Wisconsin Milk Marketing Board UWA9702 & UWA9801

### Dates

July 1996 - December 1997

## Objectives

Develop a whey applications system to support developing new markets.

Work with 3-5 Wisconsin companies.

Execute technology development from DMI, a national base.

## Summary

Based on consumer demand, production of cheese, and therefore whey, continues to increase in the United States. CDR has responded to this growth and industry requests by developing a whey applications program. The program is designed to utilize CDR whey research and expertise, as well as research from other Centers, to assist industry in commercializing the technology and component applications. The goal of these efforts is to increase the value and use of whey and whey components in food and other products.

The Wisconsin whey and whey utilization short course was held on April 8-9, 1997. Bill Wendorff, UW Extension, put together a good overview course featuring speakers from the Department of Food Science, CDR, and industry. The short course was sold out by March and the reviews after the course were positive. The whey short course will be presented again this year.

In October 1997, Kimberlee (K.J.) Burrington was hired at CDR to build the whey program at CDR based on cheese/whey industry needs. K.J. comes to us with expertise in food product development, especially baked goods. She has been meeting with selected cheese/whey producers to discuss their needs and how CDR's applications program can address those needs.

FINAL REPORT

# Conversion of Whey Permeate to Propylene Glycol for Food and Non-Food Uses

**Personnel**

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Anita J. Shaw, Graduate Student, Department of Chemical Engineering;
Roxanne M. Smith;
Yi-Jui Wu, Students, Dept. of Chemical Engineering

**Dates**

July 1994 - June 1997

**Funding**

Dairy Management Inc. CME95

## Objectives

The overall objective of this project is to develop a fermentation process for the conversion of lactose in whey permeate to propylene glycol, a large volume commodity chemical. The fermentation process yields an optically pure product, R-propylene glycol, with added value over synthetically produced propylene glycol. The specific objectives are to:

1. Screen and select microorganisms capable of fermenting lactose to propylene glycol.

2. Optimize the medium and environmental conditions for propylene glycol production from whey permeate by the organism(s) identified in Objective 1. The effects of carbon to nitrogen ratio (C/N), trace nutrients, and medium pH will be investigated.

3. Develop technology for the removal of inhibitory acetate and lactate from whey permeate-based medium during fermentation so as to achieve increased propylene glycol concentration and productivity

## Summary

We have established that *Thermoanaerobacterium thermosaccharolyticum* (ATCC 31960) (formerly known as *Clostridium thermosaccharolyticum* produces propylene glycol from hydrolyzed whey permeate (WPH) and WPH with added yeast extract, yielding titers of 4.5 g/l in 48 h in 300 ml anaerobic flasks. Both glucose and galactose were utilized in the fermentations. To further investigate this finding, eight different whey-based media fermentations were carried out in triplicate for a total of twenty-four fermentations. The eight media were whey or whey permeate, with or without prior hydrolysis of lactose, and with or without added yeast extract. The major products of the *T. thermosaccharolyticum* fermentation are propylene glycol, acetate, lactate and ethanol. Fermentations of WPH and hydrolyzed whey gave greater propylene glycol selectivity (moles propylene glycol per moles of by-products) compared to the media with added yeast extract. However, media with yeast extract produced more propylene glycol in absolute concentration. Unhydrolyzed whey and unhydrolyzed whey permeate were converted to propylene glycol only with the addition of yeast extract, and selectivity was lower than it was for hydrolyzed medium.

During the course of this work we made a discovery of significance to Objective 1. We found that a triose phosphate isomerase mutant of the bacterium *Escherichia coli* transformed with the gene for the enzyme aldose reductase is able to ferment galactose to propylene glycol. The same strain transformed with both aldose reductase and methyglyoxal synthase genes is able to ferment both galactose and lactose to propylene

glycol. Currently titers of propylene glycol are in the range of 1 g/l, which is lower than those obtained from galactose or WPH with *T. thermosaccharolyticum*. However, this work is significant in that it provides the basis for further development of metabolic pathways to propylene glycol from lactose. Unlike *T. thermosaccharolyticum*, *E. coli* is an important industrial organism for which there are many tools for further strain improvement and modification. The finding that *E. coli* can be engineered to produce propylene glycol from sugars such as galactose and lactose provided the basis for a U.S patent application filed through the Wisconsin Alumni Research Foundation (WARF).

One of the key isssues for the production of propylene glycol with either *T. thermosaccharolyticum* or an *E. coli* strain is the inhibition of product formation by fermentation by-products such as acetate and lactate. The focus of the work on Objective 3 was to increase the productivity and final titer of the fermentation by developing new technology for removal of inhibitory products from the broth with ion exchange resins simultaneous to the fermentation. When acetate and lactate anions are not removed during the fermentation, low productivity results due to product inhibition. When acetate was added to a level of 20 g/l during the fermentation, cell mass was significantly reduced, providing evidence of strong product inhibition. Our research established that a column packed with Amberlite IRA-400 effectively removes lactate from uninoculated defined glucose medium, while controlling pH. When one anion binds to the column, one hydroxide ion was displaced, which controlled the pH without the need for adding base. In subsequent research, we used a column to extract lactate and acetate from an actual fermentation using defined glucose medium. We set-up a fermentation system where broth was continuously pumped from a water-jacketed (60°C) one-liter fermentor into a microporous hollow-fiber filter. Cells were returned directly to the fermentor, and cell-free broth passed through an anion-exchange column before it was returned to the fermentor. By establishing a technology for the simultaneous removal of acetate from the fermentation broth, we met Objective 3 of the project.

This research will provide the basis for the industrial production of a new fermentation chemical, propylene glycol, from whey permeate. Propylene glycol is a major organic chemical with extensive applications in the food industry, both as an ingredient and as an antifreeze and heat transfer fluid. Currently propylene glycol is produced entirely from propylene, a petrochemical. This synthetic propylene glycol is a racemic mixture, a mixture of left and right handed forms of propylene glycol. The fermentation process provides a unique route to enantiomerically pure R-propylene glycol. Since the other major non-volatile products of the fermentation are acetic and lactic acid, it may also be possible to concentrate the fermentation broth and make a cultured whey product containing propylene glycol and organic acids.

## Presentations

Shaw, A.J., N.E. Altaras, and D.C. Cameron. 1997. Metabolic engineering of 1,2-propanediol production in Escherichia coli. Division of Biochemical Technology, Abstract No. 47, 213th ACS National Meeting, San Francisco, CA, April 1997.

Cameron D.C., M.L. Hoffman, A.J. Shaw, and N.E. Altaras. 1997. Design, construction and analysis of metabolic pathways for the production of diols. Engineering Foundation, Biochemical Engineering X, Kananaskis, Alberta, Canada, May 18-23.

## Publications

Cameron, D.C., N.E. Altaras, M.L. Hoffman, and A.J. Shaw. In press. Metabolic engineering of propanediol pathways. Biotechnology Progress.

Shaw, A.J. 1997. Metabolic engineering of methylglyoxal metabolism in *Escherichia coli*. Ph.D. Thesis, Department of Chemical Engineering, University of Wisconsin-Madison.

## Patents

Cameron, D.C., A.J. Shaw, and N.E. Altaras. Microbial production of 1,2-propanediol from sugar. U.S. patent filed February 1997.

chapter 3

# Fluid milk

Evaluation of Bifidobacteria as bioactive adjuncts in dairy products ........ 147

Growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder ........ 149

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*FINAL REPORT*

# Evaluation of *Bifidobacteria* as bioactive adjuncts in dairy products

**Personnel**

John B. Luchansky, associate professor, Food Microbiology and Toxicology

**Funding**

Wisconsin Milk Marketing Board LCH 97

**Dates**

July 1996 - June 1997

## Objectives

Identify human isolates and commercially-available bifidobacteria with attributes useful for maintenance in foods and the intestinal tract.

Enhance the stability of select isolates of bifidobacteria in model dairy foods and evaluate efficacy in such foods and to a more limited extent in an animal model.

## Summary

In recent years information about the contributions of bifidobacteria and other lactic acid bacteria (LAB) to human health and food safety have been the subject of much debate due, in part, to a paucity of scientifically-controlled studies, particularly studies evaluating their performance in foods and in people. Considerable excitement over the health and safety benefits of foods containing bifidobacteria exists worldwide, but particularly throughout Europe and Asia where over 70 bifid-containing products are marketed and consumed. In recent years there has also been considerable interest in compounds that promote the growth of bifidobacteria, compounds referred to as bifidogenic factors or prebiotics. Prebiotics have been found in a variety of foods and feeds and have been targeted for various applications. In general, prebiotics are non-digestible by human digestive juices but are metabolized by select bacterial species, such as the bifidobacteria. Further research is warranted to identify and optimize bifidobacteria and bifidogenic factors to enhance human health.

We identified human isolates of bifidobacteria with potential for use as probiotics. The isolates were tested for enhanced growth in synthetic media in the presence of bifidogenic compounds. Select isolates were then fed to piglets and monitored for the ability to promote weight gain and improve feed conversion. In retrospect, we may be have been overambitious in setting such all-encompassing objectives for a single graduate student to realistically accomplish in one year. As such, given the constraints of time and money, as well as the complexity and magnitude of the experiments, it was not possible for us to fully evaluate candidate bifidobacteria in a model dairy food. However, as described herein, significant progress was made in identifying candidate strains for use as probiotics.

Since March of 1994 we isolated >300 *Bifidobacterium* spp. from healthy human volunteers (primarily from a single family). The clonality and succession of these strains was established using pulsed-field gel electrophoresis (PFGE). We also used PFGE to identify clonal types that predominated. In addition, we evaluated the potential of oligosaccharides for use as prebiotics. Commercially-available fructo-oligosaccharides (FOS-1 and FOS-2), xylo-oligosaccharide (XOS), and inulin were tested at levels of 0.1% to 4.0% (w/v) for the ability to enhance the growth of *Bifidobacterium* spp. in a basal

synthetic media. Twelve predominate bifidobacteria strains from a single family were evaluated, as well as *B. animalis* ATCC 27536 and *B. breve* ATCC 15698. All 14 bifidobacteria strains showed enhanced growth at 37˚C in 30 h in the presence of 1.5% of FOS-1 and FOS-2, whereas 10 of the 14 bifidobacteria strains (including both ATCC strains) showed enhanced growth at 37˚C in 30 h in the presence of 1.5% XOS. All strains tested did not display enhanced growth in the presence of 1.5% inulin. Moreover, increasing the levels of inulin or XOS to 4.0% did not result in enhanced growth of strains that grew poorly at levels of 1.5% inulin or XOS. Variable generation times among strains were noted. Addition of FOS-1 or FOS-2 at 1.5% significantly reduced the generation time of 11 bifidobacteria strains ($p< 0.05$). Also, 9 bifidobacteria strains grew significantly faster with decreased generation times when cultured with 1.5% XOS. A summary of the growth response to prebiotics of all 14 bifidobacteria strains is provided in Table 1.

For the feeding study, piglets were obtained from and cared for by the University of Wisconsin Livestock Laboratory. Within 30 minutes after birth, some animals were dosed with a cocktail of LAB (experimental) and some animals were dosed with saline (controls). At regular intervals throughout weaning and post-weaning, we monitored weight gain and feed conversion of select animals from both groups. The Statistical Analyses System (version 6.0: SAS, Cary, NC) was used to analyze the data collected. These prefatory studies indicated that the experimental animals displayed greater weight gain and feed conversion ratios than otherwise similar control animals.

Given the heightened awareness of bifidobacteria, bifidogenic factors and/or bifid-containing products and the attendant economic benefits, the proposed research is timely because it will allow the dairy industry to fully exploit the potential health benefits of such products as the demand grows. The strains identified and characterized have considerable potential for use as probiotics since select strains predominate in humans, tolerate laboratory passage, and enhance animal performance. The basic strategies outlined herein are also applicable to other probiotic cultures and prebiotic compounds for applications in a variety of dairy foods.

## Publications/ Presentations:

Tasi, S.-J., and J. B. Luchansky. 1997. Effects of prebiotics on *Bifidobacterium*. Abstracts of the Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians (T33), p62.

Luchansky, J.B. "Pulsed-field typing and mapping of bacteria associated with foods." Invited speaker at the University of Nebraska Center for Biotechnology, Lincoln, Nebraska. February 26, 1997.

Table 1. Mean extent of growth and mean generation time (h) of 14 *Bifidobacterium* strains grown with 1.5% perbiotics

| | Basal | Glucose | FOS-1 | FOS-2 | Inulin | XOS |
|---|---|---|---|---|---|---|
| **Growth**[1] | $0.23\pm0.00^{d}$ | $0.74\pm0.01^{a}$ | $0.54\pm0.01^{b}$ | $0.76\pm0.01^{a}$ | $0.26\pm0.00^{d}$ | $0.46\pm0.01^{c}$ |
| **Time**[2] | $4.15\pm0.07^{a}$ | $2.79\pm0.05^{c}$ | $2.96\pm0.04^{bc}$ | $2.26\pm0.03^{c}$ | $4.42\pm0.11^{a}$ | $3.27\pm0.06^{b}$ |

Results are mean values from 14 Bifidobacterium strains with triplicate experiments ± S.E.
Means with the same letters in a row are not significantly different ($p<0.05$)
[1]: extent of growth was monitored by spectrophotometrically measuring turbidity at 650nm
[2]: generation time (h)

*INTERIM REPORT*

# Growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder


**Personnel**

Amy C. Lee Wong, associate professor, John B. Luchansky, associate professor, Amy B. Ronner, research specialist, and Alan J. Degnan, senior research specialist, Dept. of Food Microbiology and Toxicology; Mark E. Johnson, senior scientist, Center for Dairy Research



**Funding**

Dairy Management Inc. FM18


**Dates**

July 1997-December 1998

## Objectives

Determine the potential for growth of *B. cereus* and enterotoxin production in re-hydrated infant formula and processed cheese spread at refrigeration and room temperatures.

Identify bacteria that produce antagonistic substances (bacteriocins) against *B. cereus*. Validate the effectiveness of these substances in re-hydrated infant formula during storage and in processed cheese spread during manufacture and/or storage.

## Summary


One of the first goals was to establish a sensitive and reproducible enzyme-linked immunosorbent assay (ELISA) to detect the amount of each of the three protein components (B, L1, and L2) in the *B. cereus* diarrheal enterotoxin, HBL. We also optimized assay conditions to inhibit potential interference by milk components. With the ELISA we developed, we can detect reliably as low as 0.25 ng per component/ml. Using a cocktail of three *B. cereus* strains isolated from food poisoning outbreaks, we inoculated three different infant formulas (low iron and iron-fortified ± maltodextrin) with $10^3$ spores/ml of re-hydrated formula, followed by storage at refrigeration (4, 8, and 12˚C) and abuse (25˚C) temperatures. No growth occurred during 10 days of storage at any of the refrigeration temperatures. However, at 25˚C, *B. cereus* started to grow after 4-6 h, and cell numbers increased by 2-3 log/ml after 12 h, and 5-7 log/ml after 24 h. Similar growth patterns occurred when lower inoculum levels (10 and $10^2$/ml) were used. We have begun to run enterotoxin analysis on samples where growth occurred. So far we have observed that low levels of one or more of the HBL components could be detected after 8 h storage at 25˚C, and by 24 h, all three components are present. We have some preliminary indication that growth may occur at 12˚C with a higher inoculum, and will be conducting experiments with $10^5$ spores/ml at the three refrigeration temperatures.

We have bacteriocins active against *B. cereus*. In the coming year, they will be evaluated in infant formula for their efficacy to inhibit growth. We will also begin experiments to address the potential for *B. cereus* growth in processed cheese spread at refrigeration and room temperatures.

*INTERIM REPORT*

# Identification and characterization of components of the proteolytic enzyme system of *Lactobacillus helveticus* which affect bioactive peptide accumulation

**Personnel**

James L.Steele, associate professor, UW-Madison Food Science, Bart Weimer, associate Professor, Utah State Univ., Jeff Broadbent, assistant professor, Utah State Univ., Jeff Pederson, post-doctoral researcher, UW-Madison Food Science

**Dates**

June 1997- December 1999

**Funding**

Dairy Management Inc. FF18

## Objectives

To screen strains of *Lactobacillus helveticus* for the type and level of bioactive peptides/bioactive peptide precursors which accumulate as the result of the organisms growth in milk.

Determine which components of the proteolytic systems of the selected strains of *Lb. helveticus* are essential for the accumulation of the bioactive peptides/bioactive peptide precursors from milk.

Construct strains of *Lb. helveticus* which accumulate elevated levels of the bioactive peptides/bioactive peptide precursors of interest.

## Summary

Progress towards objective one has involved development of analytical techniques for the rapid identification of peptides. Specifically, progress has been made in the area of coupling capillary chromatography with mass spectroscopy. The coupling of these pieces of equipment should allow us to rapidly screen strains of *Lactobacillus helveticus* for the type and level of bioactive peptides/bioactive peptide precursors which accumulate as the result of the organisms growth in milk.

Research towards objective two has focused on the cell-envelop proteinase specificity of strains of *Lb. helveticus*. To date, the proteinase specificity of three strains has been examined using $_{s1}$-(f1-23) as the substrate. Two strains appear to have indistinguishable specificity while the third strain, *Lb. helveticus* CNRZ32, has a different specificity. By using primers designed from the nucleotide sequence of the *Lactobacillus delbrueckii* subsp. *bulgaricus* proteinase gene, we have been able to amplify portions of the *Lb. helveticus* CNRZ32 proteinase gene. These DNA fragments have been cloned and approximately 1.7 Kb of the gene has now been sequenced. Amino acid residues likely to be involved in substrate specificity have been identified by comparing the deduced amino acid sequence for the CNRZ32 proteinase to previously characterized proteinases from *Lactobacillus casei*, *Lb. delbrueckii* subsp. *bulgaricus*, and *Lactococcus lactis*. This information will allow us to construct a proteinase-negative derivative of CNRZ32 by gene replacement and ultimately to construct derivatives of CNRZ32 which express proteinases with different substrate specificities. These derivatives will allow us to determine if proteinase specificity has an essential role in the accumulation of the bioactive peptides/bioactive peptide precursors from casein during growth of *Lb. helveticus* in milk.

This study will address industry needs outlined in the National Dairy Research Plan for fluid milk and dried milk products. Specifically, objective 5, identify and pursue the health and nutritional benefits of milk; goal 5.1, to leverage bioactive peptides in milk for positioning or potential positioning; tactic 4, investigate microbial enzymatic activities leading to the formation of bioactive compounds in milk, will be addressed. The intent of this project is to begin developing the knowledge required to select/construct strains of lactic acid bacteria which will enhance the level of casein-derived bioactive peptides produced by digestion of fermented milk products.

# chapter 2, section3

# Cheese whey

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Process modification of starter cultures for flavor enhancement in low fat cheese .......... 140

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*FINAL REPORT*

# Fractionation of whey proteins using ion exchange membranes

**Personnel**

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**Funding**

Wisconsin Milk Marketing Board 93-9

**Dates**

November 1993 - November 1997

## Objectives

The overall objective of this research is to develop an economical large-scale technology for producing pure individual whey proteins so that existing whey protein concentrate manufacturers can convert over without having to invest in a new plant. This new technology is needed to exploit the unique nutritional and functional properties of dairy proteins not found with other proteins derived from soy beans and eggs. The specific objectives are to:

Show that glycomacropeptide, lactoferrin and lactoperoxidase can be fractionated from whey using ion-exchange membranes.

Show that beta-lactoglobulin, alpha-lactalbumin and immunoglobulin G can be fractionated into pure products using multicomponent adsorption behavior of the ion-exchange membrane.

Optimize the fractional properties by modifying processing conditions such as whey pH and loading volume, and eluant pH.

Scale-up the process by collecting pilot-plant data needed to design, build and operate a successful commercial-scale process.

## Summary

Lactoferrin and lactoperoxidase were fractionated from whey using a bench-top scale sulfopropyl ion exchange membrane cartridge (model S-100, Sartorius Corp., Edgewood, NY) having a membrane volume of 2 ml. Using filtered whey (0.7 µm filter paper, Micro Filtration Systems, Dublin, CA), flow rates of 10 and 50 ml/min were used without significant pressure drop or loss of performance. Up to 250 ml of whey was loaded before breakthrough of lactoperoxidase or lactoferrin (Fig. 1). Loading more whey caused the lactoperoxidase activity of the effluent to exceed the lactoperoxidase activity of the inlet whey because lactoferrin, which binds more tightly than lactoperoxidase, displaced lactoperoxidase from the membrane.

Using a 250 ml loading volume of whey, essentially all the lactoferrin and lactoperoxidase were recovered from the whey. Two elution peaks were collected. In the first peak, using 0.3 M NaCl, pure lactoperoxidase was obtained. In the second peak, using 0.9 M NaCl, pure lactoferrin was obtained. Repeated loading and elution cycles were performed in sequence without cleaning between cycles, and performance was stable (Table 1).

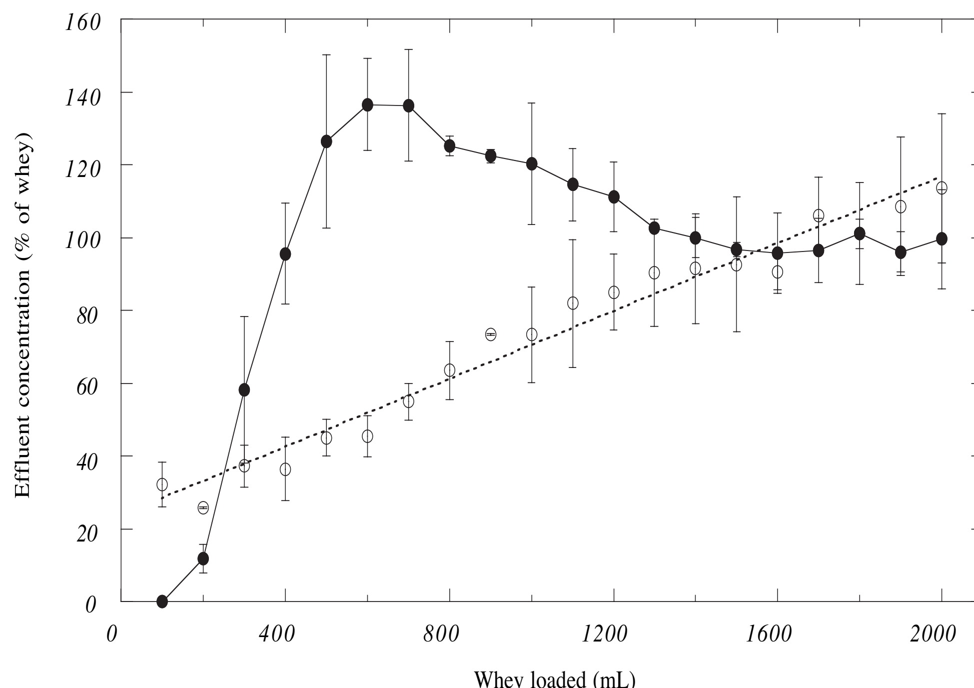


**Figure 1**. Breakthrough curves of lactoperoxidase (solid circles) and lactoferrin (hollow circles) for loading whey into the S-100 membrane. Each point is the average of two trials, and error bars span ± one standard deviation.

Based on these successful laboratory-scale experiments, the process was scaled-up 8-fold by using a pilot-plant-scale membrane having a volume of 16 mL. The cycle time was 9 min, the flow rate was 400 ml/min, and the loading volume was 2000 ml. Matching performance was observed. Thus, the membrane system was more rapid, smaller in size, and used a higher flow rate than traditional bead-based systems.

Next, attention was centered on fractionating kappa-casein glycomacropeptide (GMP) from whey. GMP is the moiety cleaved from kappa-casein by chymosin during cheese making. It occurs at a concentration of 1.2 to 1.5 g/L in sweet whey, comprising 15 to 20% of the total protein. Glycomacropeptide is an example of a purified whey protein that has unique medical or health benefits e.g. control of satiety and phenylketonuria, properties not found with other proteins. Developing a technology for fractionation of GMP from whey will allow production of a high-value nutraceutical product from whey.

We found that GMP could be fractionated from whey using a quaternary methyl amine ion exchange membrane cartridge (model PSC10-QM, BPS Separations Ltd., Spennymoor, County Durham, U.K.) having a 10-mL membrane volume and a large pore size. Optimal conditions were: whey pH of 5.0-6.0, loading volume of 100-150 ml, flow rate greater than 15 ml/min, and 0.3 M NaCl for elution. The process was scaled-up by a factor of four using the optimal conditions. Ten cycles were successfully conducted, yielding 3.2 glycomacropeptide from 4.8 L of whey, or ~ 50% recovery.

Further research was aimed at increasing the purity of the GMP using a second adsorption step and scaling up the process. Both goals were met. This work was the subject of a patent application (P97071US) and an ongoing partnership with a company for commercialization of the technology.

Lastly, the fractionation of alpha-lactalbumin (ALA) and beta-lactoglobulin (BLG) from whey using ion exchange was investigated. We discovered a means to produce both products in a highly pure and undenatured form. This has not before been possible. Pure undenatured ALA is of keen interest to the infant formula industry because bovine milk and whey are deficient in ALA compared to human milk. Supplementing infant formula with pure undenatured ALA would "humanize" infant formula and promote infant health through better nutrition. BLG is a potent gelling agent for use in foods e.g. to replace egg white. Our process uses one unit operation to produce both products and has been disclosed to WARF.

**Table 1**. Lactoperoxidase (LP) activity and lactoferrin (LF) content of the effluent, LP peak, and LF peak (% of total loaded) for repeated cycles using the S-100 module without cleaning between cycles.

| Cycle | Effluent | | 0.3 M NaCl | | 0.9 M NaCl | | Total | |
|---|---|---|---|---|---|---|---|---|
| number | LP | LF | LP | LF | LP | LF | LP | LF |
| 1 | 5 | 50 | 60 | 1.3 | 1.0 | 35 | 66 | 86 |
| 2 | 6 | 45 | 71 | 2.3 | 1.5 | 41 | 78 | 89 |
| 3 | 7 | 47 | 78 | 2.0 | 1.4 | 40 | 86 | 89 |
| 4 | 8 | 43 | 65 | 3.0 | 0.8 | 44 | 73 | 90 |
| 5 | 7 | 43 | 65 | 2.3 | 0.9 | 41 | 73 | 86 |
| 6 | 8 | 41 | 86 | 2.0 | 1.0 | 40 | 95 | 83 |
| 7 | 8 | 41 | 73 | 1.8 | 1.0 | 39 | 83 | 82 |
| 8 | 9 | 41 | 71 | 1.3 | 1.1 | 41 | 81 | 84 |
| 9 | 9 | 41 | 82 | 1.7 | 0.7 | 42 | 92 | 84 |
| 10 | 9 | 35 | 63 | 1.4 | 0.7 | 39 | 73 | 75 |
| 11 | 10 | 39 | 70 | 1.5 | 0.7 | 39 | 81 | 79 |
| 12 | 10 | 42 | 77 | 2.0 | 0.5 | 38 | 87 | 82 |
| Average | 8 | 42 | 72 | 1.9 | 0.9 | 40 | 81 | 84 |
| Std. Dev. | 2 | 4 | 8 | 0.5 | 0.3 | 2 | 9 | 4 |

Although the U.S. market for high value, purified whey proteins is small at present, several factors indicate it will grow. Other major dairying countries are developing markets for these purified individual whey protein products. In the future, the food industry will demand proteins with higher nutritional and functional properties because of the trend towards foods with enhanced health benefits, lower fat content and lower lactose content.

The proposed ion exchange membrane process offers numerous advantages. Evaluating and optimizing the process should provide the stimulus for the U.S. dairy industry to expand into the new domestic and international markets for whey protein products with enhanced nutritional and functional properties.

## Publications/Presentations

Adisaputro, I.A., Wu, Y.-J., and Etzel, M. R. Strong cation and anion exchange membranes and beads for protein isolation from whey. *J. Liq. Chrom. & Rel. Technol.*, 19(9), 1437-1450 (1996).

Chiu, C.K. and Etzel, M.R. Fractionation of lactoperoxidase and lactoferrin from bovine whey using a cation exchange membrane.*J. Food Sci.* **62**(5), 996-1000 (1997).

Chiu, C.K. Fractionation of casein glycomacropeptide, lactoperoxidase and lactoferrin from bovine whey using ion exchange membranes or beads. M.S. thesis, Univ. of Wisconsin, Madison (1996).

Etzel, M.R. Production of k-casein macropeptide for nutraceutical uses. Pat. Appl. 97071US (1997).

*INTERIM REPORT*

# Fractionation of κ-casein glycomacropeptide from whey for nutraceutical uses: scale up of the ion exchange membrane technology

**Personnel**

Mark. R. Etzel, associate professor, Shinta Dermawan, graduate student, Dept. of Food Science, Laura T. Marschoun, graduate student, Dept. of Biological Systems Engineering

**Funding**

Dairy Management Inc. CW47

**Dates**

July 1997 - July 1999

## Objectives

Increase the use of whey proteins for health and nutrition applications by developing processing, fractionation, and modification technologies, specifically:

Scale up the anion exchange membrane technology for fractionation of k-casein glycomacropeptide from whey.

## Summary

Glycomacropeptide (GMP) is the moiety cleaved from κ-casein at the $Phe^{105}$-$Met^{106}$ position by chymosin during cheesemaking. GMP occurs at a concentration of 1.2 to 1.5 g/L in sweet whey, comprising 15 to 20% of the total protein. Known biological functions of GMP, as consolidated from several nutritional and biological research reports, include the utilization of GMP in dietetic foods and pharmaceuticals. Aromatic amino acids (phenylalanine, tryptophan and tyrosine) are absent from GMP. Thus, GMP may be useful for the treatment of phenylketonuria, a hereditary disorder in which aromatic amino acids cannot be metabolized. Furthermore, consumption of as little as 25 mg of GMP stimulates the release of cholecystokinin, a hormone that regulates food intake by creating the sensation of satiety. Thus, GMP may be useful in the billion-dollar weight loss or diet foods marketplace.

The objectives of our past research were to develop a new and more economical method for the large-scale fractionation of GMP from bovine whey based on ion exchange membranes. The basis of the ion exchange method is that GMP has a negative net charge in whey at acidic pH, while other whey proteins are charged positive. Thus, when whey is contacted with anion exchange membranes or beads at acidic pH, GMP binds to the ion exchange media while the other whey proteins remain in the fluid phase. The bound GMP can be eluted from the media using a salt solution. Experiments were conducted to: 1) determine the effect of operating conditions on the purity and recovery of GMP, 2) operate consecutive cycles of loading and elution to simulate an industrial process, and 3) compare the performance of ion exchange membranes to ion exchange beads, the conventional media.

In this follow-up project, we have discovered a process to increase the purity, lower the cost of manufacture, and increase the commercial value of GMP. Our process for producing a GMP having nutraceutical properties utilizes whey and two ion exchangers of opposite polarity in series. A hydrolyzed GMP nutraceutical food product having less that about 4% total of the hydrophobic aromatic amino acids phenylalanine, tryptophan

and tyrosine can also be produced using our process. This invention was the subject of a patent application (P97071US) and an ongoing partnership with a company for commercialization of the technology.

The nutraceutical, κ-casein glycomacropeptide, has been shown to have special medical benefits. GMP comprises 15 to 20% of the protein in whey, making it plentiful in supply. However, prior attempts to purify it from whey have resulted in low recovery percentages, low purity and unfavorable economics. Recently, we developed an ion exchange membrane process that overcomes this limitation and that has a higher relative throughput than prior processes. In this research, we will further develop and scale up this new ion exchange process.

## Publications/Presentations

Etzel, M.R. Production of κ-casein macropeptide for nutraceutical uses. Pat. Appl. 97071US (1997).

*FINAL REPORT*

# Process modification of starter cultures for flavor enhancement in low fat cheese

**Personnel**

Mark. R. Etzel, associate professor, Brian Chi-Shung Brian To, graduate student, Dept. of Food Science

**Dates**

July 1994 - June 1997

**Funding**

Dairy Management Inc. ETZ 95

## Objectives

The overall objective of this research is to develop more flavorful low-fat Cheddar cheese by using process-modified starter culture adjuncts that accelerate the ripening process. Processing conditions are to be developed which reliably modify the culture characteristics to permit flavor enhancement in low-fat cheese. This allows the manufacture of more consistent and flavorful low-fat cheeses, thus increasing the demand for cheese and milk. The specific objectives are to:

Establish methods for small-scale production of cell pastes of candidate cultures.

Freeze, freeze dry and spray dry the cell paste solutions using processing conditions which range from attenuating to preserving of metabolic activity.

Analyze the cell pastes, solutions and cell powders for metabolic activity, specifically cell survival, lactic acid production, and b-galactosidase and aminopeptidase activity.

Select the cultures and processing conditions which are best for flavor enhancement in low-fat cheese, and then produce large amounts of adjunct needed for cheesemaking.

## Summary

The culture *Brevibacterium linens* ATCC 9174 was selected as the first candidate for investigation. *B. linens* is responsible for the ripening of surface-ripening cheeses such as Limburger and Brick. Incorporation of this obligate aerobe into Cheddar cheese, as an adjunct to the normal starter culture, has been shown to enhance the rate and extent of flavor development. Inexpensive methods for producing, preserving, and distributing these adjuncts cultures must be developed before widespread use in the dairy industry is economical. For this reason, the effects of processing (freezing, freeze drying and spray drying) on the characteristics of *B. linens* were measured in this research.

Survival was 100% for *B. linens* after freezing and freeze drying. For spray drying, survival was halved for every 5°C increase in the outlet air temperature. Heat was the sole mechanism decreasing cell survival during spray drying. Thermal resistance was measured vs. moisture content and temperature, resulting in D-values ranging from 120 min at 25.3% solids and 44.0°C to 2.6 min at 46.2% solids and 55°C. This is much more heat sensitive than alkaline phosphatase, an indicator of proper pasteurization of milk. However, thermal inactivation of *B. linens* during spray drying can be eliminated by proper selection of the outlet-

air temperature. By extrapolation of the data, 100% survival would occur at an outlet air temperature of 57°C. The freeze dried and spray dried cultures were stable during prolonged storage at 4°C in the absence of oxygen and moisture.

In the second part of this investigation, five lactic acid bacteria, *Lactococcus lactis* ssp. *cremoris* D11, *Lactobacillus casei* ssp. *pseudoplantarum* UL137, *Lactobacillus delbrueckii* ssp. *bulgaricus* CH3B, *Lactobacillus acidophilus* NCFM and *Streptococcus salivarius* ssp. *thermophilus* CH3TH, were separately frozen, freeze dried or spray dried, and tested for survival and lactic acid production before and after processing. Virtually all the cells survived freezing. Of the survivors, 60 to 70% survived the dehydration step of freeze drying, except for *Lb. bulgaricus*, for which one fifth survived. Survival after spray drying was greatest for *S. thermophilus* and lowest for *Lb. bulgaricus*. In contrast to freezing and freeze drying, spray drying caused a significant delay in lactic acid production and reduction insurvival. After spray drying, survival was greatest for *S. thermophilus* and lowest for *Lc. cremoris*.

The results of this research will help advance the cheese industry. The demand for low fat cheese is growing. This new technology will help to develope low fat cheeses with a flavor and texture comparable to full fat cheeses. These low fat cheeses will be popular for producing spray-dried powders for use in low fat convenience foods such as cheese nachos and frozen dinners, and will appeal to consumers on low-fat diets who may have reduced their consumption of traditional full fat cheeses. This new technology will help to accelerate the already increasing demand for cheese, which will directly result in an increased demand for milk. In addition, the results of this research will help to advance the starter culture industry, most of which is based in Wisconsin.

## Publications/Presentations

To, B.C.S. 1996. Properties of *Brevibacterium linens* and five different lactic acid bacteria attenuated by spray drying, freeze drying, or freezing. M.S. thesis, Univ. of Wisconsin, Madison.

To, B.C.S. and Etzel, M.R. 1996. Spray drying, freeze drying, or freezing of three different lactic acid bacteria species. *J. Food Sci.,* 62(3): 576-578 & 585 (1997).

To, B.C.S. and Etzel, M.R. 1996. Survival of *Brevibacterium linens* (ATCC 9174) after spray drying, freeze drying, or freezing. *J. Food Sci.,* 62(1): 167-170 & 189 (1997).

To, B.C.S. and Etzel, M.R. 1996. Survival of *Brevibacterium linens* ATCC 9174 after spray drying, freeze drying, or freezing. Am. Inst. Chem. Eng. Conf. Food Eng., Nov. 2-3, 1995, poster 8.4.

*APPLICATIONS PROGRAM REPORT*

# Whey applications program

### Personnel

J. Russell Bishop, director, Kimberlee J. Burrington, associate researcher, Tom Szalkucki, assistant director, Center for Dairy Research

### Funding

Wisconsin Milk Marketing Board UWA9702 & UWA9801

### Dates

July 1996 - December 1997

## Objectives

Develop a whey applications system to support developing new markets.

Work with 3-5 Wisconsin companies.

Execute technology development from DMI, a national base.

## Summary

Based on consumer demand, production of cheese, and therefore whey, continues to increase in the United States. CDR has responded to this growth and industry requests by developing a whey applications program. The program is designed to utilize CDR whey research and expertise, as well as research from other Centers, to assist industry in commercializing the technology and component applications. The goal of these efforts is to increase the value and use of whey and whey components in food and other products.

The Wisconsin whey and whey utilization short course was held on April 8-9, 1997. Bill Wendorff, UW Extension, put together a good overview course featuring speakers from the Department of Food Science, CDR, and industry. The short course was sold out by March and the reviews after the course were positive. The whey short course will be presented again this year.

In October 1997, Kimberlee (K.J.) Burrington was hired at CDR to build the whey program at CDR based on cheese/whey industry needs. K.J. comes to us with expertise in food product development, especially baked goods. She has been meeting with selected cheese/whey producers to discuss their needs and how CDR's applications program can address those needs.

FINAL REPORT

# Conversion of Whey Permeate to Propylene Glycol for Food and Non-Food Uses

**Personnel**

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Anita J. Shaw, Graduate Student, Department of Chemical Engineering;
Roxanne M. Smith;
Yi-Jui Wu, Students, Dept. of Chemical Engineering

**Dates**

July 1994 - June 1997

**Funding**

Dairy Management Inc. CME95

## Objectives

The overall objective of this project is to develop a fermentation process for the conversion of lactose in whey permeate to propylene glycol, a large volume commodity chemical. The fermentation process yields an optically pure product, R-propylene glycol, with added value over synthetically produced propylene glycol. The specific objectives are to:

1. Screen and select microorganisms capable of fermenting lactose to propylene glycol.

2. Optimize the medium and environmental conditions for propylene glycol production from whey permeate by the organism(s) identified in Objective 1. The effects of carbon to nitrogen ratio (C/N), trace nutrients, and medium pH will be investigated.

3. Develop technology for the removal of inhibitory acetate and lactate from whey permeate-based medium during fermentation so as to achieve increased propylene glycol concentration and productivity

## Summary

We have established that *Thermoanaerobacterium thermosaccharolyticum* (ATCC 31960) (formerly known as *Clostridium thermosaccharolyticum* produces propylene glycol from hydrolyzed whey permeate (WPH) and WPH with added yeast extract, yielding titers of 4.5 g/l in 48 h in 300 ml anaerobic flasks. Both glucose and galactose were utilized in the fermentations. To further investigate this finding, eight different whey-based media fermentations were carried out in triplicate for a total of twenty-four fermentations. The eight media were whey or whey permeate, with or without prior hydrolysis of lactose, and with or without added yeast extract. The major products of the *T. thermosaccharolyticum* fermentation are propylene glycol, acetate, lactate and ethanol. Fermentations of WPH and hydrolyzed whey gave greater propylene glycol selectivity (moles propylene glycol per moles of by-products) compared to the media with added yeast extract. However, media with yeast extract produced more propylene glycol in absolute concentration. Unhydrolyzed whey and unhydrolyzed whey permeate were converted to propylene glycol only with the addition of yeast extract, and selectivity was lower than it was for hydrolyzed medium.

During the course of this work we made a discovery of significance to Objective 1. We found that a triose phosphate isomerase mutant of the bacterium *Escherichia coli* transformed with the gene for the enzyme aldose reductase is able to ferment galactose to propylene glycol. The same strain transformed with both aldose reductase and methyglyoxal synthase genes is able to ferment both galactose and lactose to propylene

glycol. Currently titers of propylene glycol are in the range of 1 g/l, which is lower than those obtained from galactose or WPH with *T. thermosaccharolyticum*. However, this work is significant in that it provides the basis for further development of metabolic pathways to propylene glycol from lactose. Unlike *T. thermosaccharolyticum*, *E. coli* is an important industrial organism for which there are many tools for further strain improvement and modification. The finding that *E. coli* can be engineered to produce propylene glycol from sugars such as galactose and lactose provided the basis for a U.S patent application filed through the Wisconsin Alumni Research Foundation (WARF).

One of the key isssues for the production of propylene glycol with either *T. thermosaccharolyticum* or an *E. coli* strain is the inhibition of product formation by fermentation by-products such as acetate and lactate. The focus of the work on Objective 3 was to increase the productivity and final titer of the fermentation by developing new technology for removal of inhibitory products from the broth with ion exchange resins simultaneous to the fermentation. When acetate and lactate anions are not removed during the fermentation, low productivity results due to product inhibition. When acetate was added to a level of 20 g/l during the fermentation, cell mass was significantly reduced, providing evidence of strong product inhibition. Our research established that a column packed with Amberlite IRA-400 effectively removes lactate from uninoculated defined glucose medium, while controlling pH. When one anion binds to the column, one hydroxide ion was displaced, which controlled the pH without the need for adding base. In subsequent research, we used a column to extract lactate and acetate from an actual fermentation using defined glucose medium. We set-up a fermentation system where broth was continuously pumped from a water-jacketed (60$^{o}$C) one-liter fermentor into a microporous hollow-fiber filter. Cells were returned directly to the fermentor, and cell-free broth passed through an anion-exchange column before it was returned to the fermentor. By establishing a technology for the simultaneous removal of acetate from the fermentation broth, we met Objective 3 of the project.

This research will provide the basis for the industrial production of a new fermentation chemical, propylene glycol, from whey permeate. Propylene glycol is a major organic chemical with extensive applications in the food industry, both as an ingredient and as an antifreeze and heat transfer fluid. Currently propylene glycol is produced entirely from propylene, a petrochemical. This synthetic propylene glycol is a racemic mixture, a mixture of left and right handed forms of propylene glycol. The fermentation process provides a unique route to enantiomerically pure R-propylene glycol. Since the other major non-volatile products of the fermentation are acetic and lactic acid, it may also be possible to concentrate the fermentation broth and make a cultured whey product containing propylene glycol and organic acids.

## Presentations

Shaw, A.J., N.E. Altaras, and D.C. Cameron. 1997. Metabolic engineering of 1,2-propanediol production in Escherichia coli. Division of Biochemical Technology, Abstract No. 47, 213th ACS National Meeting, San Francisco, CA, April 1997.

Cameron D.C., M.L. Hoffman, A.J. Shaw, and N.E. Altaras. 1997. Design, construction and analysis of metabolic pathways for the production of diols. Engineering Foundation, Biochemical Engineering X, Kananaskis, Alberta, Canada, May 18-23.

## Publications

Cameron, D.C., N.E. Altaras, M.L. Hoffman, and A.J. Shaw. In press. Metabolic engineering of propanediol pathways. Biotechnology Progress.

Shaw, A.J. 1997. Metabolic engineering of methylglyoxal metabolism in *Escherichia coli*. Ph.D. Thesis, Department of Chemical Engineering, University of Wisconsin-Madison.

## Patents

Cameron, D.C., A.J. Shaw, and N.E. Altaras. Microbial production of 1,2-propanediol from sugar. U.S. patent filed February 1997.

chapter 4

# Communications

# Center for Dairy Research Communications

### Personnnel

Tim Hogensen, program asst.
Karen Paulus, editor
Mary Thompson, outreach specialist

### Dates

July 1996- June 1997

### Funding

Wisconsin Milk Marketing Board UWA9702

## Outreach Events

Wisconsin Cheese Industry Conference
April 2-3, 1997
CDR, along with the Wisconsin Cheese Makers Association, presented a full day and half of practical and timely seminars for people in the dairy foods industry. Graduation ceremonies for Wisconsin's first Master Cheese Makers™ were one of the highlights of the conference banquet.

WI Farm Progress Days
July 1997
Mary Thompson attended and spoke about CDR's research and applications programs

Producing Safe Dairy Foods Workshop
September, 1997
This workshop focuses on the nature and control of dairy foods pathogens. Although over 40 people attended this year, we think we have reached a plateau and will repeat the workshop every other year.

World Dairy Expo - October 1997
CDR supports a booth at this annual event—we see it as a chance to meet and talk to our funders.

World Food Expo - November 1997
Chicago, IL
CDR supported a booth at this first ever show, the largest dairy foods trade show. Samples of reduced fat cheese and chocolates made with milkfat fractions assured a steady stream of visitors.

Artisan Cheese Course - French Cheeses
November 4 - 5, 1997
The latest in a series of Artisan Cheese Workshops

## Public Service Announcements

Four, 3 minute public service announcements about CDR's Application Programs were recorded and broadcast to 78 radio stations in WI.

## Industry Teams

The members of the cheese industry team met twice in the past year to develop the Center's research program and to hear about the progress of research projects. The safety industry team met this year to hear about research progress, to discuss current safety concerns, and to identify future research topics.

## Publications

CDR still publishes an annual report, although we have moved the publication date to March to coincide with Dairy Management Inc.'s calendar year deadline. Our technical annual report summarizes all current research projects and is distributed to funding agencies and scientists in academia, industry and at other dairy research centers around the world.

We also publish a shorter, general report which we now call the Cutting Edge. We use it to describe CDR and highlight several research projects in an easy-to-read style. We have taken the Cutting Edge to the Dairy Expo, IFT, and the joint meeting with Wisconsin Cheesemakers. Volume 2 of the Cutting Edge, which is published every other year, will debut in April 1998.

The Dairy Pipeline continues to be a practical and dependable training publication for the dairy industry. The Pipeline, including back issues, is now available through our web site.

We use technical fact sheets to simply and efficiently describe and explain completed research projects and to update information about our applications programs.

## CDR Online

We continued adding to the web site, including making back issues of the Pipeline available. A complete makeover of the web site is planned for 1998.