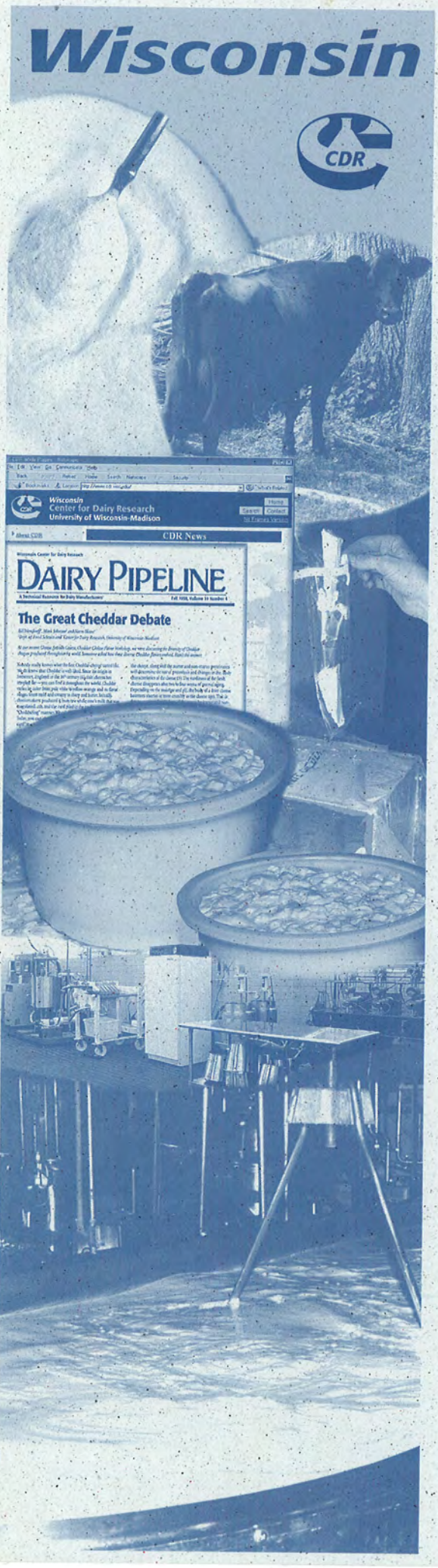
Wisconsin Center for Dairy Research



Annual Report 1998

Wisconsin
Center for Dairy Research

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CDR Annual Report

Published March 1, 1999, 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 1998. 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.

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

Cover Design by Tim Hogensen

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.

Objectives

To conduct an aggressive, balanced, basic and applied research program in the following ways:

Exploring and understanding the functional properties of cheese and cheese products to increase the use of both.

Improving the value and expanding the use of milkfat.

Recovering and/or modifying nonfat solids, especially whey components, to enhance their value for food and nonfood uses.

Maintaining and enhancing consumer confidence by developing technologies that will strengthen dairy food safety and quality systems.

Providing dairy product marketing information, business management tools and policy analysis to improve the economic health of both individual dairy processors and the Wisconsin dairy industry.

Support research and develop programs in traditional and novel areas.

Creating a closer working relationship/partnership with industry.

Effectively communicating research/technical information.

Providing education to industry and university students.

Increasing and diversifying funding resources.

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Chapter 2, Cheese

Chapter 4, Communications

Reports unavailable at publication date:

Milkfat

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Robert Lindsay

Improvement of functionality, flavor, and stability of butter
Robert Lindsay

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Robert Lindsay

Cheese

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

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Robert Lindsay

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John P. Norback

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Carol M. Chen

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Mark. R. Etzel

CDR structure

CDR is organized into three functional areas — research, applications, and communications. Three committees composed of both industry and academic representatives assist with administration and research planning.

CDR staff

J. Russell Bishop, Ph.D., director
Tom Szalkucki, administrative officer
Joe Bissonnette, network administrator
Carmen Huston, financial specialist 2
Lisa Lokken, financial specialist
Jackie Utter, program assistant 2
Sandra Sekel, program assistant 2

Research/Application Staff

Gene Barmore, research specialist
Kim Burrington, whey applications program coordinator
Carol Chen, researcher
Amy Dikkeboom, research specialist
Brian Gould, senior scientist
Kristen Houck, research specialist
John Jaeggi, associate researcher
Mark Johnson, senior scientist
Kerry Kaylegian, researcher
Jim Path, outreach specialist
Juan Romero, associate researcher
Leesa Stefano, research specialist
Karen Smith, associate researcher
Marianne Smukowski, research specialist
William Tricomi, assistant researcher
Matt Zimbric, research specialist

Communications

Joanne Gauthier, communication specialist
Tim Hogensen, program assistant
Karen Paulus, editor
Mary Thompson, outreach specialist

The Research Program

CDR sponsors a diverse range of research on dozens of dairy topics including disciplines from genetic engineering to economics. However, the CDR research program focuses on four areas: 1) demand and use for milkfat, 2) developing new applications for nonfat milk components, 3) cheese technology, and 4) dairy foods safety and quality.

Communications

Information and technology transfer is an essential component of CDR. CDR's communications program provides publications, workshops, seminars, conferences, and scientist exchanges.

Committees

Administrative Committee

The Administrative Committee is responsible for policy formulation and appointment of the CDR Director. Its members (FY 1998) 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 1998) include:

Aimutis , Bill, Land O' Lakes Inc.
Bishop, J. Russell, Wisconsin Center for Dairy Research
Blaska, Gregory, DMI and WMMB
Dentine, Margaret, College of Agricultural & Life Sceinces
Dobson, William D., Dept. of Agricultural Economics
Etzel, Mark, Dept. of Food Science
Geyer, James, Foremost Farms
Haines, Bill, DMI
Hartel, Richard, Department of Food Science
Hill, Charles, Dept. Chemical Engineering
Johnson, Eric, Dept. of Food Microbiology & Toxicology
Johnson, Mark, Center for Dairy Research
Krug, David, DMI and WMMB
Lindsay, Robert, Department of Food Science
Mathison, Matt , WMMB
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, Wisconsin Center for Dairy Research

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 1998):

Aimutis, Bill, Land O' Lakes Inc.
Balmer, Tom, National Milk Producers Federation
Bell, Larry, Schreiber
Bhowmik, Tarun, Tastemaker
Bishop, J. Russell, Wisconsin Center for Dairy Research
Burrington, David, Chr. Hansen's Laboratory
Byrne, Rob, National Cheese Inst/Amer Butter Inst.
Bumbalough, John, Land O'Lakes Inc.
Campbell, Larry, Hershey Foods
Carr, Jim, SKW Biosystem Inc.
Clark, Warren, American Dairy Products Institute
Crawford, Robert, Plymouth Cheese/DFA
Everson, Tom, Grande Cheese Co.
Fuhrmann, Dave, Foremost Farms
Geyer, Jim, Foremost Farms
Gregorich, LaVerne, Grassland Dairy Products Inc.
Haines, Bill, DMI
Hanrahan, Jim, Saputo Cheese/Stella
Helm, Tom, Grande Cheese
Keel, Tom, Foremost Farms
Kozak, Jerry, NMPF
Legreid, Bradley, Wisconsin Dairy Products Association, Inc
Leitner, Ken, Alto Dairy Coop
Mathison, Matt, Wisconsin Milk Marketing Board
Mehnert, David, Kraft
Middleton, Jerry, Simplet/Arpin
Moran, Jim, Kraft-General Foods Inc.
Muck, George, Dean Foods
Narasimmon, Raj, Schreiber Foods, Inc.
Sellars, Robert, R. L. Sellars & Associates, Inc.
Skatrude, Tom, IFI
Sommer, Dean,Alto
Umhoefer, John, Wisconsin Cheese Makers Assoc.
Van Boxtel, Joep, , Gist-Brocades Dairy Ingredient Group
Van Dyke, Deborah, Schreiber Foods, Inc.
Wallace, Don, Rhodia
Willnett, Doug, Rhodia
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
Quality and Safety—Eric Johnson, Food Research Institute, University of WI-Madison

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chapter 1

Milkfat

INTERIM REPORT

Effects of milkfat source and composition on crystallization kinetics

Personnel: R.W. Hartel, professor, Colleen Kubitz, research assistant, Dept of Food Science

Funding

Dairy Management Inc.

Dates

June 1997 —June 1999

Objectives

The overall objective is to correlate the variability in anhydrous milkfat with fractionation efficiency.

1. 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).

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

Summary

Milkfat samples have been collected from various sources throughout the year. In particular, AMF produced from fresh cream obtained from the same source (in Minnesota) has been analyzed over the past year. In addition, AMF samples produced from whey cream and butter were obtained from the same supplier. Further, several AMF samples were obtained from German, Irish and New Zealand sources for comparison. The AMF samples were analyzed for compositional differences (fatty acid profile, acyl carbon profile and minor lipid content) and then crystallization kinetics evaluated using a turbidity technique.

Somewhat surprisingly, the AMF samples obtained from the same origin throughout the year showed no significant differences in either chemical composition or crystallization behavior. The AMF prepared from fresh cream obtained in January had identical properties to the AMF prepared from fresh cream from the same source obtained in August. Thus, no seasonality was observed in this milkfat, probably due to the practice of feed supplementation throughout the year.

The samples of AMF obtained from whey cream and butter exhibited only slight differences in chemical composition and crystallization behavior. Analysis of the data is still underway to correlate the differences in chemical composition with the differences in crystallization kinetics. Similarly, the international AMF samples, in general, only showed slight differences in chemical composition and crystallization behavior. However, the sample obtained from Ireland had an induction time for nucleation about twice that of the other fats. Data analysis continues.

It has been well documented that the composition of anhydrous milkfat (AMF) can vary based on source and processing conditions, and that these differences can have considerable effect on crystallization kinetics and fractionation efficiency. However, our understanding of these variations, and their impact on crystallization kinetics and fractionation is severely lacking. A better understanding of the variables that influence crystallization of milkfat will allow us to control the fractionation process. Furthermore, these results will help us understand the seasonal and regional differences in milkfat products.

FINAL REPORT

Improvement of thin-layer fractionation technology

Personnel: RW Hartel, professor, Dept of Food Science, J. Ulrich, professor, University of Bremen

Funding

Dairy Management Inc.

Dates

June 1997—June 1998

Objectives

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

Summary

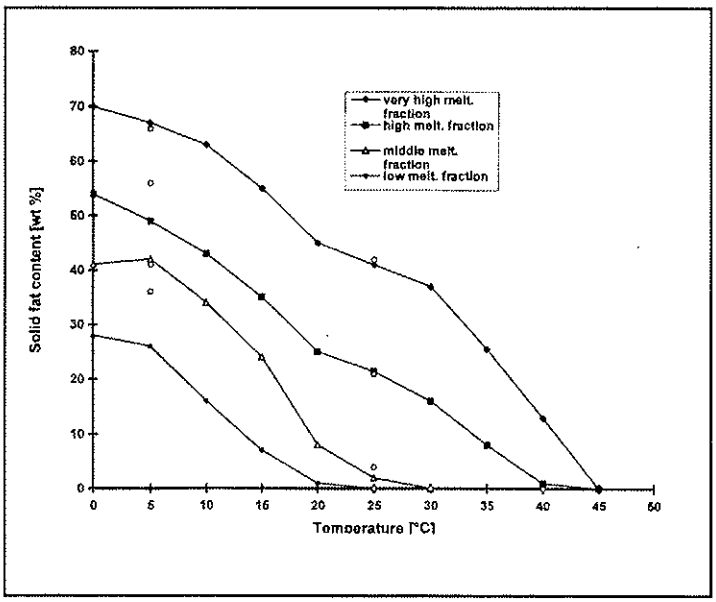
In previous phases of this work, it has been shown that milkfat can be fractionated using solid-layer (thin layer) crystallization technology. In this technology, a layer of milkfat is crystallized on a cooled metal surface and separation is accomplished by simply draining the remaining liquid fat. Solid fat curves for solid-layer fractionation, shown in Figure 1, generally look the same as those for fractions obtained using suspension crystallization technology. Chemical composition of these milkfat fractions followed the typical pattern, with higher-melting fractions containing increased levels of long chain, saturated fatty acids and lower-melting fractions having higher levels of short chain and unsaturated fatty acids. However, the slow growth rates of milkfat resulted in high surface area requirements for solid-layer fractionation. Thus, the anticipated cost savings of this technology over commercial suspension crystallization technologies was not realized.

In this phase of the work, our goal was to evaluate various techniques for improving the quality of the separation and the economy of the technique. These techniques included (1) changing the temperature of the bulk fluid, (2) deposition of a primary crystalline layer on the metal surface, (3) sweating with warm fluid inside the tube and (4) sweating with hot gas external to the solid layer.

As can be seen in Fig. 2, the clear point, or melting point, of the product can be raised by increasing the temperature of the melt in the bulk. The temperature of the cooled tube was kept constant in this case. Moreover, increasing the bulk temperature produces smoother crystal layers. Low bulk temperatures lead to an agglomeration of needle-like crystals that formed on the cooled surface. These agglomerates entrap higher amounts of liquid oil and resulted in lower clear points of the solidified fraction.

Besides crystal growth, the effects of a pre-nucleation step on the solid layer were examined in more detail. In previous studies, nucleation on the cooled tube at the beginning of a crystallization process was found to be problematical, because fast growing crystals were shaped like needles. These needles tended to trap a lot of mother liquor. Therefore a primary layer (seed layer) was produced on the cooled surface by plunging the cooled tube into the molten product. After a strong cooling of this layer outside the molten product, the actual crystallization was started in the feed melt.

Figure 1



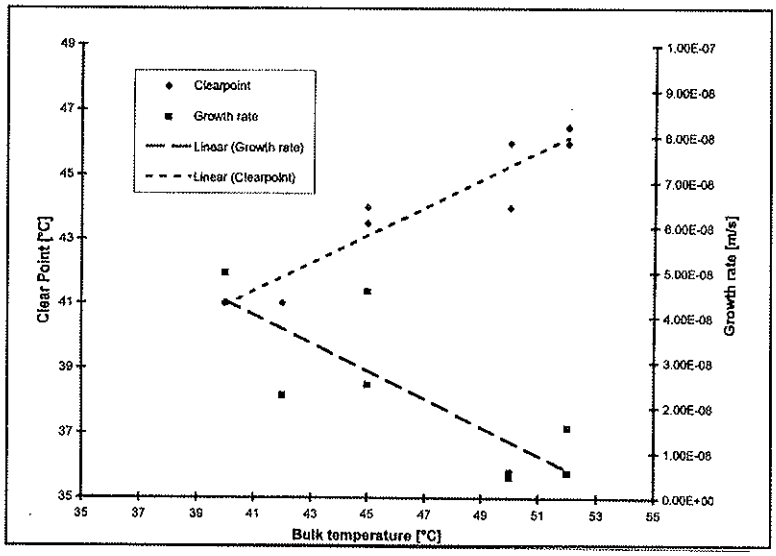


Figure 2

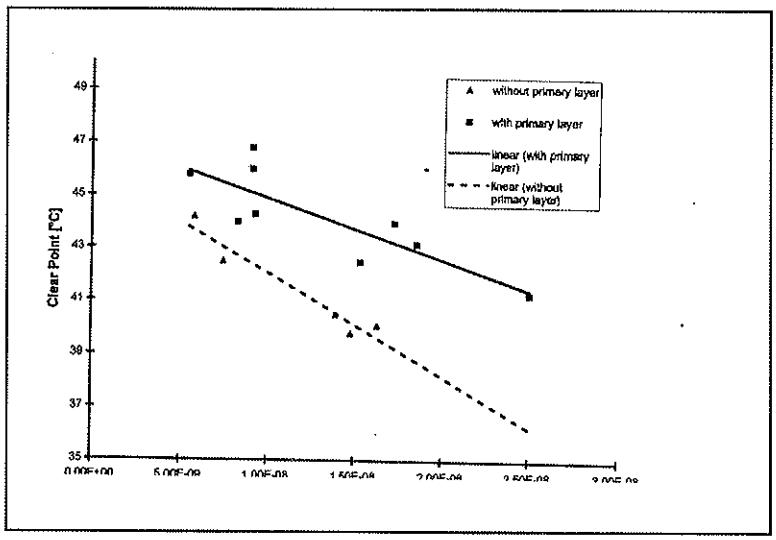


Figure 3

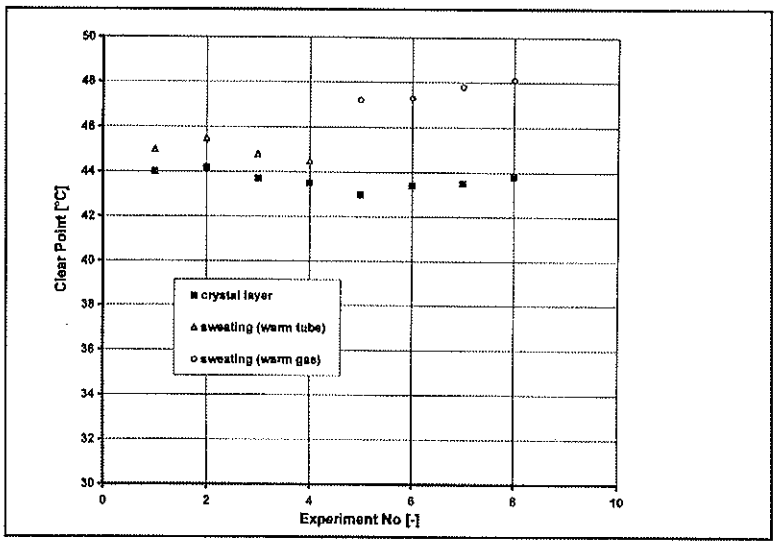


Figure 4

As shown in Fig. 3, the clear point of the solid fraction generally decreased while growth rate was increased with a prenucleation step. Obviously, the use of a primary layer leads to higher clear points of the high-melting fraction, although the growth rate was the same. It also can be observed that the use of primary layers lead to more homogeneous crystal growth than without it.

Furthermore, it is possible to improve the quality of crystalline products by ***post crystallization treatments*** like "sweating" and "washing." Due to the softness of milkfat layers, "sweating" is a favorable process. In principle, the crystal layer is separated from the melt after crystallization by draining the liquid fat. The crystal layer is then heated up to a temperature near the melting point of the original, natural milkfat. The adhering mother liquor or the impurities enclosed in the pores of the crystalline layer can be removed by this sweating step.

Heating the tube (crystallization surface) is one possibility for sweating. Another possibility is to use an atmosphere around the crystalline layer, like a a warm gas, while the cooled tube is set at a temperature lower than the melting point of the product.

The results of both kinds of post treatments are presented in Fig. 4. The differences concerning clear point of the original, natural milkfat, the crystal phases and the sweating phases are shown. Obviously, the "sweating by warm gas" leads to higher clear points of the high-melting fraction than "sweating by heating up the tube."

Capital and operating costs for both dynamic and static solid-layer crystallization technology were compared to the commercial fractionation technology based on suspension crystallization. Due to the relatively slow growth kinetics of milkfat, the surface area required for solid-layer

crystallization techniques was significantly larger than expected. Thus, the capital costs for solid-layer fractionation are almost twice as high as for suspension crystallization. However, operating costs for static layer technology and suspension crystallization are equivalent and maintenance costs are less for the solid-layer technique. The primary advantage of the solid-layer technology is that a higher melting point solid fraction of equivalent yield can be produced. Thus, if this fraction is most highly desired, fractionation by the solid-layer technology may be worth the additional capital costs.

Despite the fact that the surface-layer fractionation technique, as we have developed it to date, does not meet our economic goals, it still may have potential in certain circumstances. The economics of surface-layer technology for milkfat fractionation can be improved using post-crystallization techniques. The choice of surface-layer technologies for milkfat fractionation may be best when the high-melting fraction is most desired.

Publications

Peters, S., Ulrich, J. and R.W. Hartel, Milkfat Fractionation by Solid Layer Melt Crystallization, J. AOCS (submitted).

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).

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

Tiedtke, M., S. Niehorster, J. Ulrich and R.W. Hartel, Separation of Milkfats by Solid Layer Crystallization, proceedings of Bremen International Workshop for Industrial Crystallization (BIWIC), J. Ulrich (ed.), University of Bremen Press, pp. 32-39 (1994).

INTERIM REPORT

Rheological and structural properties of dairy-based lipid mixtures

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

Funding

Dairy Management Inc.
Wisconsin Milk Marketing Board 9703

Dates

June 1997— June 1999

Objectives

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

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

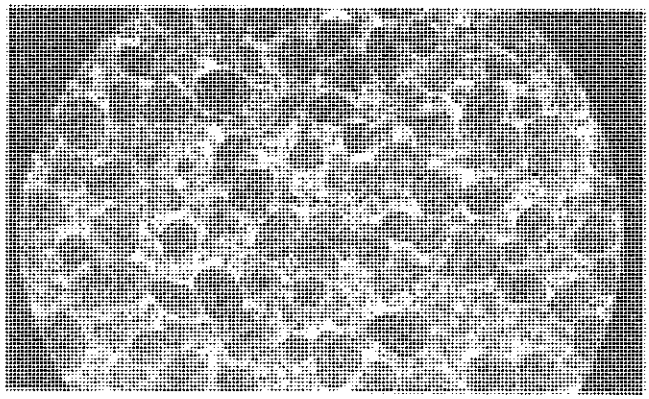
Summary

The effects of lipid composition and processing conditions on lipid crystalline microstructure are being studied. Various mixtures of milkfat fractions and canola oil are melted at 60°C for one hour to remove any crystal memory. The molten fat is cooled to crystallization temperature where crystallization is allowed to proceed for several hours in the presence of agitation. The crystal slurry is then allowed to cool to 10°C and set into a semi-solid product. This process simulates commercial processing of lipids although the conditions are not exactly translatable to commercial conditions.

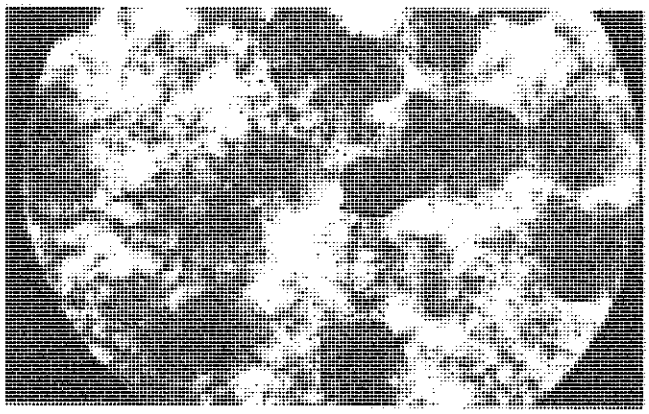
The variables being studied in this experiment include:
- rate of cooling to crystallization temperature (fast and slow),
- crystallization temperature (25 to 30°C),
- agitation rate (50 to 300 RPM),
- lipid formulation:
  - 30, 40 and 50% high-melting in low-melting milkfat fractions,
  - 30, 40 and 50% high-melting milkfat fraction in canola oil,
- storage time at 10°C (1 day to 3 weeks).

The following analyses are performed on the either the slurry or the semi-solid sample.
- solid fat content of slurry (NMR),
- solid fat content of semi-solid product (NMR),
- optical microscopy of slurry crystals,
- confocal microscopy of crystals in semi-solid product,
  - a small amount of dye is added to the molten product prior to crystallization to enhance the image from confocal microscopy,
- mechanical properties of semi-solid product (DMA).

Figure 1
Confocal laser scanning microcsope image of a 30% mixture of high melting milkfat fraction in low melting fraction. Sample crystallized at 25°C for 3 hours and cooled to 10°C with analysis after 24 hours.



Intermediate agitation rate (150 RPM)



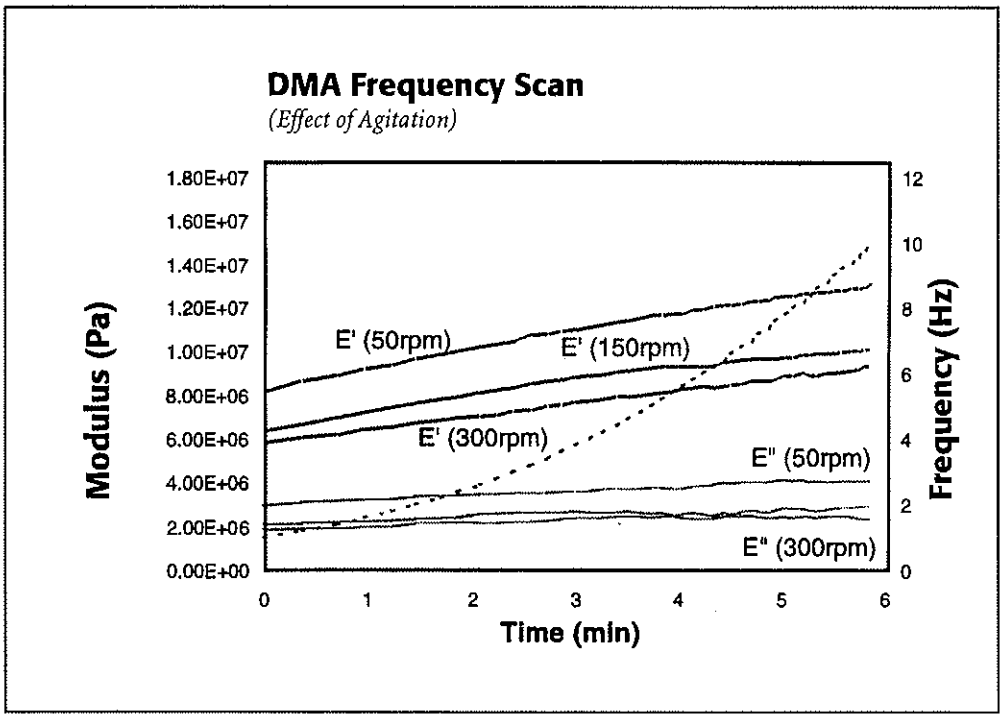
Low agitation rate (50 RPM)

Representative confocal microscope images of the crystalline structure in the semi-solid product are shown in Figure 1. These images clearly show two crystalline phases. The dense primary crystals are those that were formed at the elevated crystallization temperature under agitation. These are surrounded by a more diffuse secondary crystalline structure, which undoubtedly was formed as the slurry cooled under stagnant conditions to molding temperature of 10°C. The interaction between these crystalline structures, and the liquid matrix in which they are contained, gives rise to the characteristic mechanical properties of that semi-solid material. For comparison, the DMA frequency scan for the samples crystallized at different agitation rates is shown in Figure 2. These clearly show that crystalline structure influences mechanical properties (elastic modulus) even though all of the samples had identical solid fat content at 10°C. Note that elastic modulus (e') represents the solid-like nature of the semi-solid material. Higher elastic modulus generally correlates with harder, more solid-like materials.

The results to date may be summarized as follows. Increasing RPM gives rise to more smaller primary crystals and this leads to a decrease in elastic modulus. Rapid cooling also led to higher numbers of smaller primary particles and this also resulted in higher elastic modulus, although differences were fairly small. The lower crystallization temperature (25°C) led to smaller primary crystal sizes, which, again, led to lower elastic modulus. In contrast, addition of higher levels of high-melting milkfat fraction (up to 50%) resulted in more, larger and more dense primary crystals. This led to higher elastic modulus; however, the higher level of high-melting fraction also meant higher solid fat content.

Similar studies are currently underway on mixtures of high-melting milkfat fraction and canola oil. We are also working on techniques to quantify the crystalline microstructure from the confocal microscope images for correlation with the mechanical properties. Other researchers are currently promoting use of a fractal dimension to characterize the irregularity of the crystalline structure. They have found that the fractal dimension measured from polarized light microscope images is identical to the fractal dimension found from mechanical property analysis. Thus, there appears to be some correlation between crystalline microstructure and mechanical properties through the fractal dimension. However, we are exploring other measures from the confocal microscope images (primary particle size and density, ratio of primary to secondary crystals, surface structures, etc.) for correlation with mechanical properties.

Figure 2



FINAL REPORT

Mechanisms of milkfat nucleation

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

Funding
Wisconsin Milk Marketing Board UW 9503

Dates
June 1995—June 1998

Objectives

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

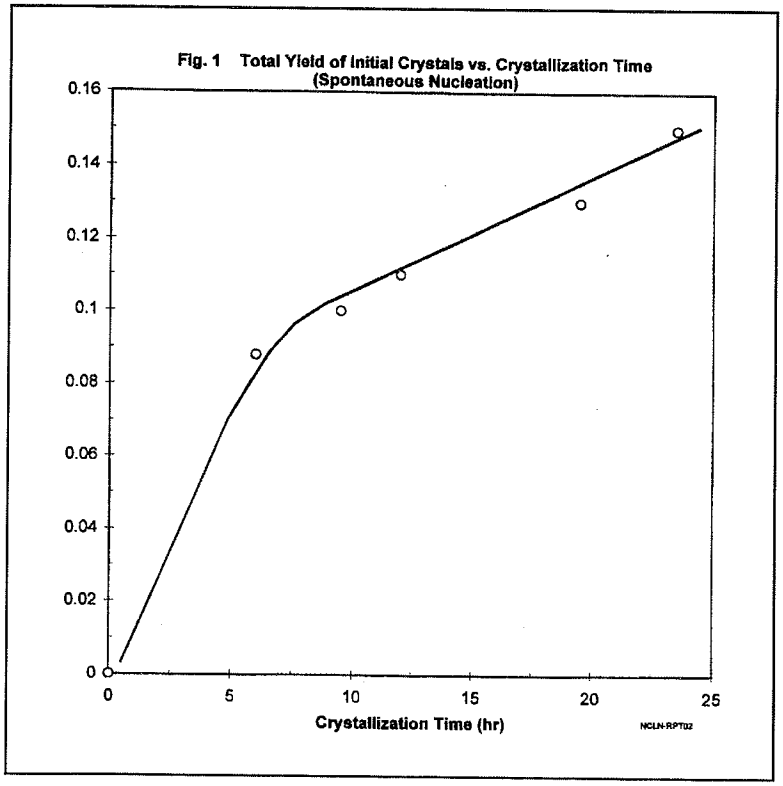
2. Determine the effects of milkfat composition, agitation rate and temperature on kinetics of milkfat nucleation in melt crystallization.

Summary

Two anhydrous milkfats (AMF) were used in this study. One was obtained during the winter feeding season (WAMF) and the other during the summer feeding season (SAMF). These milkfats had slightly different melting (clear) points: WAMF - 35.0°C and SAMF - 36.1°C.

The AMF was melted at high temperature (80°C) for at least one hour to destroy any crystal memory. The melt was then cooled statically to nucleation temperature, where either spontaneous or induced nucleation occurred. For spontaneous nucleation, the milkfat was left undisturbed until nuclei appeared. For induced nucleation, the metastable melt was agitated intensely for a brief period of time. After nucleation, the temperature of the melt was raised to allow crystal growth (incubation temperature), either statically or dynamically (with stirring). Solid fractions were collected periodically during the process by vacuum filtration of the slurry through Whatman #4 filter paper. All experiments were performed in a constant temperature and vibration free (walk-in) chamber constructed specifically for this study.

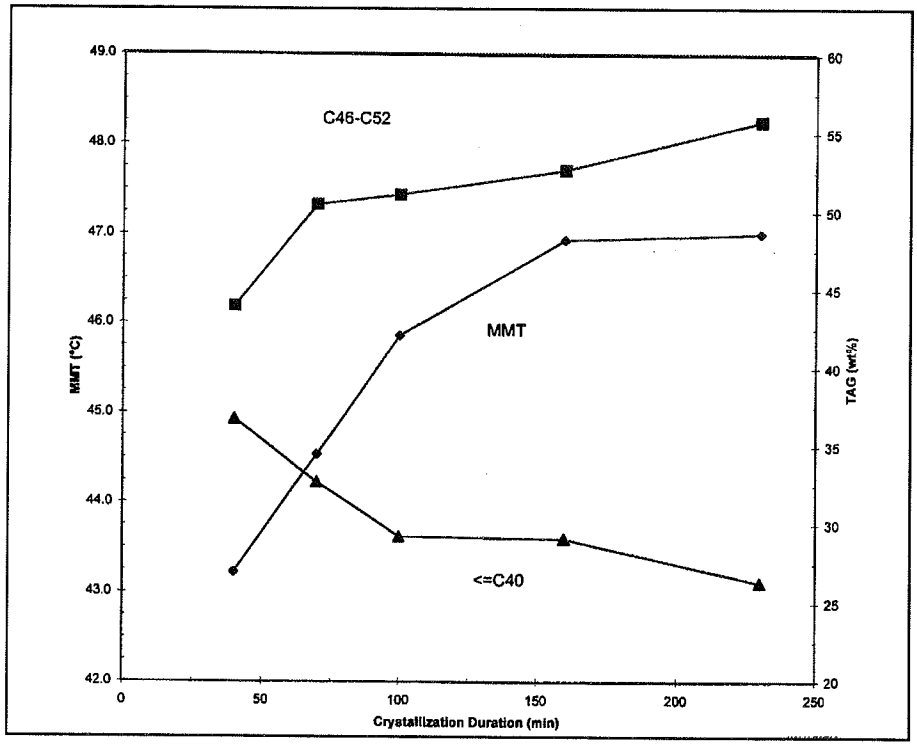
The weight of the solid fraction on the filter paper gave the yield of fractionation at that point in the crystallization process. Each solid fraction was analyzed for melting profile (DSC) and chemical composition (fatty acid and acyl carbon profiles by GC). The number of nuclei formed per unit volume was measured by using optical microscopy. A known volume of the slurry was placed into a chamber on a slide and all crystals in this volume counted. To obtain nucleation rate, the rate of change in number of nuclei with time was calculated.



Variable parameters were studied as follows: nucleation temperature (26 - 34°C); agitation intensity and duration at nucleation temperature; incubation temperature (28 - 35°C). To characterize the agitation intensity, a Reynolds number based on the impeller diameter was calculated.

Significant differences in both nucleation rates and crystalline characteristics were observed when nucleation was allowed to occur either spontaneously (no agitation) or induced (with agitation). Under stagnant

Figure 4
Change of TAG content and MMT of solid fraction with cystallization duration



content of TAG with acyl carbon number less than 40. The ratio of acyl carbon numbers between C46-C52 to those less than C40 in the nuclei increased with time of crystallization. During the crystallization process, the ratio (C46-C52)/(<C40) increased from 1.20 after about 40 minutes of crystallization to about 2.12 after 4 hours of crystallization.

Based on these results, we have hypothesized a mechanism for nucleation of milkfat when induced from the subcooled liquid. Cooling the AMF below its melting point sets up a metastable, supersaturated solution depending on the nature of the fat and the temperature. When the metastable melt is agitated for a brief period of time, what appears to be a nematic phase, resembling a liquid crystal, appears. Although the milkfat melt still appears to be clear, the molecules are orienting in a liquid crystal-like state. After sufficient time, crystalline units begin to appear although the melt is still generally clear. Nucleation occurs when these embryos begin to transform into nuclei with true crystalline structure. These nuclei continue to grow by incorporating the embryonic crystalline units in the surrounding vicinity until the units are fully consumed and crystallization ceases.

Many factors can influence nucleation and crystallization of milkfat. The factors that influence the type and amount of crystalline units formed at the induction point include the composition of the milkfat, time and temperature of the milkfat in the metastable zone, the extent and intensity of agitation while in the metastable zone. The temperature-time history and agitation rate during the prenucleation period influence the survival and development of these nucleation units. Finally, the melt temperature and agitation rate influence the extent of crystal structure transformation. Of these parameters, the nucleation temperature, agitation intensity and melt temperature for growth play the most important roles in controlling crystallization of milkfat. In order to show the effects of these parameters, experiments were performed holding the time at nucleation temperature constant at 5 minutes and a duration of agitation of 30 s. Nucleation temperature was varied from 26.5 to 36.0°C with incubation temperature varied from 27.5 to 33.0°C for each nucleation temperature. Agitation rate, as defined by an agitation Reynolds number, during the nucleation period was varied from 0 to 400 to determine the effects of agitation on nucleation. Incubation was carried out at stagnant conditions.

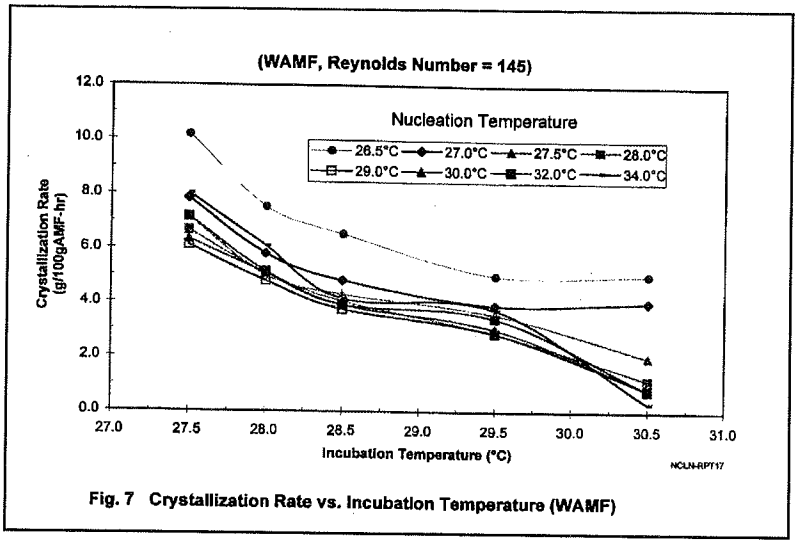


Fig. 7 Crystallization Rate vs. Incubation Temperature (WAMF)

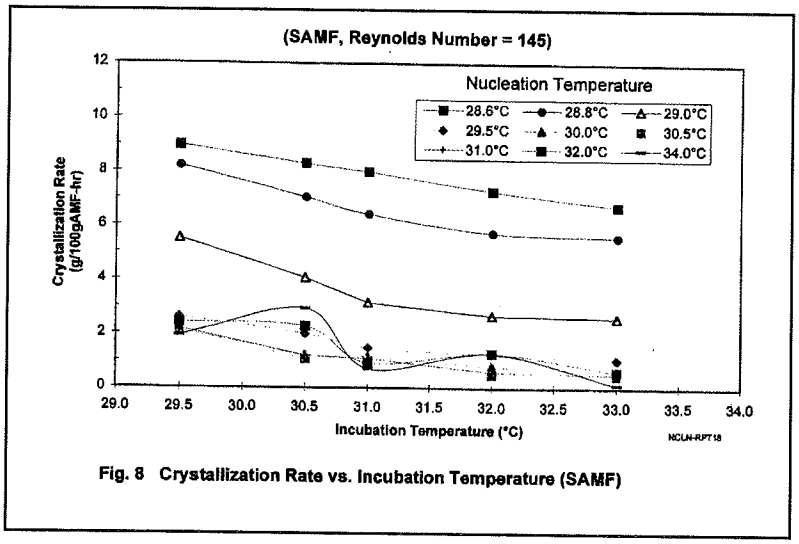


Fig. 8 Crystallization Rate vs. Incubation Temperature (SAMF)

Based on these results, optimal conditions for nucleation and crystal growth of any milkfat sample can be obtained. These principles are described in our patent for a new fractionation technology.

Presentations

Shi, Y., RW Hartel and B. Liang, Kinetic Characteristics of Milkfat Melt Crystallization with Induced Nucleation, poster presentation at AOCS Annual Meeting, Chicago, IL (May, 1998).

Liang, B., Y. Shi and RW Hartel, Fractionation of Anhydrous Milkfat by Use of Efficient Cooling, Nucleation and Crystal Growth Techniques, poster presentation at AOCS Annual Meeting, Chicago, IL (May, 1998).

Shi, Y., B. Liang and RW Hartel, Mechanism and Kinetics of Melt Crystallization of Milkfat, poster presented at Annual AOCS Meeting, Seattle, WA (1997).

Shi, Y., R.W. Hartel and B. Liang, Relationship Between Chemical Composition and Melting Properties of Milkfat Fractions, paper presented at Annual AOCS Meeting, Seattle, WA (1997).

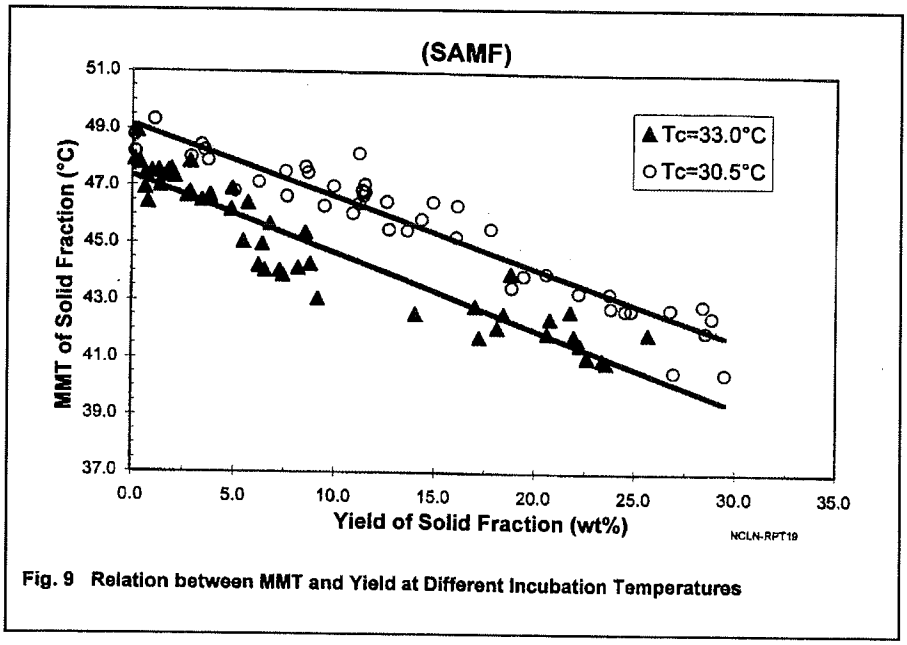


Fig. 9 Relation between MMT and Yield at Different Incubation Temperatures

Table 1

Compostion of neutral lipids in cocoa butter, milkfat and milkfat fractions

Fat	TAG[c]	FA[c]	1,3-DAG[c]	Sterols	1,2-DAG[c]	MAG[c]
ICCB[d]	96.8 ± 0.1	1.8 ± 0.0	1.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	trace
SAMF[d]	98.0 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	trace
WAMF[d]	98.0 ± 0.3	0.7 ± 0.1	0.7 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
VHM[d]	97.6 ± 0.1	1.8 ± 0.1	0.4 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	trace
HM[d]	97.6 ± 0.2	1.6 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0
MM2[d]	97.4 ± 0.2	1.5 ± 0.3	0.7 ± 0.1	0.2 ± 0.0	0.2 ± 0.2	0.1 ± 0.0
MM1[d]	98.0 ± 0.2	0.8 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.1	0.1 ± 0.1
LM[d]	97.8 ± 0.8	1.2 ± 0.6	0.3 ± 0.1	0.2 ± 0.0	0.4 ± 0.3	trace

Table 2

Fatty acid content of combined minor lipids separated from cocoa butter, milkfat and milkfat fractions.

Fatty Acid	ICCB[c]	WAMF[c]	VHM[c]	HM[c]	MM2[c]	MM1[c]	LM[c]
C4:0	0.0	1.9	2.1	2.2	2.3	1.9	2.2
C6:0	0.0	4.0	0.6	0.9	1.0	0.6	1.1
C8:0	0.0	0.8	0.8	0.8	0.8	0.9	1.0
C10:0	0.0	2.1	1.6	2.0	1.7	1.5	1.9
C12:0	0.0	4.4	2.8	7.2	8.2	3.0	3.3
C14:0	10.9	13.2	23.0	23.8	21.7	27.8	16.8
C14:1	0.0	1.6	0.0	0.0	3.2	0.0	3.0
C16:0	29.5	34.4	37.5	33.7	33.3	34.8	33.1
C16:1	9.4	2.6	4.2	4.7	8.9	6.5	7.9
C18:0	25.7	13.3	13.8	11.8	10.7	11.4	10.7
C18:1	24.48	21.67	13.66	12.82	14.91	11.59	19.1

Table 3

Acyl carbon content of minor lipids separated from cocoa butter, milkfat, and milkfat fractions.

Acyl Carbon	ICCB[a]	WAMF[a]	VHM[a]	HM[a]	MM2[a]	MM1[a]	LM[a]
C12	0.2	3.4	1.7	1.4	1.4	1.5	3.4
C18	1.1	4.6	5.5	2.5	4.3	2.9	5.7
C24	22.1	18.5	12.1	11.1	16.6	6.2	22.8
C26	0	2.4	1.8	3.3	3.5	2.2	1.2
C28	0	4.0	6.8	4.4	6.7	1.8	2.6
C30	0	6.1	5.5	8.6	4.8	1.9	5.3
C32	0	8.7	6.8	1.4	0.8	4.7	4.3
C34	1.5	6.0	7.9	8.4	8.3	2.4	0.4
C36	8.2	1.2	3.7	3.0	1.1	5.7	2.4
C38	6.2	0.5	0.6	4.0	2.3	0.8	3.2
C40	0.4	2.5	2.2	0.8	3.4	1.3	1.2
C42	0	1.4	1.5	2.0	2.7	1.8	1.4
C44	0.5	3.9	4.7	4.3	4.9	3.5	1.7
C46	7.7	9.8	7.5	10.6	9.3	8.1	11.6
C48	21.3	13.4	11.0	14.9	12.6	15.8	17.6
C50	22.3	5.8	13.8	14.0	9.7	17.8	8.7
C52	7.6	7.1	5.1	4.0	4.8	17.2	6.0
C54	1.0	0.7	1.9	1.4	1.9	6.9	0.7

a. Acyl carbon values based on the average of three trial

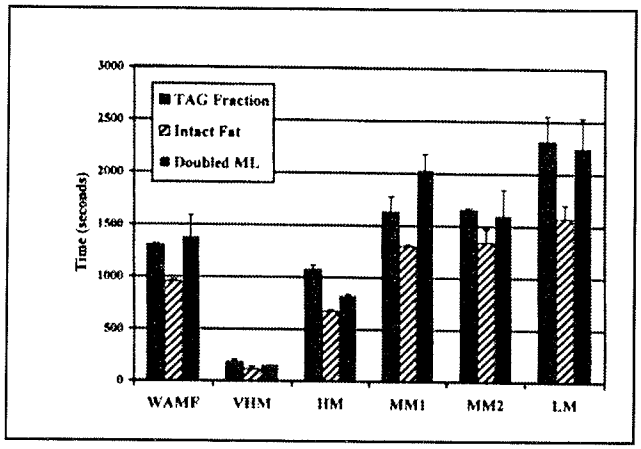


Figure 3
Crystallization induction times for 10% milkfat-cocoa butter blends with varying levels of minor lipids (ML): no milkfat ML (TAG fraction), normal levels of milkfat ML (intact fat), and twice the level of milkfat ML (Doubled ML). WAMF and milkfat fractions; very high melting (VHM), high melting (HM), middle melting1 (MM1), middle melting2 (MM2), and low melting (LM). Samples were crystallized with agitation at 25 °C. Induction time deteremined as the time when absorbance began to increase.

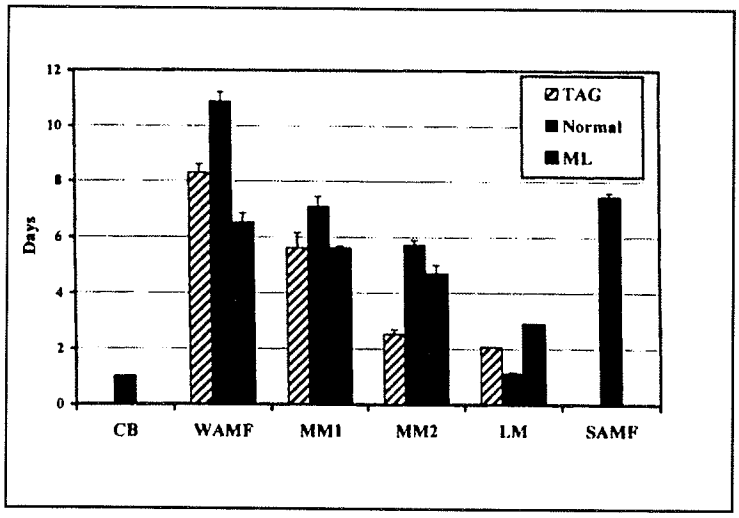


Figure 4
Bloom induction time for chocolates made with 10% milkfat fractions with varying levels of minor lipids (ML). No milkfat ML (TAG), normal levels of ML (Normal) and twice the level of ML (ML).

Cocoa butter (CB), WAMF, SAMF, milkfat fractions:middle melting1 (MM1), middle melting2 (MM2), and low melting (LM). Very high melting and high melting fractions did not show visual bloom after 60 days and were not included.

The crystallization behavior of mixtures of the milkfat fractions (at 10% addition level), with and without the minor lipids, with cocoa butter and palm kernel oil was also studied. In this case too, both removal and addition of twice the level of milkfat minor lipids resulted in a decreased crystallization rate (increased induction time).

In chocolate, milkfat plays an important role in product texture and stability. Of particular importance to the dairy industry is the ability of milkfat and certain milkfat fractions to inhibit fat bloom formation in chocolate. However, a full understanding of the mechanisms by which milkfat fractions inhibit bloom formation in chocolate is still lacking. One hypothesis is that milkfat and milkfat fractions alter the crystalline structure of the cocoa butter, and thereby, influence the migration of liquid cocoa butter to the surface of the chocolate where recrystallization can occur. In particular, the minor lipids of milkfat may play a role in this mechanism. In this study, we investigated the effects of milkfat minor lipids on the crystallization structure of cocoa butter and on subsequent bloom formation in chocolate. Blends of milkfat fractions (10%) containing different levels of minor lipids with cocoa butter were crystallized at 25°C for 1 hour under agitation and then cooled to 10°C to set into a semi-solid product. In all cases, the cocoa butter crystalline structure was composed of dense, primary crystals (formed at 25°C) of relatively large (200-500 μm) size surrounded by a matrix secondary crystalline structure formed during cooling to 10°C. Interestingly, the most compact crystalline structures (both primary and secondary) were formed at the normal levels of minor lipids in the milkfat fractions. Note that the full complement of minor lipids were still present in the cocoa butter. Both removing milkfat minor lipids and adding twice the normal level disrupted both primary and secondary crystalline structure. Even greater disruption of the cocoa butter crystalline structure was seen with the very high-melting milkfat fraction. Similar results were

INTERIM REPORT

Interactions of milkfat fractions in foods – Ice cream

Personnel: R.W. Hartel, professor, R.C. Bradley, Jr., professor, Rachel Adleman, research assistant, Dept. of Food Science

Funding

Wisconsin Milk Marketing Board UW 9604

Dates

June 1997—June 1999

Objectives

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

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

Summary

Milkfat is an important component of ice cream—it influences mouthfeel, taste and texture. Emulsifiers are added to ice cream to interact with fat phase to promote these properties. However, our understanding of the mechanisms that lead to development of proper structure and quality is severely lacking. By providing a better understanding of the mechanisms by which lipid-emulsifier interactions lead to desired structure and quality, the use of milkfat fractions in ice cream can be enhanced.

A variety of ice creams were produced with different milkfat fractions and with different emulsifier combinations. These ice creams were evaluated for the important physical properties during manufacture and after hardening. Parameters measured included:

Development of air cell distribution during freezing

An optical microscope technique was used to measure air bubble distribution. A thin slab of ice cream was placed on a microscope slide. A layer of solvent (kerosene) was placed on top of the ice cream sample along with a cover slide. The ice cream was warmed slowly in a controlled temperature environment to allow the air bubbles to appear. Images of the air bubbles were then analyzed for size distribution using image analysis software.

Extent of fat destabilization during freezing

A standard spectrophometric method was used to follow changes in extent of fat destabilization during freezing. Samples were taken at various times during freezing and diluted prior to measurement of turbidity. Turbidity of the melted ice cream was compared to that of the initial ice cream mix to determine the extent of fat destabilization.

Development of overrun during freezing

Overrun was measured by filling a standardized volume cup with a level of ice cream and weighing the cup. The difference in weight (density) from the ice cream mix allows calculation of the overrun.

Ice crystal size distribution after freezing and during storage

A slab of the ice cream was placed on a microscope slide and a few drops of solvent added. A second slide was placed on top of the sample and force applied to produce a thin section of the ice cream for optical microscopy. Ice crystal size distribution was determined from the images using image analysis software. Once the ice creams were hardened, they were stored at -15°C for several months. Ice crystal size distributions were measured during storage.

Interfacial tension between lipid and serum

The interfacial tension between the serum phase and each of the milkfat fractions with each of the emulsifiers was measured using the Wilhelmy plate method with the lipid at 60°C to ensure that the lipid was melted.

The variables for this experimental design were 4 milkfat components, 3 emulsifiers and 3 emulsifier levels. Milkfats used were anhydrous milkfat (AMF), and high- (HMF), middle- (MMF) and low-melting (LMF) milkfat fractions. Mono- and diglycerides (MAG/DAG), polysorbate 80 (PS80) and a commercial blend (80% MAG/DAG and 20% PS80) were used as emulsifiers. Emulsifiers were used at 0.1, 0.2 and 0.3% addition levels, except for the PS80 which was used at 0.01, 0.02 and 0.03% due to high its high emulsifying activity.

creams made with AMF and the different emulsifiers. Increasing the level of emulsifier caused a delay in the development of overrun during freezing. Interestingly, the changes in overrun correlated quite well with air cell size. In general, as overrun increased, the mean air cell size decreased. When overrun began to decrease due to overwhipping, the mean air cell size began to increase (Figure 3). Apparently, overwhipping resulted in coalescence of air bubbles, leading to larger mean size.

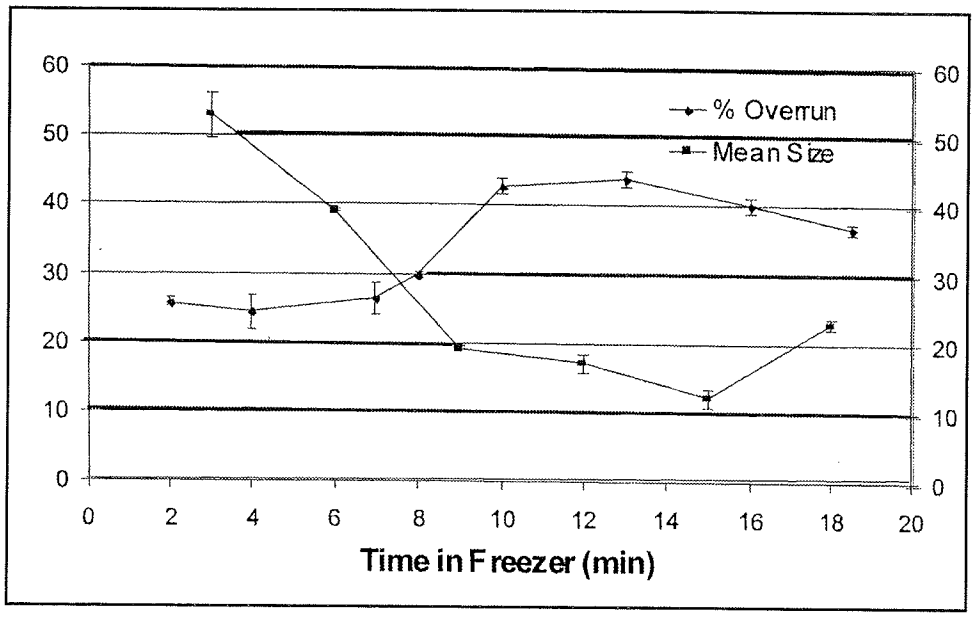


Figure 3
Overrun and mean air cell size vs. time during freezing of ice cream made with low melting milkfat fractions and 0.2% commercial blend.

The extent of destabilization generally increased linearly with time during freezing, as shown in Figure 4 for ice creams made with AMF. The type of fat used played an important role in determining the extent of fat destabilization. Ice creams made with LMF had significantly higher levels of destabilization than those made from any other milkfat component. In fact, the extent of destabilization correlated inversely with melting point of the milkfat component. Apparently, the solid fat content of the lipid during freezing plays an important role in extent of destabilization. That is, a certain amount of solid fat is necessary to promote stability of the air cells as the mix is being frozen.

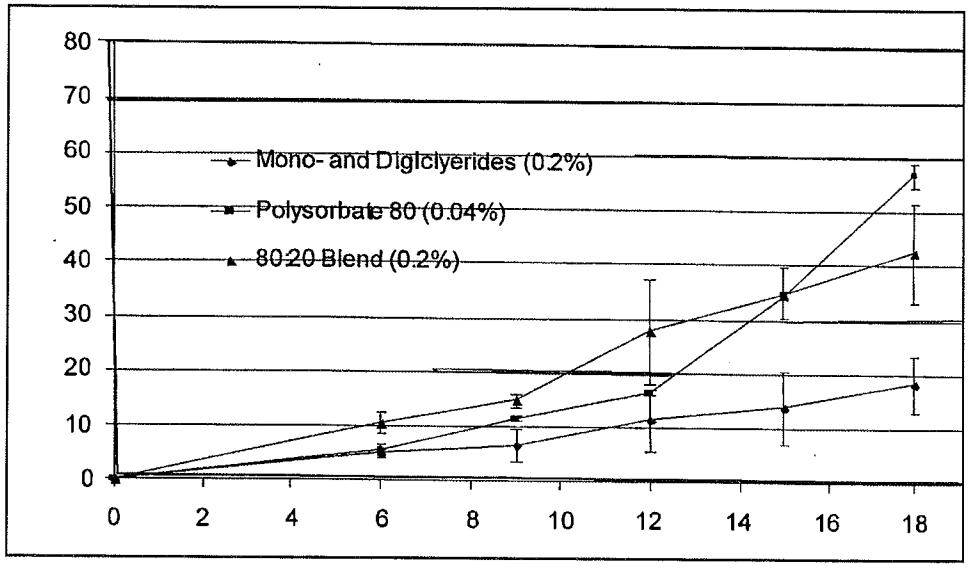


Figure 4
Extent of fat destabilization during freezing of ice cream made with varying emulsifiers and anhydrous milkfat

As expected, the PS80 caused greater destabilization than the MAG/DAG. However, there was a synergistic effect where the commercial blend of MAG/DAG and PS80 generally had the highest level of destabilization. Increasing the level of emulsifier also increased the extent of destabilization, in accordance with the corresponding decrease in interfacial tension.

FINAL 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—July 1998

Objectives

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

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

3. Determine the effect of milkfat source on milkfat crystallization.

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

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

6. Investigate the effects of scale of operation.

Summary

There are many parameters that influence crystallization and filtration of milkfat in a batch, suspension crystallizer. Empirical results over the past 3 decades of milkfat fractionation have documented the variability of milkfat fractionation due to different operating conditions. However, this project is the first to correlate the operating parameters with crystallization and filtration kinetics.

In the first phase of this project, mixing parameters were studied for crystallization at a single temperature. These results have been reported in part in past reports, but will be reported in full here. This is based on the MS thesis of Daniel Patience. The remaining objectives of this project were carried out by a team of graduate and undergraduate researchers. They investigated the effects of different crystallization temperatures, milkfat source and pretreatments on crystallization and filtration kinetics.

Effects of crystallization temperature on milkfat crystallization and crystal separation

Winter anhydrous milkfat (WAMF) was crystallized and fractionated at temperatures from 22.5 to 30°C. Milkfat samples were melted at 60°C and cooled to crystallizer temperature in a time of one hour under agitation conditions (at the optimal condition found for Objective 4). The crystallizer slurry after 24 hours of crystallization was filtered at the same temperature in a pressure filtration rig at 5 bar (gauge). Filtration curves were obtained as the weight of liquid (filtrate) produced per unit time.

In addition, the effects of crystallization temperature on a second stage fractionation were evaluated. The liquid fraction from crystallization at 25°C in the CDR Tirtiaux pilot plant was crystallized at 21, 19 and 17°C to determine the effect of temperature on the second fractionation step. All experiments were performed as described in previous reports (see Objective 4 for more details).

Effects of pretreatment and cooling rate

Several pretreatment steps were evaluated. These included one set of experiments to determine the effect of cooling rate, one set to determine the effect of prefiltration of the milkfat, and one set to determine the effect of holding at 33°C for 1 hour, then filtering, prior to crystallization.

For summer anhydrous milkfat (SAMF) crystallized at 27°C, the effect of cooling rate was studied. For most of the experiments, the milkfat was cooled from 60°C to crystallization temperature in about an hour. In the rapid cooling experiments, the milkfat was cooled to 27°C in under 30 minutes.

Most of these experiments were performed on milkfat that was filtered upon receipt. The milkfat was melted, filtered on coarse filter paper, separated into the desired

liquid portion, so that any color found in the filter cake must come from liquid entrained in the filter cake. We have found this to provide a reasonable estimate of the liquid entrainment. Filtration efficiency is given, so that the amount of liquid entrainment can be calculated as 100-efficiency.

Crystallization kinetics were characterized by plotting geometric mean size of the crystal size distribution (L') as it changed with time. This curve was fitted to an equation of the form:

$$L'(t) = L_s \exp \{ (t - L_D)/t_c \} \qquad (4)$$

The parameters L_s, which represents a steady state mean size, L_D, which represents a lag time for crystal growth and t_c, a growth rate time constant, were determined by fitting data from duplicate experiments to Eq. (4) with a nonlinear regression program.

Effects of processing variables on physical properties and yields of fractions

For each of the fractionation experiments, the yield of solid fraction was calculated from the weight of the filter cake. The percent liquid entrainment was calculated by the method of Evans, based on spectrophotometric studies of yellow color. This assumes that the yellow color is contained entirely in the liquid phase.

Effects of scale of operation

The mixing study of Objective 4 was performed at two different scales of operation (0.6 and 3.6 L).

Effects of crystallization temperature on milkfat crystallization and crystal separation

As expected, decreasing crystallization temperature resulted in substantial changes in crystallization rate of milkfat as measured by spectrophotometry. Decreasing crystallization temperature caused a decrease in induction time for crystallization (onset of nucleation) from 120 min at 30°C to 60 min for 22.5°C. In general, a decrease in crystallization termperature resulted in a linear decrease in induction time. Lower crystallization temperatures also resulted in faster crystallization after nucleation had occurred. As crystallization temperature was lowered, the rate of change of turbidity (equivalent to mass rate of crystallization) increased substantially due to the more rapid rate of nucleation and growth. These differences in crystallization rate resulted in substantial differences in crystal size distribution.

Crystallization temperature also caused substantial changes in product yield, filtration efficency and rate, and amount of liquid entrained in the filter cake (Table 1). As temperature decreased, the solid fraction yield increased from 12.8% at 30°C to 42.4% at 22.5°C. However, a portion of this increase was due to the increased level of liquid entrainment in the filter cake. Filtration resistance, as measured by pressure filtration, generally increased as crystallization temperature increased. One interesting note is that crystallization at 22.5°C resulted in higher fraction yield, but lower liquid entrainment than might be expected. This is unusual, especially since the filtration resistance for crystallization at 22.5°C was the highest of all temperatures, probably because of the higher solid fat content in the slurry.

The fatty acid composition of the resulting solid fractions also generally followed the expected trend (Figure 1). With decreasing crystallization temperature, levels of

Table 1. Effects of crystallization temperature for first stage fractionation.

Temperature (°C)	Liquid Entrainment (%)	Yield (%)	Filtration Efficiency (%)
30	50.9	12.8	50.6
28	51.1	19.8	49.1
27.5	54.4	20.6	49.5
27	65.3	25.7	36.6
25	70.1	32.1	33.3
22.5	45.9	42.4	59.1

Table 4. Effects of prefiltration on fractionation.

Prefiltration	Liquid Entrainment (%)	Yield (%)	Filtration Efficiency (%)
no	90.2	31.8	9.9
yes	45.6	29.7	60.7

Table 5. Effects of holding at 33°C for 1 hour and filtering prior to fractionation.

Hold at 33°C	Liquid Entrainment (%)	Yield (%)	Filtration Efficiency (%)
yes	51.2	19.8	51.2
no	65.0	22.5	35.2

yield associated with higher liquid entrainment and reduced filtration efficiency, as shown in Table 3. These results were due to the formation of a greater number of smaller crystals at the rapid cooling rate. These crystals must have become trapped within the interstitial spaces in the filter cake and reduced liquid flow through the cake. This was born out by the filtration resistance, which was higher in the case of rapid cooling.

The majority of our studies were performed on milkfat that was prefiltered prior to crystallization. Several studies were performed on unfiltered milkfat to determine the effect on fractionation. Although the results for this series were somewhat inconsistent, the averages (of 3 replicates) show that prefiltration helped to decrease the amount of liquid entrainment and increase filtration efficiency (Table 4). These differences occurred even though there was no apparent difference in crystallization kinetics of these AMF samples. Filtration resistance in pressure filtration was also generally not affected by prefiltration. Again, these results may be somewhat unreliable due to the inconsistent nature of the individual results.

One additional series of experiments with prefiltration was performed to assess the effects of removing high-melting components from the milkfat prior to fractionation. To evaluate the influence of these high-melting components (minor lipids or high-melting TAG) on crystallization and filtration, several samples were held at 33°C for one hour and filtered prior to cooling to crystallization temperature. The purified samples had higher induction time (delayed nucleation), 105 min as compared to about 60 min, although subsequent crystallization rate was not affected. Removal of these high-melting components (these may be trisaturated TAG, liquid crystals of minor lipids, etc.) caused an increase in yield, probably due to the increased level of liquid entrainment in the filter cake, and caused the filtration efficiency to decrease (Table 5).

Effects of milkfat source

Significant differences in crystallization and filtation of samples of winter (WAMF) and summer (SAMF) milkfat at 27 and 28°C were found. SAMF, which had slightly lower melting point and solid fat profile, had a slower crystallization rate at 27°C, although the induction time was only slightly lower for the WAMF. Induc-

Table 6. Differences in fractionation between summer and winter milkfats.

AMF	Temperature (°C)	Liquid Entrainment (%)	Yield (%)	Filtration Efficiency (%)
WAMF	27	45.6	29.7	60.7
SAMF	27	65.3	25.7	36.6
WAMF	28	75.7	28.7	24.3
SAMF	28	51.2	19.8	49.1

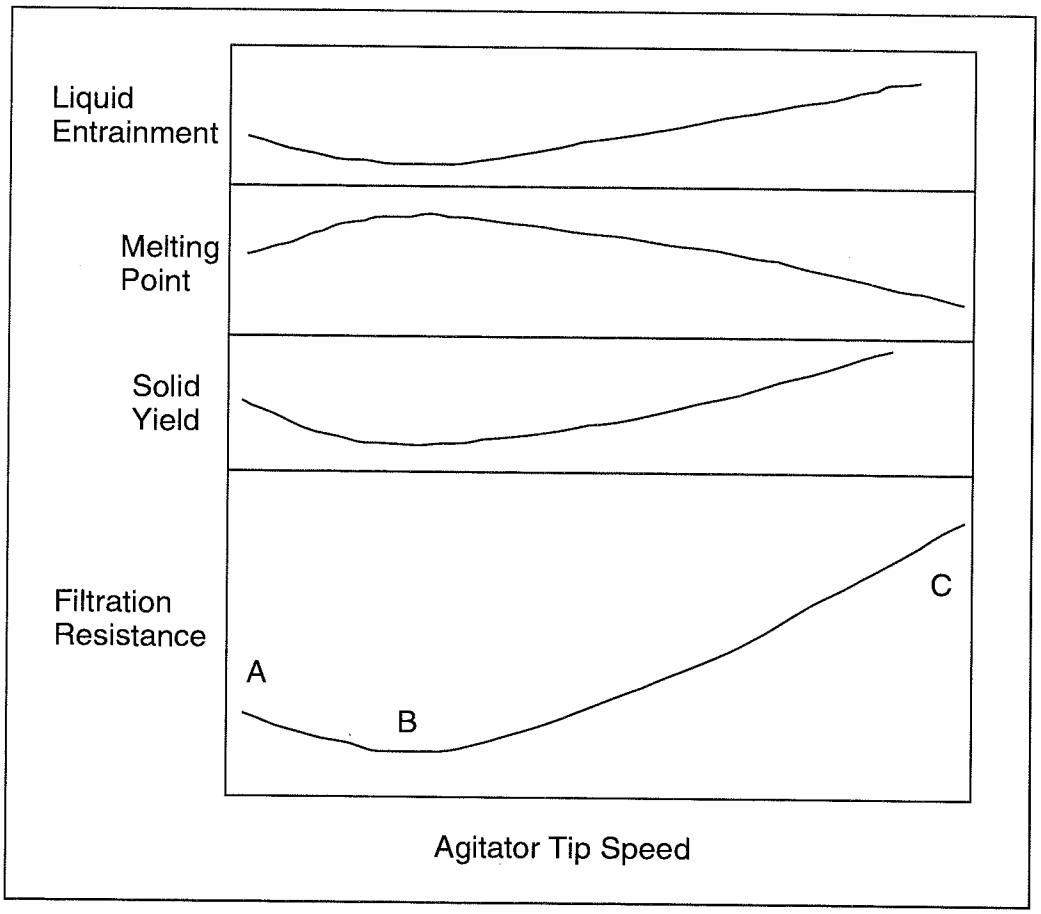
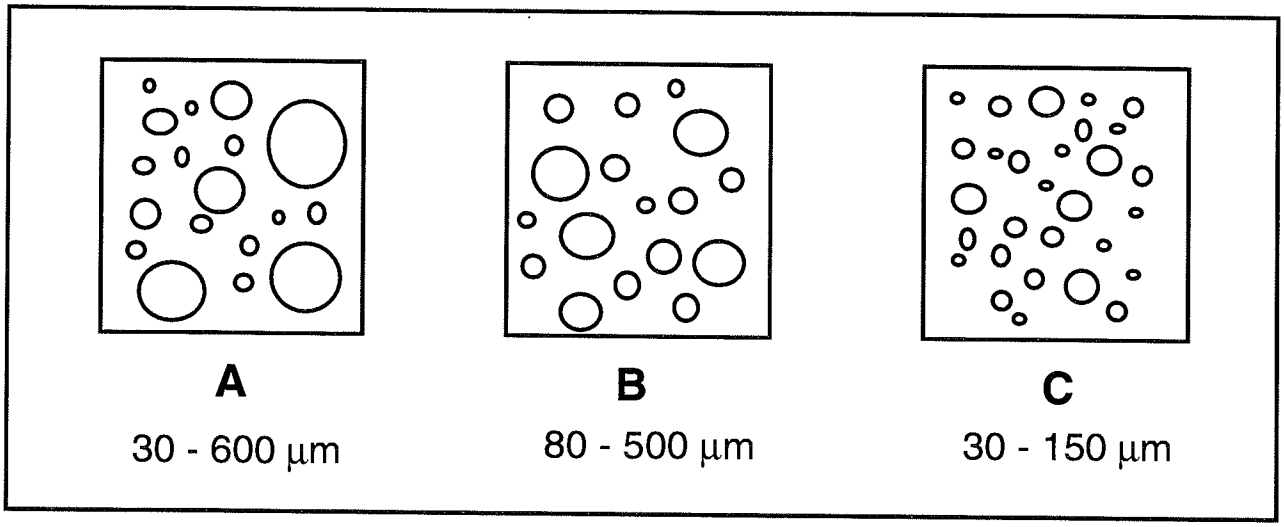


Figure 3
Effect of mixing speed on filtration resistance and physical properties of milkfat.



able to characterize the effect of mixing on crystallization and subsequent filtration.

Figure 3 schematically shows the effect of different agitation rates (characterized by tip speed) on filtration resistance and the characteristics of the size distribution of milkfat crystals. These results suggest that there are optimum operating conditions for this crystallizer that provide the fastest filtration time, with minimum liquid entrainment. At low agitation speeds (N=12.5 RPM), the crystallizer operated under laminar conditions. No settling occurred in these experiments. The milkfat crystals had little contact with the agitator and tended to collide more frequently with other milkfat crystals agglomerating to form large particles. A significant amount of very small crystals were also present.

be formed, and crystal growth kinetics do not correlate with that distribution. This is an area that deserves further attention.

Effects of scale of operation.

For fractionation at 3.5 L, filtration rate increased as scaled tip speed increased in the range of operating conditions studied. At the same time, melting point and filtration efficiency went down while yield of solid fraction went up. These results were slightly different than those found for the 0.6 L scale of crystallization, where a minimum in filtration resistance was found. Apparently, tip speed is not a good scaling factor for sizing crystallizer operations. Further studies on different scales of operation are warranted.

Publications and Presentations

Patience, D.B., R.W. Hartel and D. Illingworth, Crystallization and Pressure Filtration of Anhydrous Milkfat: Mixing Effects, J. AOCS (submitted).

Patience, D.B. and R.W. Hartel, Crystallization Kinetics and Pressure Filtration of Anhydrous Milkfat, paper presented at AOCS Annual Meeting, Indianapolis, IN (1996)

Patience, D.B. and R.W. Hartel, Crystallisation and Pressure Filtration of Anhydrous Milkfat, poster presentation at 13th Symposium on Industrial Crystallization, Toulouse, France (1996)

Illingworth, D. and R.W. Hartel, Crystallization Kinetics of Anhydrous Milkfat, abstract submitted for 1999 International Society of Fats (ISF) meeting, Brighton, UK (1999).

Patience, D.B., Melt Crystallization Kinetics and Pressure Filtration of Anhydrous Milkfat, MS Thesis (1996).

Consortium requests for samples, pilot plant time, and product evaluations were addressed as they were received; although requests this year were minimal. The Consortium will continue on an as needed basis.

The CDR Milkfat Group provides research facilities, data, and technical support to assist both manufacturers and users of milkfat ingredients as the industry begins to commercialize milkfat fractionation in the U.S. Fractionation provides an opportunity to tailor milkfat for specific applications that may benefit from the flavor of milkfat, but where the use of milkfat is hindered by its physical properties. The fractionation of milkfat to produce specialty ingredients will increase the value of milkfat and expand its use in the food industry.

Publications and Presentations

Kaylegian, K.E. Milkfat Applications Laboratory. Invited presentation at the Dairy Management Inc. National Milkfat Technology Forum, Rosemont, IL. February 1998.

Kaylegian, K.E. Lectures and laboratory sections at the CDR Milkfat as a Food Ingredient Short Course, Madison. March 1998.

Kaylegian, K.E. Milkfat Research at the Center for Dairy Research. Invited presentation to the UW Food Science Club, Madison. April 1998.

Kaylegian, K.E. Functional Properties and Applications of Fractionated Dairy Fat. Invited presentation at the American Oil Chemists Society Annual Meeting, Chicago, IL. May, 1998.

Kaylegian, K.E. Properties of Milkfat Fractions. Laboratory demonstration at the UW Applied Dairy Chemistry Short Course, Madison. May 1998.

Kaylegian, K.E. Radio interview for Ag Facts Program by the UW Dept. of Ag Journalism, Madison. October 1998.

Kaylegian, K.E. The Flexible Fat. Dairy Ind. International. Vol. 73, No. 11, p. 24-25. November 1998.

Kaylegian, K.E. Milkfat Applications Research Program. Invited presentation at DMI Symposium, IFT Chicago Supplier's Night, Chicago, IL. November 1998.

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 and Melanie Tudhope, research assistants, Department of Food Science

Funding

Dairy Management, Inc.

Dates

January 1996 —December 1999 (extended date)

Objectives

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

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

Summary

The project has evolved to encompass two critical areas needed to successfully accomplish our objectives and reach a satisfactory conclusion. One area is evaluating the nature of the physiochemical relationships that govern stabilization of oil/water interfaces, focusing on issues specific to using milkfat (and fractions thereof) as the sole lipid component in reduced fat table spreads. The other area involves developing the means to screen and bench-test table spread formulations that will predict the efficacy of processing into a satisfactory product on a pilot scale Gerstenberg & Agger texturizer.

We have developed and standardized analytical and experimental techniques for both project areas. We will use solid fat content, interfacial tension and contact angle analyses to evaluate physicochemical properties. To evaluate formulations for efficacy at the pilot scale, emulsion stability (by a centrifugal technique), color/appearance (reflectance colorimetry), morphology (photomicroscopic) and lipid crystal dynamics (differential scanning calorimetry) will be used to evaluate stability and suitability of bench-top produced dairy emulsions. 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.

Pilot scale trials with similar products have served to establish processing variables (homogenization speed, residence time and temperatures) that will be used for the pilot scale studies.

Relationships between reduction of interfacial surface tension and % MAG included in the oil phase have been established. Compared to other oils used for spreads (as a standard or control sample), non-classical behavior was repeatedly observed when butteroil was used as the lipid phase. We have confirmed the presence of phospholipid components in some of the milkfat fractions and speculate that these phospholipids are responsible for the non-classical behavior. We are currently developing the means to separate/isolate phospholipid impurities to evaluate their effect, both individually and in combination with added MAG. Butteroil-derived MAG preparations are also being prepared from milkfat (and fractions) by an enzymic technique developed previously in our laboratory. This is to determine if MAG derived from butteroil has any unique functionality based on the unusual fatty acid composition in butteroil compared to other common food oils (from which MAG is commercially prepared). Analyses of interfacial tension and contact angle in butteroil/emulsifier mixtures are being evaluated in an attempt to identify formulations that render greatest stabilizing power in emulsions under conditions where variable amounts of liquid/solid lipid exist.

A bench-top apparatus has been adapted to prepare small scale (100-200 ml) emulsions for evaluation of formulation effects and emulsion stability. The apparatus is essentially a scraped-surface heat exchanger with a paddle agitator that simulates the pilot scale texturizer. A formulation of 82.5% 8L fraction and 17.5% 21S fraction (both from butteroil) was found to be a suitable base for the spreads, due to the temperature-solidity profile of this mixture. Initial experiments revealed that using 1-3% (based on the lipid phase) MAG affected distinct morphologies and properties in the finished spreads. The 2% MAG spreads were

superior in stability (centrifugal test) and all had different appearance and morphology. The 2% MAG formulations had the smallest globules for the dispersed oil phase, whereas the 3% MAG formulation had a greater range in droplet size and some of the solid crystals were large spherulites. We are currently evaluating cooling rate (influenced by temperature nadir used) and agitator speed on emulsion/spread properties. In addition, current experiments are designed to provide an understanding on how features of dairy-spread formulations impact quality and stability of the finished product. The most (and least) successful formulations based on bench-scale testing will be tested on the pilot scale apparatus, to confirm the ability of the bench-top procedure for predicting efficacy of processing various table spread formulations into finished products of high quality.

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 increase the use of milkfat. Our approach in this project attempts to optimize the use of butteroil, butteroil fractions and functional derivatives of butteroil in table spread products.

INTERIM REPORT

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

Personnel: Charles G. Hill, Jr., professor of Chemical Engineering; Dr. Hugo S. Garcia, visiting scientist, associate professor, Department of Food Technology, Centro de Graduados, 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.

Dates

July 1997— June 1999

Objectives

1. Generate the experimental data necessary to characterize the rates of 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.

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

3. 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).

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

Summary

This project represents an extension of an earlier project funded in part through the Wisconsin Center for Dairy Research and the National Science Foundation. Our efforts have focused on using 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 SKW Biosystems. 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 that differ in fatty acid composition 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), capric (C10) and longer-chain fatty acid residues. None of these enzymes released significant amounts of intermediate length or long chain fatty acids. While lamb and kid lipases give high ratios for the C4 to C6 fatty acids released by lipolysis, the 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 utilized as indicators of soaplike (undesirable) flavors. In studies with an immobilized kid goat lipase we employed variations in 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. Variation of the pH and temperature at which the reactor operates permits additional manipulation of the chemical composition (and hence the apparent flavor notes) of the product mixture. The results obtained to date clearly demonstrate that our immobilized pregastric esterase reactor can permit one to tailor the product composition for specific applications via proper selection of operating conditions and the source of the enzyme.

Much of our effort in recent months has focused on preparation of manuscripts and theses to thoroughly document the experimental and modeling work carried out by participants in the project.

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

This research project is intended to establish a rational scientific basis for employing immobilized enzyme technology to manufacture lipolyzed dairy products with specified free fatty acid profiles and unique sensory and functional characteristics. This research has direct implications with respect to the production of lipolyzed dairy products which find applications as flavoring agents within the food industry.

Publications/presentations

"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., poster presented at the 1998 national meeting of the Institute of Food Technologists, June 24-28.

"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 Charles G. Hill, Jr., paper presented at the 1998 national meeting of the Institute of Food Technologists, June 24-28.

"Product Inhibition in the Hydrolysis of Anhydrous Milkfat over an Immobilized Calf Pregastric Esterase," by L.P. Lessard and C.G. Hill, Jr., paper presented at the 1998 national meeting of the American Institute of Chemical Engineers, November 15-20.

"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., *Biotechnology Letters,* 20, 403-405.

"Enzymatic Synthesis and Hydrolysis Reactions of Acylglycerols in Solvent-Free Systems," by C. Otero, J. A. Arcos, H. S. Garcia, and C. G. Hill, Jr., invited manuscript submitted for publication in the next volume of the series of books entitled *Methods in Biotechnology.*

INTERIM REPORT

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

Personnel: Charles G. Hill, Jr., professor 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; Kurt Keough, undergraduate, Department of Chemical Engineering.

Funding

Dairy Management Inc.

Dates

July 1996—June 1999

Objectives

1. 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.

2. 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.

3. 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

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. We have completed the experimental component of this phase of the research and submitted a manuscript describing the results of our experiments to *Biotechnology and Bioengineering*. This manuscript focuses on the dependence of the total quantities of fatty acids released under various experimental conditions. A subsequent manuscript will focus on the quantities of the various individual fatty acids released by hydrolysis over the same range of conditions.

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 significant improvement in the yield of the desired biologically active cis-9, trans-11 isomer of CLA. Work in this area is continuing with a view towards obtaining further improvements in the yield of the bioactive isomer. This phase of the research will be followed by studies (with concomitant economic

implications) involving immobilized cells. A paper describing the experimental work was presented at the national meeting of the American Institute of Chemical Engineers in November of 1998. In addition we have fabricated two chemostats that can be operated either independently or in a series flow configuration. These units will be employed in studies of the kinetics of cell growth and CLA production. Preliminary studies have been completed, and we anticipate extensive use of this apparatus in coming months.

Studies of the kinetics of the reactions of CLA with glycerol and butterfat in the presence of immobilized lipases in order to determine the reaction conditions and types of reactors that will be most useful for commercial implementation of this technology. We have persuasively demonstrated the technical feasibility of both routes for obtaining glycerides that can be incorporated in any dairy product that contains milkfat. This work is continuing, but publications in archival journals and presentations at professional society meetings are being used to disseminate the results that we have obtained to date.

Publications/presentations

"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., poster presented at the 1998 national meeting of the Institute of Food Technologists, June 24-28.

"Kinetics of the Lipase-Catalyzed Esterification of Glycerol with Conjugated Linoleic Acid," by J. A. Arcos and C. G. Hill, Jr., paper presented at the 1998 national meeting of the American Institute of Chemical Engineers, November 15-20.

"Increasing Production of Conjugated Linoleic Acid from Linoleic Acid Using Free Bacterial Cells," by C. Crowley and C. G. Hill, Jr., paper presented at the 1998 national meeting of the American Institute of Chemical Engineers, November 15-20.

"Immobilized Enzyme Technology for the Production of a Food Grade Linoleic Acid Feedstock from Corn Oil," by P. S. Sehanputri and C. G. Hill, Jr., paper presented at the 1998 national meeting of the American Institute of Chemical Engineers, November 15-20.

"Enzymatic Synthesis of Glycerides Enriched in Conjugated Linoleic Acid: Batch and Packed Bed Reactor Studies," by J. A. Arcos and C. G. Hill, Jr., accepted for presentation at the 1999 annual meeting of the American Oil Chemists' Society.

"Production of a Food-Grade Linoleic Acid via Hydrolysis of Corn Oil in a Hollow Fiber Reactor Containing an Immobilized Lipase," by P. S. Sehanputri and C. G. Hill, Jr., submitted for presentation at the 1999 annual meeting of the Institute of Food Technologists.

"Enrichment of Butteroil in Conjugated Linoleic Acid Residues in a Continuous Flow Reactor Containing an Immobilized Lipase." By H. S. Garcia, K. J. Keough, J. A. Arcos, and C. G. Hill, Jr., submitted for presentation at the 1999 annual 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., *Biotechnology Letters*, 20, 393-395 (1998).

"Rapid Enzymatic Production of Acylglycerols from Conjugated Linoleic Acid and Glycerol in a Solvent-Free System," by J. A. Arcos, C. Otero, and C. G. Hill, Jr., *Biotechnology Letters*, 20, 617-621 (1998).

"Biotechnology for the Production of Nutraceuticals Enriched in Conjugated Linoleic Acid: 1. Uniresponse Kinetics of the Hydrolysis of Corn Oil by a *Pseudomanas sp* Lipase Immobilized in a Hollow Fiber Reactor," by P. S. Sehanputri and C. G. Hill, Jr., submitted to *Biotechnology and Bioengineering.*

"Enzymatic Synthesis and Hydrolysis Reactions of Acylglycerols in Solvent-Free Systems," by C. Otero, J. A. Arcos, H. S. Garcia, and C. G. Hill, Jr., invited manuscript submitted for publication in the next volume of the series of books entitled *Methods in Biotechnology.*

INTERIM REPORT

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

Personnel: Denise M. Ney, professor, Dept of Nutritional Sciences

Funding

Wisconsin Milk Marketing Board UW9606

Dates

July 1996—December 1999

Objectives

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

2. 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)

Summary

The Center for Dairy Research provided 5 kg of a very low melting milkfat fraction (dropping point < 10° C) in August 1998. This fraction contains approximately 10% of fatty acids with less than or equal to 10 carbon atoms. This liquid milkfat fraction is being used to conduct a feeding study to compare the apparent lipid digestibility of diets containing corn oil, liquid milkfat, intact milkfat and medium chain triacylglycerols in rats.

We published a paper which demonstrates that gastric digestion of milkfat modifies lipid absorption in rats. Previous studies using intestinal infusion of milkfat have demonstrated lower apparent efficiency of absorption of milkfat compared to unsaturated fats. Our data indicate that overall digestion and absorption of saturated fatty acids from milkfat may be underestimated due to the artifact introduced by intestinal infusion in previous studies.

Publications

Hui-Chuan Lai and Denise M. Ney. Gastric digestion modifies absorption of butterfat into lymph chylomicrons in rats. J. Nutr. 128:2403-2410, 1998.

chapter 2

Cheese

INTERIM REPORT

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

Personnel: J. Russell Bishop, director, Center for Dairy Research, 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

1. Determine the effective dosage level of natamycin on shredded cheese to prevent the growth of *Penicillium roqueforti* and *Aspergillus niger*

2. Investigate the antimycotic effects of a 2% cellulose treatment on shredded cheese when used with natamycin.

3. Determine if natamycin breaks-down on cheese surfaces over time under modified atmosphere and opened packaged conditions.

4. Establish a procedure for esterification of natamycin.

5. Compare the solubility of natamycin and natamycin methyl ester.

6. Determine the effective dosage level of natamycin methyl ester on shredded cheese in preventing the growth of *Penicillium roqueforti* and *Aspergillus niger* and compare that to the native antibiotic.

7. Establish a procedure for quantifying natamycin methyl ester content on cheese surfaces.

Summary

Natamycin has been used as a food preservative since the early 1960's and is the most widely used antimycotic agent in cheese production. Although the application and usage of natamycin is great, the research concerning this antibiotic is limited. A substantial amount of research has been performed on the mode of action for polyene macrolide antibiotics, the antibiotic family for which natamycin belongs, but little research has been conducted on how to improve the efficacy or broaden the applications of these chemical constituents. It is the objectives of this project to better understand effective dosage levels of natamycin for common spoilage organisms and investigate the way in which this antibiotic is affected by the addition of cellulose and storage time in modified atmosphere and opened packaged conditions. In addition, the effect of natamycin's solubility will be investigated along with the effects of a natamycin methyl ester (ME) derivative.

In order to test the efficacy of natamycin on common spoilage organisms in cheese production it was necessary to investigate which organisms are the most problematic. It was found that the genus *Penicillium* accounts for approximately 87% of all cheese spoilage. Of the common *Penicillium* species, *Penicillium roqueforti* is the most resistant and thus was chosen to be an indicator organism when testing natamycin dosage levels. It was also found that the genus *Aspergillus* accounts for 5-7% of cheese spoilage. The species *Aspergillus niger* is easily the dominant strain for spoilage. Therefore, *Aspergillus niger* was chosen as a second indicator organism.

The first step we took was to obtain pure, well identified strains of these indicator organisms. Previous researchers at Virginia Tech had used these two specific strains and kept cultures cryogenically stored at -80°F. *Penicillium roqueforti* and *Aspergillus niger* cultures were rehydrated and grown on Czapek Solution Agar (Difco Laboratories, Detroit, Michigan). The morphology of these cultures were identified and examined for purity under magnification. Both cultures were identified as viable and pure.

The next step was to harvest and lyophilize *Penicillium roqueforti* and *Aspergillus niger* spores for use when inoculating shredded cheese. The organisms were cultured in Malt Extract Broth (Difco Laboratories, Detroit, Michigan) and then plated on Malt Extract Agar (Difco Laboratories, Detroit, Michigan). Conidia and

ascospores were harvested by flushing each plate with 10 ml of sterile phosphate/magnesium chloride (PMC) + 1% Tween 80 dilution water and gently rubbing the surface with a sterile bent glass rod.

The suspensions were pipetted into sterile test tubes. Spore suspensions were vortexed and then decanted through sterile glass wool into sterile centrifuge tubes. Tubes were centrifuged on low speed for 10 min and allowed to stop without breaking. The PMC + Tween 80 dilution water was decanted and spores were resuspended in 10 ml of Malt Extract Broth (Difco Laboratories, Detroit, Michigan). The spore suspensions were then vortexed and transferred into sterile 125 ml flasks with stir bars. The combined spore suspensions were mixed and pipetted into 2.0 ml cryogenic screw-cap tubes. Tubes were frozen under liquid nitrogen for 24 hrs, then placed in a subzero storage unit. Spores were lyophilized through sublimation in a freeze dryer for 24 hrs. Spore load will be determined on a per gram basis by surface plating multiple samples on Dichloran Rose Bengal Chloramphenicol (DRBC) Oxoid Agar. DRBC Agar was chosen for its superior enumeration qualities of yeasts and molds associated with food spoilage.

International Dairy Federation (IDF) procedure 140A:1992, Determination of Natamycin Content on Cheese and Cheese Rind by Molecular Absorption Spectrometry and by High-Performance Liquid Chromatography, was investigated to see if we could test the natamycin content on cellulose added to cheese. Determination of natamycin content on a commercially available cellulose added shredded cheese was performed using the molecular absorption spectrometry method. Results showed that cellulose added products do not interfere with this procedure. Natamycin content on cheese will be determined using molecular absorption spectrometry method due to its greater accuracy and precision when compared to the HPLC method.

The other phase of this project is the development of a natamycin ME derivative. Natamycin belongs to the polyene macrolide antibiotic family which includes amphotericin B and nystatin, commonly used in the medical field to treat skin mycoses and systemic infections. Medical research shows that polyene antibiotic methyl ester derivatives are water-soluble, exhibit good stability, and are less toxic and show greater activity then their parent antibiotic. Therefore, Virginia Tech wants to explore the antimycotic efficacy of natamycin in its methyl ester form.

The procedure for the esterification of natamycin is an adaptation of a method the U.S. Environmental Protection Agency employs for methyl esterification of acidic herbicides and other related compounds. These environmental samples must undergo methyl esterification for analysis by gas chromatography. This procedure involves the base-catalyzed decomposition of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Diazald®) in an Aldrich(MNNG diazomethane generation apparatus. The types and quantities of some reagents have been changed to account for specific solubility and chemical behavior of natamycin. The adapted procedure will be tested in the near future.

The esterification of the carboxylic group of natamycin will be confirmed by NMR spectra in dimethyl sulfoxide-d6. Once a reference sample is established, subsequent natamycin ME derivatizations will be confirmed by TLC silica gel.

Finally, the experimental designs for determining effective dosage level of natamycin and natamycin ME in preventing the growth of *Penicillium roqueforti* and *Aspergillus niger*, determining the antimycotic effects of a 2% cellulose treatment when used in conjunction with natamycin and natamycin ME, and determining if natamycin and natamycin ME break-down on cheese surfaces over time under modified atmosphere and opened packaged conditions has been established. Pilot plant trials will begin in September.

In conclusion, this project will provide additional information on the antimycotic effectiveness of natamycin and a natamycin ME derivative. By better understanding the effect of natamycin and its methyl ester derivative on common spoilage organisms, it will enable food scientists to explore additional uses of natamycin in a multitude of food systems. It is the belief of these researchers that the inhibitory spoilage action of natamycin can be expanded to commodities other than cheese.

FINAL REPORT

High moisture, shelf-stable grated cheese processing and packaging

Personnel: Joseph E. Marcy, associate professor, Food Science and Technology, Virginia Tech, J. Russell Bishop, director, Mark Johnson, senior scientist, Center for Dairy Research

Funding

Dairy Management Inc.

Dates

January 1996—December 1997

Objective

1. Develop a commercially acceptable procedure to produce a Parmesan cheese with an a_w = .86 which retains the desirable aroma components and functional properties removed during conventional processing and drying.

Summary

Under current production procedures, Parmesan cheese is produced with an a_w=.90 and then dried to a final a_w=.75 to produce a shelf-stable cheese. While this does produce a stable product it also removes the desirable aroma components of fresh grated Parmesan and adversely affects the functional properties of the cheese.

In this study, Parmesan cheese aged 5, 9, and 12 months was dried using an alternative drying method to produce a high quality, shelf-stable Parmesan cheese. Dehydration was achieved by placing cheese in an airtight anaerobic chamber supplied with a constant flow or nitrogen and stored at 45° F. Over a period of 6-12 weeks 10.7% moisture by weight was removed, reducing water activity to 0.86. In subsequent drying periods the addition of drie-rite to the system allowed for equivalent moisture removal in 2-3 weeks.

Sensory evaluation using a simple preference test was performed to determine potential consumer acceptance of the three ages of alternatively dried Parmesan when compared to commercially available Kraft Parmesan ($p< 0.05$). Alternatively dried Parmesan, aged 5 and 11 months, did not show significant preference. Alternatively dried Parmesan was challenged with *Staphylococcus aureus* under modified atmosphere conditions over an eight-week period to ensure shelf stability. Age did not have a significant effect ($p>0.05$) on the growth of *S. aureus*. Time had a significant effect ($p<0.05$) on the growth of *S. aureus*. Headspace gas analyses indicate sufficient oxygen barrier properties of the packaging materials with less than 2% oxygen ingress over eight weeks.

A high moisture, shelf-stable grated cheese has several distinct benefits to the cheese industry. First, a cheese with the full flavor and aroma for fresh grated Parmesan (or other hard grated cheese) will be available in shelf-stable form. Consumers have increasingly shown a preference for full flavor cheese as shown by the rapid increase in the sale of refrigerated MAP shredded cheeses.

Because the proposed new processing method will produce a cheese without drying, a process step has been eliminated in the cheese making operation. Not only does this eliminate one part of the process, it greatly increased the yield, by allowing the cheese manufacturer to sell a cheese with higher moisture content.

High moisture, shelf-stable cheese also affords new use opportunities for hard grated cheese thereby increasing the total sales of hard grated cheeses. This is particularly true for institutional uses for an ingredient. Because the cheese has not been dried with hot air, the expected result is improved functional properties of cheese made by the proposed method.

APPLICATIONS PROGRAM REPORT

Cheese Applications Program

Personnel: Carol Chen, researcher, Amy Dikkeboom, research specialist, Bill Hoesly, research cheese maker, Kristen Houck, research specialist, John Jaeggi, associate researcher, Mark Johnson, senior scientist, Juan Romero, associate researcher, William Tricomi, assistant researcher, Matt Zimbric, research specialist

Funding

Wisconsin Milk Marketing Board UWA9901-2

Dates

January 1998—December 1998

Objectives

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

2. Conduct industry directed cheese applications research; modifying manufacturing processes or ingredients during cheese making to produce a functionally specific cheese.

3. Direct contact with industry to meet informational needs.

Summary

In 1998, the Cheese Applications program worked with 62 Wisconsin companies: 43 cheese manufacturers, 9 ingredient suppliers, 4 organizations, 2 contract laboratories, 2 end users and 2 equipment manufacturers. We have manufactured cheese for research, visited cheese manufacturers and provided technical information concerning commodity and specialty cheeses. The large number of interactions demonstrates the commitment between the Wisconsin Center for Dairy Research and the Wisconsin cheese industry.

Our most rewarding experiences have been in the commercial production of CDR developed technologies. We have provided the technology and assisted in the scale-up of reduced-fat Cheddar cheese for a large Cheddar cheese manufacturer. Over the past 2 years, the CDR has been working with a northern Wisconsin agricultural Co-op to develop yogurt cheese and fruit flavored yogurt cheeses. In 1998, CDR personnel assisted in the scale-up of these cheeses, which are now commercially available. We have also worked with a multi-regional cheese manufacturer on the affects of milk storage on the physical properties of low moisture, part-skim Mozzarella cheese. We are currently working with two manufacturers on the scale-up of CDR pizza cheese. This work has been challenging, as we need to customize the CDR Pizza manufacturing process to meet the needs of the end user's specifications (machinability, cooking conditions, and end user preferences).

The Cheese Applications program works directly with the cheese industry as a technical resource. We provide assistance for tailor manufacturing of cheese for specific end uses, general cheese technology questions, and cheese quality issues. A significant portion of our work also involves cheese meltability. This involves defining how to measure cheese meltability, setting specifications, and eventually manufacturing a cheese with the desired melt characteristics. We also assist the cheese industry by addressing general cheese technology issues, cheese process control, cheese defects, milk standardization or cheese yield.

A major portion of our research cheese making involved evaluation of starter cultures systems and/or starter adjuncts. The starter systems / adjuncts were used in the production of Cheddar, Mozzarella or cottage cheese, and their effect on the manufacturing process was evaluated. Resulting physical and sensory properties of the cheese were also correlated.

Publications and Presentations

Members of the Cheese Applications Program team provided technical information at several national and regional meetings or conferences. The staff plays an important role in the Cheese Technology Short Course held in March and October. Throughout the year, the CDR provides tours for various journalists, councils, academia and industry groups. Pizza cheese and /or

reduced-fat Cheddar technology has been shown at 4 of these tours.

"Improving the Functionality of Lower fat Cheese." by Carol Chen at the American Dairy Products Institute Annual Meeting, Chicago, IL. April 28, 1998.

"Cheese as a Food Ingredient" by Amy Dikkeboom for Herb Kohl and staff. June 2, 1998.

"Manufacturing skim milk Mozzarella cheese." by Matt Zimbric, Carol Chen, Amy Dikkeboom, John Jaeggi, Mark Johnson and Bill Tricomi at the 1998 Annual ADSA Meeting, Denver, CO. July 1998.

"Cheese as a Food Ingredient: Partnership between the Wisconsin Center for Dairy Research and the Cheese Industry," by Carol Chen at the 35th Annual Marschall's Italian and Specialty Cheese Seminar, Madison, WI. September 17, 1998.

"Cheese making possibilities and potential impacts." by Mark Johnson at the Impact of CODEX on Cheese making Conference, Madison, WI. October 28, 1998

"The Wisconsin Center for Dairy Research Cheese Applications Program." by Carol Chen at the Using Cheese as a Food Ingredient Conference, Madison, WI. October 29, 1998.

"The Science of Cheese making." by Mark Johnson at the Using Cheese as a Food Ingredient Conference, Madison, WI. October 29, 1998.

"Cheese making Solutions: Wisconsin Center for Dairy Research make the science of cheese making easier" by Rachael Robinson, Dairy Field Magazine. November 1998.

(21°C). Statistical significance of the data was tested by a multifactor analysis of variance.

Cheese making in the CDR pilot plant	• Worked with 12 companies (6 manufacturers, 6 ingredient suppliers) Forty-eight cheese making dates or 171 vats/batches of cheeses (20% manufactures, 80% ingredient suppliers) • Manufactured a wide variety of cheeses: Cheddar, Lower fat Cheddar, LMPS Mozzarella, Lower fat Mozzarella, Muenster, Cottage, Feta, Pro cessed, and Parmesan cheeses
Applications or analytical testing	• Worked with 14 companies (7 manufacturers, 5 ingredient suppliers, 1 equipment manufacturer, 1 organization) • Applications work included evaluation of cheese physical properties: melt, stretch, slice, browning. Evaluation of cheese under different cooking conditions (forced air ovens, convection ovens, microwave ovens, deep frying) • Chemical analysis of cheese • Sensory evaluation of cheese
CDR onsite visits	• Visited 14 cheese manufacturers (18 different trips) • Assisted in the scale-up of CDR cheese technologies, discussed tailor manufacturing of cheese for specific end use, cheese quality or general cheese technology issues
Phone consultations	• Worked with 53 companies (39 manufacturers, 4 ingredient suppliers, 2 contract laboratories, 2 end users, 2 equipment manufacturers, 4 organizations) • Discussed general cheese technology issues, milk standardization, cheese yield, controlling the meltability of cheese, cheese defects, cheese process control

INTERIM REPORT

Optimizing the standardization of milk to manufacture 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, research cheesemaker, 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 —December 1998

Objectives

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

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

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

Summary

The cheese making experiments conducted in the first year of this project evaluated milk standardized to a casein:fat ratio of 1.70 and 10% total solids in the manufacture of 50% reduced-fat Cheddar cheese. Whole milk was standardized with either condensed skim (40% solids), reconstituted NDM (20% solids) or skim milk. It was necessary to add water to obtain the 10% solids. A complete fat and nitrogen analysis was completed. 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 increased from 74.9 to 76.1. Proteolysis in the cheese was assessed by 12% TCA soluble nitrogen at 2, 6, 12, and 24 weeks of aging. Cheeses made with standardizing whole milk by NDM resulted in 10-20% less proteolysis at all sampling points as compared to the control. Standardization of milk with condensed skim did not affect the levels of 12% TCA soluble nitrogen. Cheeses standardized with NDM had less cheddar flavor and were firmer than the control, while standardization of milk with condensed skim did not affect the flavor or texture.

In the second year of this project, whole milk used to manufacture 50% reduced fat Cheddar cheese was standardized to a casein:fat ratio of 1.65, by addition of skim (control) or reconstituted NDM (20% solids) or condensed skim (CS, 40% solids). The experimental vats (milk standardized by addition of NDM or CS), required the addition of water. Control milks had 10% total solids, while in the experimental vats, the solids levels were varied to approximately 10%, 12% or 14%. Traditional no-wash cheddar manufacturing protocol was followed. To control moisture, control vats were set for 50 minutes, while experimental vats were set for 40 minutes. Cheeses made with the addition of NDM had increased moisture compared to the control, while cheeses made with addition of CS had a decrease in moisture compared to the control. At 10% solids level, there was less fat recovered in the cheese made from milk standardized by the addition of NDM or CS, compared to the control. These results are consistent with the results from the first year of cheese making. At the higher solids level, there were no differences in the fat recovered in the cheese made from milk standardized with NDM, when compared to the control. At higher solids levels, the milk standardized with CS had more fat recovered in the cheese than the control. At 10% solids level, there was similar percentages of nitrogen recovered in the cheese regardless of the method of standardization. At higher solids levels, the milk standardized with either NDM or CS (when compared to the control), had increased percentage of nitrogen recovered.

The higher solids milks contained higher levels of lactose, the cheese contained higher levels of lactic acid, and consequently a lower pH. However the ratio of converting lactose (expressed as equivalent moles of lactic acid in Table 2) to lactic acid in the cheese remains fairly constant, regardless of the initial solids content in the milk. Thus, if we know the initial percentage of lactose in the milk, we can predict the final lactic acid level in the cheese. Proteolysis in the cheese was assessed by 12% TCA soluble nitrogen at 2, 6, 12, and 24 weeks of aging. 12% TCA soluble Nitrogen (as a percent of total cheese nitrogen) was similar in all cheeses made from milk standardized to the 10% solids level. These results differ from experiments conducted in year 1. Cheeses made from higher milk solids level appear to have less 12% TCA soluble nitrogen (as a percent of total cheese nitrogen).

In this project we analyzed milk samples for levels of denatured whey proteins using a HPLC method. Test results were inconsistent and inconclusive. We are currently taking another approach to access levels of denatured whey proteins in the initial milk. We plan to correlate levels of denatured protein to percentage of fat and nitrogen recovery in cheese and cheese quality.

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 1998. 93rd Annual ADSA Meeting, Denver CO.

Comparative study of milk standardization methods and initial milk solids levels in the manufacture 50% reducedfat Cheddar cheese. C.M. Chen, A.L. Dikkeboom, M.E. Johnson. July 1999. 94th Annual ADSA Meeting, Memphis, TN.

Table 1. Percentage of fat and nitrogen recovery, R value and cheese yields for 50% reduced-fat Cheddar cheese made from milk standardized with reconstituted NDM or condensed skim milk.

Treatment (milk solids level)	% Fat Recovery	% Nitrogen Recovery	R - value	Actual Yield	Adjusted Yield[1]
NDM					
whole + skim (10%)	89.15	74.69	1.13	8.08	8.39
whole + NDM (10%)	86.48	74.83	1.15	7.99	8.06
whole + NDM (12.25%)	88.55	75.39	1.15	9.59	9.82
whole + NDM (14.5%)	89.01	75.73	1.16	11.22	11.59
Condensed skim					
whole + skim (10%)	88.68	75.08	1.16	7.93	8.04
whole + CS (10%)	87.95	75.52	1.14	8.44	8.66
whole + CS (12.25%)	89.61	76.13	1.14	10.25	10.69
whole + CS (14.5%)	90.04	75.86	1.16	12.13	12.63

[1] *Adjusted to 47% moisture, 1.7% salt*

Table 2. Percentage of equivalent lactic acid in milk and cheese.

Treatment (milk solids level)	Cheese pH (45 days)	% eq Lactic Acid in Milk[1]	% eq Lactic Acid in Cheese[2]	Ratio eq Lactic Acid[3]
NDM				
whole + skim (10%)	5.15	0.81	1.06	0.77
whole + NDM (10%)	5.14	0.81	1.02	0.81
whole + NDM (12.25%)	5.07	0.98	1.23	0.81
whole + NDM (14.5%)	5.02	1.17	1.49	0.81
Condensed skim				
whole + skim (10%)	5.15	0.80	1.04	0.77
whole + CS (10%)	5.13	0.86	1.07	0.80
whole + CS (12.25%)	5.08	1.05	1.34	0.79
whole + CS (14.5%)	5.05	1.23	1.56	0.79

[1] *Equivalent Lactic Acid in Milk (eq moles lactic acid in milk / moles water in milk)*

[2] *Equivalent Lactic Acid in Cheese (eq moles lactic acid in cheese / moles water in cheese)*

[3] *Ratio of equivalent Lactic acid in milk to equivalent lactic acid in cheese (Column 2 divided by Column 3)*

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, research cheesemaker, 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

A technology developed at the CDR resulted in a non-stretched, non-brined stirred curd Pizza cheese manufacturing protocol, which offers several advantages to the cheese industry. This protocol enables manufacturers of stirred curd, pressed cheeses the ability to make a cheese with composition, melt and stretch similar to LMPS Mozzarella cheese. However, Pizza cheese differs from LMPS Mozzarella in that it is whiter in color, after baking is more opaque, oils off less, produces fewer blisters, is less chewy, and does not brown. These characteristics make Pizza cheese ideal for use as an ingredient in baking applications. The first project developed a manufacturing protocol for a cheese suitable for use on pizzas. However, to enhance the use of Pizza cheese as a food ingredient, several important details need to be addressed; i.e. stability of desirable physical characteristics, and adapting the manufacturing protocol to obtain cheeses with any physical characteristic desired (color, melt, flow, stretch, flavor). To address these issues we plan to do the following:

1. Determine the minimal and maximal aging for optimal physical and sensory characteristics of Pizza cheese when used on pizzas.

2. Evaluate shelf-life stability and physical properties with respect to:
starter culture alterations
milk standardization methods
coagulant level
denatured whey protein addition

Summary

Pizza cheese (non-pasta filata) was made with *Lactococcus lactis sp.* or *Streptococcus thermophilus.* In addition, the milk was either pasteurized at 180°F, or 164°F. As expected, the higher pasteurization temperature resulted in cheese with a higher moisture content. Through the first 60 days of storage, the cheeses made with *Streptococcus thermophilus* were higher in pH than cheeses made with *Lactococcus lactis.* Meltability of the cheese was greatly reduced with the higher pasteurization temperature (regardless of the higher moisture content, and increased pH). Preliminary results also indicate a restricted melt due to a high salt content (>2.3%).

Milk for pizza cheese was standardized with reconstituted non-fat dry milk. Casein to fat ratios were held constant, but the total milk solids level of the milk varied from 11.0 to 14.5%. Initial tests indicate no significant differences in melt due to the use of non-fat dry milk or to solids level of the milk.

Pizza cheese was made with reduced coagulant levels. There does not appear to be any significant reduction in melt or free oil formation with a reduced coagulant level. However, there was a significant reduction in free oil in the pizza cheese compared to pasta filata cheeses (Table 1). Composition was similar between the cheeses (see Table 2). There appears to be no significant difference in proteolysis among the pizza cheeses, even with the reduced coagulant level (see Table 2).

Table 1: Percent free oil per gram of cheese fat

treatment	3-4 days	10 days	30 days	60 days
pasta filata	29.4	36.9	38.3	36.9
pasta filata	26.9	36.6	37.0	35.1
double rennet level	8.3	12.8	18.7	17.4
double rennet level	6.9	14.6	16.9	22.2
normal rennet level	5.1	14.7	15.7	14.2
normal rennet level	4.2	11.2	10.6	12.2
half rennet level	5.3	12.9	25.7	20.6
half rennet level	5.7	11.8	15.7	16.8

Table 2

treatment	Moisture	FDM	% TCA sol. N / % total N in cheese 10 Day	1 Month	2 Months
pasta filata	46.17	43.15	2.8	4.7	4.9
pasta filata	46.76	43.02	3.7	6.6	9.1
double rennet level	46.33	42.25	3.6	6.4	8.8
double rennet level	47.45	43.91	3.5	6.4	9.7
normal rennet level	45.63	42.21	2.7	5.0	7.9
normal rennet level	46.27	41.80	3.0	5.2	8.5
half rennet level	46.46	41.57	3.1	4.9	7.9
half rennet level	47.61	41.83	2.9	4.6	8.1

FINAL REPORT

Lower-fat swiss cheese: evaluation of free fatty acid concentrations on the development of flavor

Personnel: Carol M. Chen, researcher, Amy L. Dikkeboom, research specialist, William Hoesly, research cheesemaker, Kristen B. Houck, research specialist, John J. Jaeggi, associate researcher, Mark E. Johnson, senior scientist, Juan Romero, associate researcher, William A. Tricomi, assistant researcher, Matthew G. Zimbric, research specialist

Funding

Dairy Management Inc.

Dates

July 1997 to December 1998

Objectives

1. To explore the use of flavor enhancing starter adjuncts, such as *Lactobacillus helveticus* CNRZ 32, succinic acid producing *Lactobacillus sp. (RL 3)* and esterase positive *Lactobacillus casei* (Lila) to increase the flavor of lower fat Swiss cheese.

Summary

An informal survey of lower fat Swiss cheese varieties collected from Madison area grocery stores yielded a disappointing array of unpleasant flavors, poor eye development and poor bodied lower fat Swiss cheeses. We also received inquires about these problems from both industry consultants and manufacturers of lower fat Swiss cheese—emphasizing the need to study body and flavor problems. The quality of Swiss-type cheese depends on an array of fermentations and chemical reactions such as lactic acid fermentation, fat hydrolysis, and proteolysis. This project proposed to analyze key flavor components such as acetic, propionic, lactic acid and branched-chain free fatty acids to help define the chemistry behind quality lower fat Swiss cheese flavor.

Since we had already demonstrated that we could make an acceptable 25% reduced fat Swiss cheese, we decided to concentrate on a 50% reduced fat Swiss cheese for this project.

The manufacturing schedule that we followed can be observed in Table 1. The variables used in the different trials are as follows:
Amount of bacterial adjunct per 550 pounds of milk
LH 32 was a commercial frozen pellet, containing 10^{10} CFU / g of *Lactobacillus helveticus*, and the RL 3 and Lila strains were grown in MRS broth overnight. Both the RL 3 and Lila strains had counts of approximately 10^9 CFU per ml.

Composition, pH, cheese microbiology and free fatty acid analysis for selected cheeses are given in Tables 2, 3 and 4.

Vat 1	Control (no adjunct added)
Vat 2	5.5 g LH 32
Vat 3	110 ml RL 3
Vat 4	5.5 ml Lila
Vat 5	Control (no adjunct added)
Vat 6	110 ml RL 3, 5.5 g LH 32
Vat 7	110 ml RL 3, 5.5 ml Lila
Vat 8	110 ml RL 3, 5.5 ml Lila, 5.5 g LH 32
Vat 9	Control (no adjunct added)
Vat 10	5.5 g LH 32
Vat 11	110 ml RL 3
Vat 12	110 ml RL 3, cheese ripened at 45°F
Vat 13	Control (no adjunct added)
Vat 14	110 ml RL 3, 5.5 g LH 32
Vat 15	55 ml RL 3, 2.5 g LH 32
Vat 16	55 ml RL 3, 2.5 g LH 32, cheese ripened at 45°F

Actual percent fat reduction was 40-47% based on the minimum legal description of a full fat Swiss cheese. Sensory Analysis was conducted by a small group of experienced cheese graders to initially test the quality of the cheeses. Based on a consensus of the small group, four cheeses (ages ranging from four to seven months) were picked for evaluation in a consumer panel, consisting of 160-177 individuals.

Cheeses from vats 15 and 16 were chosen for evaluation because of their high Swiss flavor and body characteristics. Cheeses from vats 3 and 8 were chosen to demonstrate whether the consumer panel would accept a reduced fat Swiss that (in the opinion of the

Lower Fat Swiss Project 50% Reduced-Fat Swiss Cheese

Table 1: Manufacturing Schedule

Operation	Time (min)		pH or TA
Initial Milk	**550 lb**	Control TA pH	 0.21 6.23
Preacidify milk - Acetic Acid (day before) Diluted 1:4 with acetic acid : water		Temp pH	----- 6.20
Add Annatto (dbl str)	-5		1 drop
Add CaCl2 - 3 oz/1000 lb	0		49 ml
Add Starter CHL 970 DVS - Lot # 17067 65 ml/1000 lb	 0 36 g	Temp TA pH	90.2°F 0.21 6.23
Add Propionibacterium shermanii Chr Hansens PS-1 type - Lot # 19067 8 ml/1000 lb	 0 4.4 ml		
Add Adjunct ..... varied levels			
Add TiO2	0		15 g
Add Coagulant Marschalls Chymostar (dbl str) 0.92oz/1000 lb	 15 15 ml	Temp TA pH	90.1°F 0.21 6.21
Cutting very firm - 3/8" knives	55	TA pH	0.13 6.21
Pre-draw 45% of original volume	70	Temp	89.5°F
Add Water Back 100-110°F / Cooking 45% of original volume	80	Temp	94.0°F
Reach Cooking Temperature ~100°F	 105	Temp TA w-pH c-pH	100.1°F 0.05 6.31 6.18
Pump Curd To Pressing Vat **Begin** **End**	 115 125	TA w-pH c-pH	0.05 6.28 6.16
Start Draining Whey **Finish Draining Whey**	125 135		
Hooping	145	c-pH	6.06
Horizontal Press - In **- Out**	145 325	c-pH	 -----
Resting at Room Temp, overnight		AM - pH	5.10
Brining Time (saturated, 50°F)	24 hours		
Precooling Time 45°F	10 days		
Time in Warm Room 72°F	21 days		

Table 2: Cheese Composition

Actual Cheese Yield	vat 1	vat 2	vat 3	vat 4	vat 5	vat 6	vat 7	vat 8
Milk (lb)	550	550	550	550	550	550	550	550
Cheese (lb)	36.4	37.3	37.1	37.7	39.7	39.9	39.8	40.9
% Cheese Yield	6.6	6.8	6.7	6.9	7.2	7.3	7.2	7.4
Cheese Composition	**vat 1**	**vat 2**	**vat 3**	**vat 4**	**vat 5**	**vat 6**	**vat 7**	**vat 8**
% Moisture	47.60	47.60	47.08	47.02	48.27	47.59	47.98	47.95
% Fat (Mojonnier)	13.80	13.69	14.09	13.89	13.76	14.20	14.11	13.53
% Salt	0.08	0.08	0.08	0.07	0.07	0.08	0.07	0.07
% Protein (N x 6.36)	34.81	33.46	34.08	34.71	34.23	34.43	34.10	33.57
% MNFS	55.22	55.15	54.80	54.61	55.97	55.47	55.86	55.46
% FDM	26.34	26.13	26.62	26.23	26.59	27.09	27.12	26.00
% S/M	0.17	0.17	0.17	0.15	0.15	0.17	0.15	0.15
Cheese Composition	**vat 9**	**vat 10**	**vat 11**	**vat 12**	**vat 13**	**vat 14**	**vat 15**	**vat 16**
% Moisture	44.19	46.51	45.05	44.51	43.47	44.45	44.85	44.45
% Fat (Mojonnier)	15.17	14.48	15.23	15.14	14.78	14.27	14.62	14.28
% Salt	1.35	2.025	1.51	1.53	1.57	1.15	0.93	1.17
% Protein (N x 6.36)	34.77	34.02	34.92	34.62	34.92	34.39	35.55	35.41
% MNFS	52.10	54.38	53.15	52.45	51.01	51.85	52.53	51.85
% FDM	27.19	27.07	27.72	27.29	26.15	25.70	26.51	25.71
% S/M	3.05	4.35	3.35	3.44	3.61	2.59	2.07	2.63
Cheese pH	**vat 1**	**vat 2**	**vat 3**	**vat 4**	**vat 5**	**vat 6**	**vat 7**	**vat 8**
1 day	----	----	----	----	----	----	----	----
pH into warm room (10 d)	5.36	5.33	5.37	5.41	5.48	5.43	5.54	5.37
pH out of warm room	----	----	----	----	----	----	----	----
2 months	5.46	5.49	5.52	5.53	5.63	5.51	5.56	5.49
4 months @ 35°F storage temp	5.62	5.69	5.59	5.59	5.87	5.66	5.91	5.64
4 months @ 45°F storage temp	5.63	5.61	5.68	5.59	6.07	5.58	5.73	5.75
6 months @ 35°F storage temp	5.65	5.84	5.66	5.76	5.95	5.78	5.85	----
6 months @ 45°F storage temp	5.55	5.59	5.57	5.59	6.01	5.78	5.70	5.75
Cheese pH	**vat 9**	**vat 10**	**vat 11**	**vat 12**	**vat 13**	**vat 14**	**vat 15**	**vat 16**
1 day	5.16	5.12	5.23	5.19	5.13	5.31	5.21	5.21
pH into warm room (10 d)	5.20	5.15	5.23	5.26	5.28	5.32	5.20	5.29
pH out of warm room	5.54	5.49	5.45	5.49	5.55	5.54	5.56	5.61
2 months	5.63	5.56	5.54	5.57	5.63	5.70	5.59	5.56
4 months @ 35°F storage temp	5.61	5.68	5.61	5.64	5.74	5.68	5.78	5.87
4 months @ 45°F storage temp	----	----	----	----	----	----	----	----
6 months @ 35°F storage temp	5.87	5.76	5.61	5.82	5.71	5.71	5.96	5.94
6 months @ 45°F storage temp	----	----	----	----	----	----	----	----

Table 3: Cheese micro

Cheese Micro @ 3 day	vat 1	vat 2	vat 3	vat 4	vat 5	vat 6	vat 7	vat 8
Lactobacillus (CFU/g)	7.6E+04	5.9E+08	8.5E+07	4.9E+07	1.9E+07	5.2E+07	9.8E+07	5.5E+07
Starter Organisms (CFU/g)	1.7E+09	1.9E+09	2.5E+09	2.4E+09	2.4E+08	5.7E+08	1.9E+09	6.5E+08
Cheese Micro @ 3 day	**vat 9**	**vat 10**	**vat 11**	**vat 12**	**vat 13**	**vat 14**	**vat 15**	**vat 16**
Lactobacillus (CFU/g)	8.2E+03	2.5E+07	5.6E+07	8.9E+07	8.3E+05	1.4E+08	9.7E+07	1.1E+08
Starter Organisms (CFU/g)	1.0E+09	1.3E+09	1.2E+09	1.7E+09	1.2E+07	1.0E+08	8.2E+07	1.0E+08

Table 4: Free Fatty acid analysis of cheeses tested in Consumer Panels

			ppm				
Date	Vat #	Treatment	Acetic	Propionic	IsoValeric	Lactic	Succinic
120day	vat 3	RL3	154.7	260.1	274.0	18.5	763.0
180day	vat 3	RL3	153.6	265.1	230.0	21.1	953.8
120day	vat 8	LH32, RL3, Lila	215.2	156.9	301.4	15.9	1348.7
180day	vat 8	LH32, RL3, Lila	200.3	195.8	286.5	15.6	1365.9
120day	vat 13	Control*	137.7	74.4	157.4	34.3	362.6
180day	vat 13	Control*	81.4	173.4	99.0	1.4	506.7
120day	vat 15	LH32, RL3	194.2	329.5	326.5	19.9	1153.9
180day	vat 15	LH32, RL3	186.5	332.8	285.6	20.3	1263.8
120day	vat 16	LH32, RL3@45°F	207.6	335.1	287.7	24.5	1497.4
180day	vat 16	LH32, RL3@45°F	201.1	362.1	265.4	23.6	1565.3

* *This cheese was bland and lacked Swiss flavor, was not tested in the Consumer Panels*

expert panel) were either bland (vat 8), or slightly unclean (vat 3). Both vat 3 and vat 8 were judged to have unacceptable (for a Swiss cheese) flavor by the expert panel but was superior to most of the commercial low fat Swiss cheeses tested. The experimental Swiss samples were compared to commercial full fat aged Swiss and full fat Baby Swiss samples. The commercial samples were also pre-graded to ensure their high quality (texture, body and flavor), and to confirm that they had the appropriate desirable Swiss characteristics. Different lots of the same commercial cheeses were used to maintain consistency in the consumer panels. The Baby Swiss was chosen because it employed a whey dilution step similar to that used in the manufacturing schedule that was used to make the low fat experimental Swiss cheeses.
The results of the consumer panels can be seen in Tables 5, 6, and 7. The consumer panelists were only asked to judge the acceptability of the cheeses, but were told they could also volunteer comments. Seventeen of the 175 consumer panelists voluntarily noted in panel 1 (see Table 5) that the experimental (vat 16) low fat Swiss cheese was tough and dry. Yet, two thirds of the panelists graded experimental and commercial cheese in the "Like slightly to Like very much" categories. This shows us that flavor may be more important than body to the average consumer.

In Panel 2 (see Table 6), a less acceptable cheese (according to the small expert panelists) was evaluated with the commercial samples. Consumer panelists noted that the experimental cheese lacked Swiss flavor and was too rubbery. Commercial samples had high quality flavor yet were also firm, again supporting the belief that flavor may be more important than body.
In Panel 3 (see Table 7), fifteen of the 160 panelists voluntarily commented that the CDR experimental Swiss cheese (vat 15) had excellent taste, while only 2 out of the 160 consumer panelists commented that the vat 3 cheese had excellent taste. Similarly, for the commercial cheese, only 9 of the 160 panelists thought it had excellent flavor. The voluntary panelist comments also indicated the experimental Swiss cheese (vat 15) was too dry and hard, which apparently was influential in lowering the overall consumer preference for this sample. It appeared that flavor factors were a more influential factor than body scores in establishing the overall preference for reduced fat Swiss cheese.

The goal of this trial was to test the feasibility of using adjunct cultures to improve the flavor quality of low fat Swiss cheese. Without adding adjunct cultures, the low fat Swiss cheeses lacked the Swiss cheese notes typical of full fat Swiss. However, adding individual strains of Lactobacilli as flavor adjuncts did not produce

Table 5: Consumer Panel 1
Response frequency and mean scores for the consumer preference evaluation of Swiss Cheeses

Preference Rating	Numerical Score	Vat 16 RL 3, LH 32, ripened at 45°F	Aged Swiss	Baby Swiss
Like very much	7	28	31	37
Like moderately	6	45	42	47
Like Slightly	5	38	42	29
Neither like nor dislike	4	13	11	11
dislike slightly	3	24	30	22
Dislike moderately	2	18	12	18
Dislike very much	1	9	7	11
Mean score		4.71[a]	4.82[a]	4.81[a]
F value		NS		
LSD (at 5% level)				

Total Number of Responses: n = 175
NS = not significant at the 5% level
[a] Mean scores in the same row with the same superscript are not significantly different at the 5% level

Table 6: Consumer Panel 2
Response frequency and mean scores for the consumer preference evaluation of Swiss Cheeses

Preference Rating	Numerical Score	Vat 8 LH 32, RL 3, Lila	Aged Swiss	Baby Swiss
Like very much	7	6	52	45
Like moderately	6	38	54	52
Like Slightly	5	28	36	40
Neither like nor dislike	4	9	16	12
dislike slightly	3	30	11	16
Dislike moderately	2	40	7	8
Dislike very much	1	26	1	4
Mean score		3.63[a]	5.54[b]	5.33[b]
F value		S		
LSD (at 5% level)		0.32		

Total Number of Responses: n = 177
S = significant at the 5% level
[a,b] Mean scores in the same row with the same superscript are not significantly different at the 5% level

the Swiss flavor we desired. However, a combination of two adjuncts—RL3 and LH-32— produced a 50% reduced fat Swiss cheese with typical Swiss flavor and a cheese that was as acceptable as an aged full-fat Swiss cheese. While it appears that succinic acid may accentuate flavor in cheese we still do not know the specific flavor compounds that define Swiss cheese. Further studies are needed to determine the specific microbiological and biochemical interrelationships involved in Swiss cheese flavor production.

Table 7: Consumer Panel 3
Response frequency and mean scores for the consumer preference evaluation of Swiss Cheeses

Preference Rating	Numerical Score	Vat 3 RL 3	Vat 15 RL 3, LH 32	Aged Swiss
Like very much	7	12	35	30
Like moderately	6	25	37	55
Like Slightly	5	27	32	43
Neither like nor dislike	4	11	19	9
dislike slightly	3	43	19	11
Dislike moderately	2	22	9	10
Dislike very much	1	20	9	2
Mean score		3.79[a]	4.92[b]	5.29[c]
F value			S	
LSD (at 5% level)			0.35	

Total Number of Responses: n = 160
S = significant at the 5% level
[a] Mean scores in the same row with the same superscript are not significantly different at the 5% level

APPLICATIONS PROGRAM REPORT

Dairy marketing and economics applications program

Personnel: Brian W. Gould, senior scientist, Diansheng Dong, post-doctoral research associate, Yadav, graduate research assistant, Aterido, graduate research assistant, Asanov, graduate research assistant

Funding

Wisconsin Milk Marketing Board

Dates

July 1998—June 1999

Objectives

1. Develop educational materials to assist dairy processors and farm operators to understand the use of dairy-based futures and options for managing price risk.

2. Develop business software applications to assist dairy processing sector

3. Undertake a preliminary analysis of the economics of the use of HACCP principles by dairy processors

Summary

The initial year of funding by the Wisconsin Milk Marketing Board resulted in three projects. The first project concerns the development of traditional as well as web-based information systems concerning the use of futures markets for the management of both input and output price risk by the dairy sector. A series of Dairy Pipeline articles were written, focusing on the how dairy processors can use the futures markets. These articles are available from the CDR web site (http://www.cdr.wisc.edu). We have also developed, with the cooperation of the Department of Agricultural and Applied Economics a web site containing detailed price, production, stock and other information related to the dairy industry (http://www.aae.wisc.edr/future). We are in the process of expanding this site to include an interactive tutorial on the use of dairy-based futures and options.

A second project is the continued development of business related software that will help the Wisconsin dairy processing sector make sound economic production and investment decisions. For example, we are expanding the DOS-based cheese yield/standardization program available at the Center since 1994 to a Windows-based system that more accurately reflects how the processing industry transforms raw milk into finished products under today's technological and regulatory environment.

A final project being undertaken within the dairy market and economics program is an examination of the adoption of the HACCP (Hazard Analysis of Critical Control Points) program by Wisconsin cheese manufacturers. This project will lead to an assessment of the benefits versus costs of alternative levels of adoption.

INTERIM REPORT

International dairy product demand: an evaluation of the potential for increased U.S. dairy exports

Personnel: Brian W. Gould, senior scientist, Wisconsin Center for Dairy Research, Thomas L. Cox, professor, Bradford Barham, associate professor, Department of Agricultural and Applied Economics

Funding

Babcock Institute for International Dairy Research and Development, University of Wisconsin-Madison

Dates

January 1998-Sept. 1999

Objectives

1. Identify the important determinants of the demand for dairy products in Mexico, Canada, and the former Soviet Union using household survey data.

2. Develop a data base system to undertake similar analyses for Argentina, and Brazil.

3. Use these results to generate implications of the potential for growth of U.S. dairy exports to these countries.

Summary

With the implementation of recent trade liberalization agreements such as the Uruguay round of GATT agreement, NAFTA, and MERCOSUR, the integration of Hong Kong into China, and the economic reforms occurring in the Russian Federation, there have been numerous analyses of the potential for increased U.S. agricultural-based exports. Fundamental to any of these analyses is an understanding of the structure of food demand in these foreign countries. For example, what is the impact of continued economic development on staple versus non-staple food consumption? How sensitive is food consumption to changes in price? Will the structure of food demand change with increasing household income? What is the role of household composition on such demand?
In 1996, U.S.dairy exports totaled $736 million which represents less than 15% of world exports (USDA). These exports are fairly concentrated with the top 4 countries typically representing more than 50% of total exports. In 1996 Canada represented the largest market (17%). Between 1986-1995 Mexico had been the largest market for U.S. dairy exports. Besides Canada and Mexico, another potentially important market for our dairy exports is the former Soviet Union (FSU). In 1992, Russian Federation imports accounted for 14% of U.S. dairy exports. In 1996 this had decreased to less than 3%, still representing the 7th largest U.S. export market. Prospects for increasing trade levels to the FSU may improve, however, given their domestic production of many primary foodstuffs has been decreasing. For example, raw milk production in 1993 was less than 70% of 1990's production.

Understanding how consumers react to changes in relative prices, income, and household composition and other factors is important for projecting future demand changes. This is true for domestic consumers and also for consumers in foreign markets. In an era of increased trade liberalization, the potential exists for significant increases in U.S. dairy exports (though under GATT provisions requiring decreases in the use of export subsidies, the opposite outcome of lower exports is also possible). Such exports represent one means by which the income of the U.S. (and Wisconsin) dairy sectors can be improved. There are a number of international household-level data sets that are becoming available which can be used to help predict the possible paths of future dairy product demand in foreign markets.
With the increasing integration of world dairy markets it is essential that we understand key determinants of dairy product consumption in foreign markets. Previous efforts have tended to undertake household level analyses of dairy product demand one country at a time. This has limited the ability to make comparisons across countries given differences in data, time period encompassed, methodologies employed, and commodities investigated. Such comparisons are essential for policy analysts when attempting to identify those foreign markets most likely to be amenable to receiving U.S. dairy exports.

One of the project PI's (Gould) has had a continuing program of analysis of domestic dairy product demand

since 1990 and has developed several household level database systems which have been used by the dairy industry to examine specific marketing related questions. In 1996, the principal investigators obtained funding from the Babcock Institute to undertake an initial comparison of the structure of the dairy product demand by U.S., Canadian and Mexican households. In the course of undertaking this project, household level food expenditure data for Canada (1994 Family Food Expenditure Survey) and Mexico (1994 Encuesta Nacional de Ingresos y Gastos del Hogar [ENIGH]) were obtained, the Mexican data were translated, and some preliminary models of dairy product demand were developed and estimated. The current project continues this research effort.

To date, we have undertaken analysis using food purchase data for households in Mexico, Canada and the Former Soviet Union. This has resulted in the publication of two Babcock Institute Discussion papers. In these papers we present some preliminary results of the effect of changes in household income, composition and market prices on foods purchased, including dairy products. We analyze the demand structure of a number of alternative dairy products. These responses are quantified via the estimation of a series of econometric models of dairy product demand. From these models we then calculate the response of changes in important variables on the household decision of whether to purchase dairy products.

Publications

B.W. Gould and J.S. Kim, ***Characteristics of Canadian and Mexican Dairy Product Purchases: A Comparison Using Household Expenditure Data***, Babcock Institute Discussion Paper 98-2, November 1998.

J.S. Kim and B.W. Gould, ***The Structure of Meat, Poultry and Dairy Product Demand in the Former Soviet Union***, Babcock Institute Discussion Paper 98-3, December 1998.

INTERIM REPORT

A multi-country analysis of household food demand: implications for U.S. food exports (phase I)

Personnel: Brian W. Gould, senior scientist, Diansheng Dong, post-doctoral research associate, Wisconsin Center for Dairy Research, Ron C. Mittlehammer, professor, Thomas I. Wahl, professor, Washington State University, Wen C. Chern, professor, Ohio State University, Barry G. Goodwin, professor, North Carolina State University

Funding

Department of Agriculture, National Research Initiative

Dates

July 1998—June1999 (Phase I)

Objectives

1. Review alternative methods for estimating disaggregated food demand systems that incorporate limited dependent variables.

2. Identify an appropriate methodology for undertaking a detailed analysis of disaggregated food demand in developing and developed countries that overcomes the limitations imposed by earlier two-stage estimation procedures.

3. Develop the necessary econometric software to apply this methodology to household level food expenditure data.

4. Apply this methodology to a single county (e.g., Mexico) to verify its ability to accurately describe the structure of international food demand.

5. Develop a system to extend this analysis to alternative household-based survey data sets for other countries that are currently, or may become, important export markets for both dairy and non-dairy products.

Summary

Comprehensive knowledge of food demand parameters is essential to understand the potential for development and promotion of US agricultural products in foreign markets. For example, what is the impact of continued economic development on staple versus non-staple foods? Will the developing world necessarily follow the developed countries in raising their meat and dairy consumption as their income increase? What causes the differences in food demand structure?

A good example of the importance of understanding the nature of international food demand can be found in the U.S. dairy sector where growth in domestic demand is relatively flat, the U.S. share of world dairy trade is small and competing dairy producing countries are attempting to increase their dairy export efforts. Previous research has indicated that the U.S. dairy industry will increase its export activity under trade liberalization via the passage of the North American Free Trade Agreement and the Uruguay Round Agreements of the General Agreements on Tariffs and Trade and the elimination of domestic dairy price supports after 1999. With reduced trade barriers, a better understanding of the structure of dairy product demand in potentially new export markets is needed to quantify: (i) the sensitivity of consumption levels to changes in household income; (ii) the impact of changes in market price; (iii) the role of age/sex composition of households on dairy product consumption and (iv) the implications of future changes in these variables on future consumption patterns? The present analysis will answer such questions not only for dairy products but for a wide collection of other food types as well.

The primary objective of this project is to provide a disaggregated household level analysis of food demand in countries that are currently important markets for U.S. food exports as well as countries that have the potential for increased export activity.This project will use household level data to characterize food demand. Given the number of countries to be included in this analysis, we are taking a team approach with researchers located at North Carolina State University, Ohio State University, Washington State University and University of Wisconsin-Madison. Members of the research team possess complementary skills both in knowledge of particular countries as well as use of specific demand modeling techniques. The development of this research team will foster a coordinated effort in estimating food demand parameters that are readily comparable. This

team approach will greatly increase the number of analyses that can be undertaken and coordinated given the above complementary characteristics. The research team will adopt a two-step approach to modeling food demand. Initially, we will estimate country-specific complete food demand systems composed of fairly "aggregated" commodity definitions that are appropriate for the country of concern. Secondly, in a more detailed analysis of specific commodity types we will analyze disaggregated commodities within each aggregate commodity group.

This phase of the project will entail identifying an appropriate methodology for estimating fairly disaggregated complete food demand systems, developing the software required to implement this methodology and to apply these methods to a single country's data (e.g., Mexico) as a test case. After the successful completion of this phase we will then apply the lessons learned to a more extensive analysis of international food demand.

INTERIM REPORT

Large amplitude nonlinear viscoelastic behavior of mozzarella cheese during twin-screw extrusion

Personnel: S. Gunasekaran, professor, C. Yu, A.J. Giacomin, associate professor, Mechanical Engineering, T.A. Osswald, associate professor, Mechanical Engineering, M.E. Johnson, senior scientist, CDR

Funding

Dairy Management Inc.

Dates

January 1998— December 1999

Objectives

1. Investigate the fundamental rheological behavior of Mozzarella cheese under large strain rates developed in a twin-screw extruder.

2. Study the effect of process variables/extrusion parameters on the texture of extruded cheese products to optimize operating parameters of the mixer-molder step of manufacturing Mozzarella cheese in terms of improved product yield, quality and overall productivity.

Summary

Under the large strains occurring in an extruder, the rheological behavior of the cheese is extremely nonlinear. Thus conventional techniques are unsuitable to accurately study the fundamental rheological properties. Therefore, we used a new technique, sliding-plate rheometry, to generate large, rapid transient shear deformation to study time-dependent structural changes and nonlinear viscoelastic response of Mozzarella cheese. We employed strain rates comparable to those encountered in a twin-screw extruder.

Using these rheological properties, we will analyze the mixing and flow of melted Mozzarella cheese in the extruder. This will enable us to predict the mixing, flow-induced fractionation of the fat (the dispersed phase) and protein as a function of screw design, operating parameters and cheese composition. The textural qualities of the cheese leaving the extruder will be evaluated by conventional linear viscoelastic techniques. The results of this study will give an insight into flow and mixing phenomena of cheese in a twin-screw extruder, and greatly assist in process optimization as well as new product development.

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 in three dimension (stress, τ; frequency , ω; and strain rate, γ_o). 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 includes developing constitutive equations governing LAOS behavior.

Currently, experiments are underway to study the effects of age and fat content on the rheology of full fat and fat free pizza cheese (non-stretched Mozzarella). These cheese samples were made fresh at the University dairy plant, so we know the manufacture history. Tests are being conducted at regular intervals while the cheese matures. Results will again be presented in the form of stress versus rate-of-strain loops, and an attempt will be made to evaluate the viscoelasticity of the cheese based on the network entanglements and hydrogen bonds in the cheese. Similar tests are also planned for Swiss cheese with the same pH. In collaboration with the biological systems engineering department, low fat and full fat Cheddar cheese will also be studied. Lastly, an attempt will be made to relate the uniaxial compression tests to LAOS results.

FINAL REPORT

Machinability of reduced and lowfat cheeses

Personnel: S. Gunasekaran, professor, Biological Systems Engineering, Dept. of Food Science, K. Muthukumarappan, research associate, Dept. of Food Science, Biological Systems Engineer, and N. F. Olson, professor emeritus, Dept. Food Science

Funding

Dairy Management Inc. GN096

Dates

July 1995— June 1998

Summary

Most of the cheese used as an ingredient food is in one of the following machined forms—shredded, diced, grated, and sliced. Cheeses manufactured into 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 one 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:

1. Tensile Fracture Stress using Uniaxial Tensile and Three-point Bending Tests

2. Apparent Compression Modulus using Uniaxial Compression Test

3. Specific Fracture Energy using Wire-Cutting Test as a function of composition (fat: 0-35.5% and moisture: 44-55%) and age (1-12 wk)

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 at a speed of 5.1 cm/min to characterize the mechanical properties of cheeses. The chemical composition of the cheese samples is presented in Table 1.

Table 1. Chemical composition of Cheddar cheese

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 of dimensions 19.96 mm (dia.) x 19.96 mm (height) were prepared. The samples were tested at a cross-head speed of 5.1 cm/min. Apparent compression modulus (kPa) was determined by the tangent method.

Uniaxial Tension Test

Cheese samples of dimensions 60 x 7 x 7 mm (length x width x thickness) were prepared. The cheese samples were tested at a crosshead speed of 51 cm/min. 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 (length x width x thickness) were prepared. The samples were tested at a crosshead speed of 5.1 cm/min. Critical tensile fracture stress (kPa) was calculated at the fracture point.

Wire-Cutting Test

Cheese samples of dimensions 25.4 x 25.4 x 12.5, 18.7, 25.4, 31.6, 37.9 mm (length x width x thickness) were prepared. The samples were tested at a crosshead speed of 5.1 cm/min. Stainless steel spring-tempered wires of different diameters (0.46 to 1.4 mm) were used. Specific fracture energy (J/m^2) was estimated from the force-deformation data

All the experiments were conducted in an Instron (Model 1130) Universal Testing machine equipped with a 222.4 N load cell and data logger was used. All the experiments were conducted at the room temperature

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

Age, wk	Moisture Content, %			
	44	47	50	55
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 content and age (three-point bending test)

Age, wk	Fat Content, %		
	35.5	14.5	8
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

Age, wk	Moisture Content, %			
	44	47	50	55
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 content and age (uniaxial tension test)

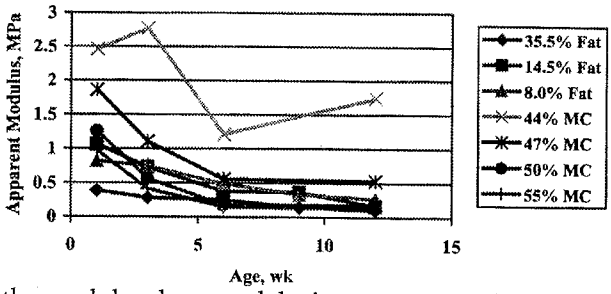
Age, wk	Fat Content, %		
	35.5	14.5	8
1	36^{a}	47^{b}	70^{c}
3	43^{a}	57^{b}	60^{b}
6	39^{a}	49^{b}	53^{b}
9	28^{a}	75^{b}	70^{b}
12	38^{a}	54^{b}	38^{a}

Table 1. Cheese applications program technical support

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.

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,

Figure 1

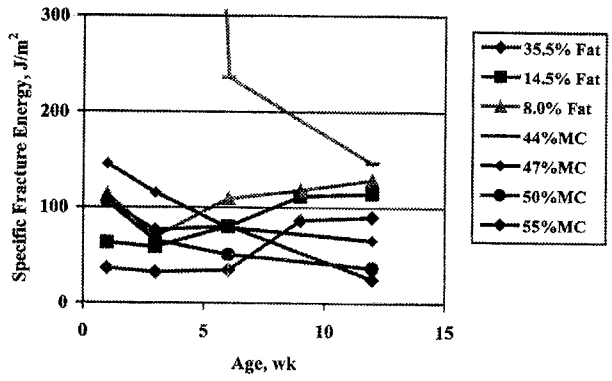


the modulus decreased during maturation (1-12 wk).

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/m^2) with decreased fat content and decreased (1366 to 25.3 J/m^2) 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.

Figure 2



FINAL 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

1. Develop method(s) to measure softening, melting and flow properties of cheeses in terms of fundamental engineering principles.

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

Summary

Controlling the softening, melting and flow of cheeses is critical to successfully use cheese as a food ingredient. This need is becoming increasingly important since many new cheese types and cheese-containing foods are being developed. There are at least two primary problems when studying the behavior of cheeses at high temperatures: 1) the physico-chemical and technological reasons for cheese behavior at high temperatures is not well known; 2) an objective method (one that is not affected by test conditions) for quantifying melt or flow of cheeses is not available.

The Schreibers test is the most widely used test for evaluating melting quality of cheeses. This test provides a satisfactory comparison of cheese types of widely varying melt properties. However, it is grossly inadequate to study minor variations in melt behavior. Also, the Schreibers test is not suitable for distinguishing cheeses that soften but do not flow. Other imitative tests such as the tube test are also limited by these drawbacks. Thus, a method based on fundamental engineering principles is needed.

An apparatus, based on the squeeze flow test principle, was designed and developed in the Food Engineering Laboratory of the University of Wisconsin. Figure 1 is a photograph of this device, named UW Meltmeter.

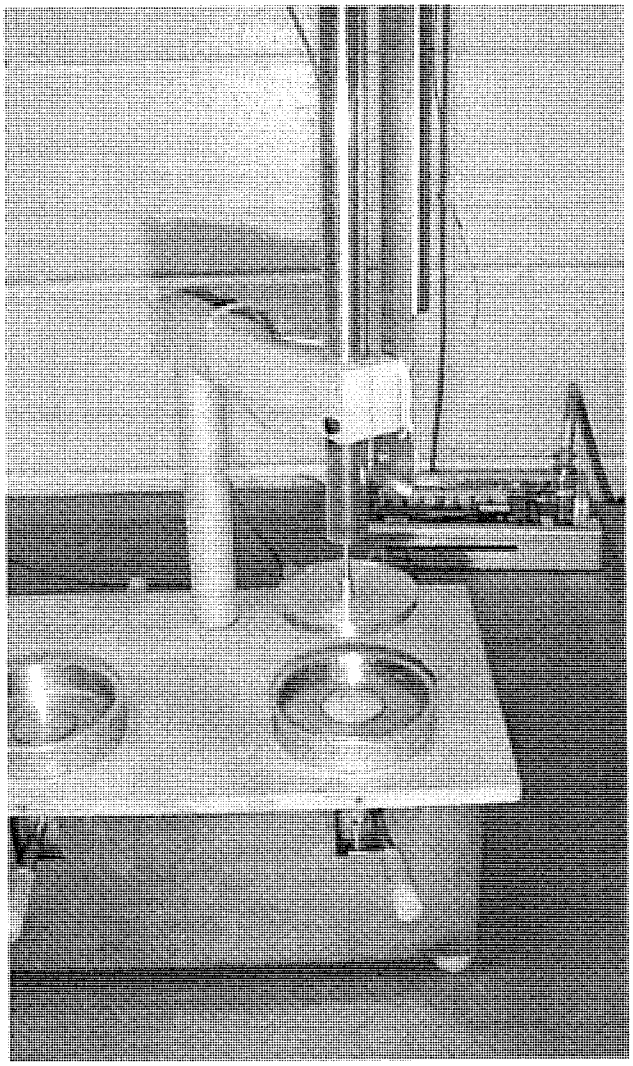


Figure 1. Picture of the UW Meltmeter designed and developed for objectively determining cheese meltability

Briefly, it is made of aluminum and has a movable outer cylinder (75-mm outer diameter; 30-mm inner diameter) equipped with an electric heater. This cylinder can be moved up and down around a 30-mm diameter stationary center piston by means of a lever. During the start of a test the outer cylinder is raised forming a 7-mm deep, 30-mm diameter sample well. A cheese sample of the same dimensions (7-mm thick, 30-mm diameter) is placed in the sample well such that the top

surface of the cheese sample is in flush with the top surface of the outer cylinder. A 66-mm diameter circular plate, attached to an LVDT (linear variable differential transformer), is lowered to cover the cheese surface and to maintain contact with the sample during the test. When the sample is heated to the preset temperature, the lever is activated lowering the outer cylinder and exposing the cheese sample to be squeezed out by the weight of the circular plate. This causes the melted cheese to flow. Additional weights can be added, as necessary, to increase the force causing the flow. A computer data acquisition system collects the sample height vs. time data.

Mozzarella cheeses used in the experiments were obtained from a local cheese company. Cheese blocks were stored in their original packaging in a refrigerator (4°C) until sample preparation. Cheese blocks were cut into slices of 7-mm thick perpendicular to the fiber orientation using a food slicer. The slices were cut with a cork borer to obtain cylindrical specimens (30-mm diameter) in a direction parallel to the fiber orientation. Hence, the compression direction was parallel to the fiber orientation in cheese. The slicing and cutting were done immediately after removing the cheese blocks from refrigerator to obtain uniform sample shape.

The factors studied were: test temperature (40 and 60°C), fat contents (14 and 43%), compression force (0.7 and 0.9 N) for constant force, and deformation rate (0.5 and 2.5 cm/min) for constant deformation rate. All the tests were replicated three times and average values are reported.

In order to compare the performance of the UW Meltmeter with the results of the Schreiber test a set of five commercial cheese samples were obtained and tested using both the tests. The Schreiber tests were conducted by heating the cheese sample (7-mm thick, 30-mm diameter) in a convective oven set at 232°C. The comparison of test results with Schreiber and UW Meltmeter tests is shown in Table 1. For UW Meltmeter, reciprocal of sample height at five seconds after the start of the test is reported. The UW Meltmeter test is clearly better than the Schreiber test. The UW Meltmeter is even more advantageous when evaluating the meltability of lower-fat cheeses.

The UW Meltmeter is a significant improvement over the current methods of empirical cheese meltability evaluation. Furthermore, the convenience of automatic data acquisition and data analysis should be considered an added advantage. The ability of the cheese makers to objectively measure and compare the melt/flow properties of cheeses will allow for a better control of cheesemake parameters. Closer control of cheese properties will promote tailor making of cheeses for a given end-use application.

Table 1. Comparison of the Meltability Evaluations of Five Commercial Cheeses by the Schreiber and UW Meltmeter Tests (based on three replications each).

Sample	Schreiber Test			UW Meltmeter Test		
	Average	Std. Dev.	CV[1], %	Average	Std. Dev.	CV, %
1	2.92	0.12	4.0	0.45	0.005	1.1
2	3.33	0.24	7.1	0.60	0.10	1.6
3	3.00	0.41	13.6	0.40	0.011	2.7
4	2.42	0.12	4.9	0.35	0.009	2.7
5	3.67	0.47	12.9	0.64	0.009	1.5
Mean CV, %			8.5			1.9

[1] *CV = Coefficient of Variation (= Average*100/Std. Dev.)*

FINAL REPORT

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

Personnel: S. Gunasekaran, professor, Biological Systems Engineering, D.J. Klingenberg, R. Subramanian, research assistant, Dept. of Food Science, and N. F. Olson, professor emeritus, Dept. of Food Science

Funding

Wisconsin Milk Marketing Board UW9504

Dates

December 1995 —December 1998

Objectives

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

2. Measure fundamental rheological parameters via transient and dynamic viscoelastic experiments.

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

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

Summary

In this project we have investigated a number of issues relating to cheese microstructure and texture/rheological properties with specific reference to cheese composition and/or heating and cooling transitions. The heating and cooling transitions were investigated to simulate the effects of such changes during preparation and consumption of cheese-containing foods.

Cheese Microstrucure

We used confocal laser scanning microscopy (CLSM) for studying the in-situ structure of fat globules in cheese. The CLSM is a significant alternative to traditional light or scanning microscopy for food structure evaluation. The main advantages are easy sample preparation, ability to examine several sequential layers in a sample unaffected by the image of out-of-focus regions above and below the plane of observation, and the ability to reconstruct the sequential two-dimensional layers into a three-dimensional image. Thus, the CLSM images can provide an excellent visualization of in-situ structure of microstructural attributes not possible with other methods.

We used the MRC-600 confocal microscope from Bio-Rad Microscience Ltd. available at the UW-Madison Integrated Microscopy Resource. Microstructure of one-month-old Cheddar cheese samples of different fat contents was observed with confocal laser scanning microscopy. An observation depth of 40 μm was used with a 0.5 μm distance between observation planes. This resulted in 81 sequential two-dimensional layered images of the cheese samples. These two-dimensional layers were digitally reconstructed using a digital image processing algorithm previously developed as a part of a previous project. Both two-dimensional and three-dimensional image analyses were performed to evaluate number (N_g for 3-D; n_g-for 2-D), size (D_S for 3-D; D_C for 2-D) and shape characteristics (sphericity, S for 3-D; circularity, C for 2-D) of fat globules in the cheese samples (Table 1). Since the fat globules are three-dimensional entities, two-dimensional views were affected by the viewing direction and location of the sample. Therefore, the three-dimensional analysis provided more accurate characterization of fat globule properties than the two-dimensional analysis. However, due to limited observation depth, many large fat globules were chopped by the image boundaries. These globules were not easily accounted for overall property characterization.

Table 1. Comparison of 3-D parameters of fat globules in Cheddar cheeses of two fat levels[1]

Cheese Sample	N_g	D_S (μm)	S (all)	S ($D_S > 2$ μm)
Low-fat cheese	452	2.5 ± 1.9	0.13 ± 0.08	0.08 ± 0.07
Very low-fat cheese	580	1. 6 ± 1.2	0.16 ± 0.08	0.09 ± 0.08

[1] *D_S and S values are reported as ± SD*

Rheological characterization

Rheologically cheese is described as a viscoelastic solid. That is, it exhibits both solid-like and liquid-like properties. The relative values of these properties depend heavily on the cheese temperature.

For this study we measured the linear viscoelastic behavior of regular (32.3% fat) and reduced fat (19.4% and 10.2% fat) Cheddar cheeses and a low-moisture, part-skim Mozzarella cheese. Cheese blocks were vacuum packaged in plastic bags and ripened at 6-8°C for rheological measurements after 2 and 6 wk from manufacture. Regular (23.8% fat) and reduced fat (4.8% fat) cheeses were stored in a refrigerator until measurements. 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 sand paper was glued to the upper plate to prevent sample slippage during measurement. Also, the exposed sides of the sample was coated with mineral oil to minimize moisture and drying during measurement.

Stress sweep measurements were performed at 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 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 reducedfat 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 an 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 cheese, 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 was lower than both Cheddar and Mozzarella cheese because of weaker protein-mineral network structure. Heating and cooling traces of dynamic viscoelastic moduli are shown in Figures 1 and 2 for six-week old regular and 33% reduced fat Cheddar cheeses. In the case of regular fat cheese, the heating and cooling transitions makes the cheese softer. However, in the case of reduced fat cheese, the differences between the heating and cooling curves were smaller, and both eventually reaching about the same value at 10(C. During the 30 min rest period between heating and cooling, the reduced fat cheese actually became tougher at 60°C.

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.

Figure 1. Dynamic moduli of Regular fat Cheddar Cheese during Heating and Cooling

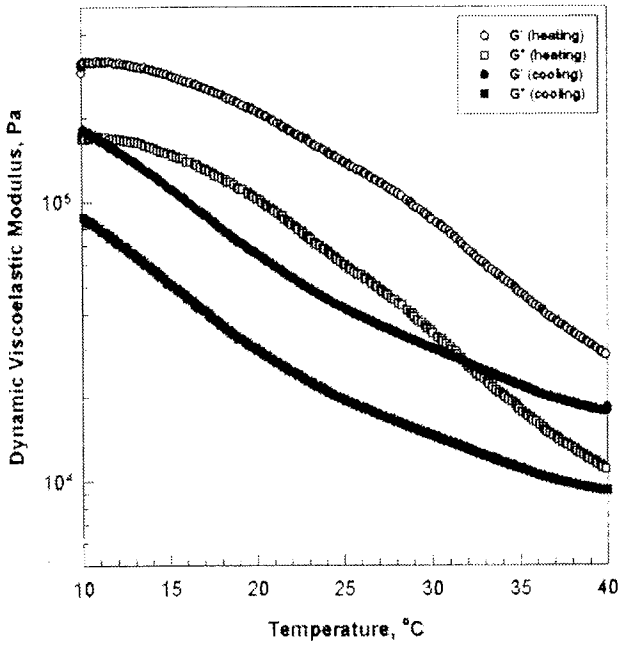
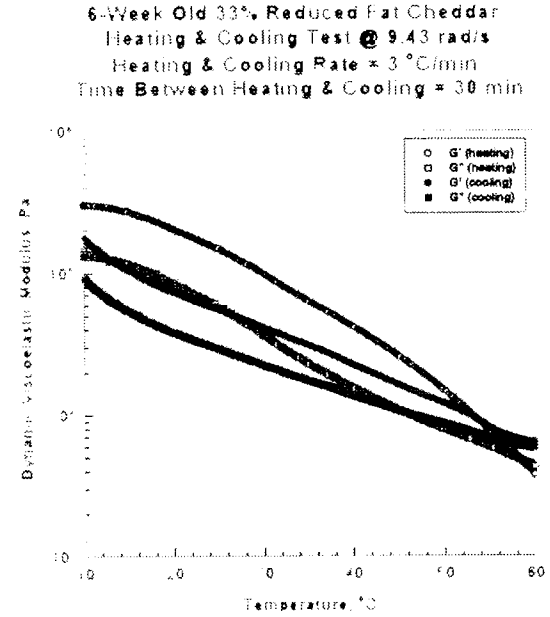


Figure 2. Dynamic Moduli of Reduced fat Cheddar Cheese during Heating and Cooling



FINAL REPORT

Temperature profiles of cheeses during melting in convection and microwave ovens

Personnel: S. Gunasekaran, professor, Biological Systems Engineering, K. Muthukumarappan, research assistant, Dept. Of Food Science, And L. T. Marschoun, Biological Systems Engineering

Funding

Dairy Management Inc. GNM97

Dates

July 1996 — June 1997

Objectives

1. 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.
2. To compare the experimentally determined thermal properties against composition based thermal properties predictive models.

Summary

Cheddar cheeses of different composition were manufactured at the CDR and their thermal properties were measured. For aging study, thermal properties of the cheese were measured repeatedly in certain time interval. Additional Cheddar cheese were bought from Pilot Plan and CDR. The composition of the cheeses was determined according to standard chemical methods. The composition of Cheddar cheese used in this study ranged from 30-60% water content, 8-37% fat content, and 22-36% protein content.

Thermal Properties

The following thermal properties were measured:
1. thermal conductivity (k) – determined by the line heat source method
2. thermal diffusivity (α) – determined by the Dickerson method
3. heat capacity (C) – determined using a differential scanning calorimeter (DSC, Netzsch) available in the Chemical Engineering Department at the UW-Madison.

The necessary probes and instrumentation for k and α measurements were designed and fabricated in the Biological Engineering Department's machine shop. In addition, density of the samples were determined to calculate α using the experimental k and C values.
The following models were developed for predicting thermal properties of Cheddar cheese based on its composition:

$k = 0.445 - 0.00415\,F - 0.00116\,W + 0.00395\,P$ ($R^2 = 0.953$)

$\alpha = -10.3 + 0.155W + 0.177F + 0.453P + 0.00061W*F - 0.00634W*P - 0.0076F*P$ ($R^2 = 0..560$)

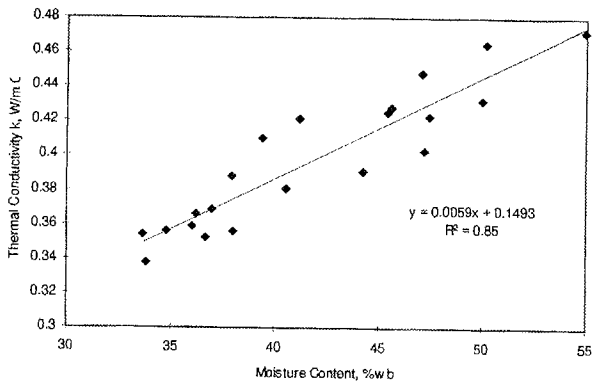
$Cp = 4.408 + 0.02742\,W - 0.01973\,F - 0.08368\,P$ ($R^2 = 0.505$)

Where W, F, and P are percent water, fat, and protein respectively.

The major findings were:

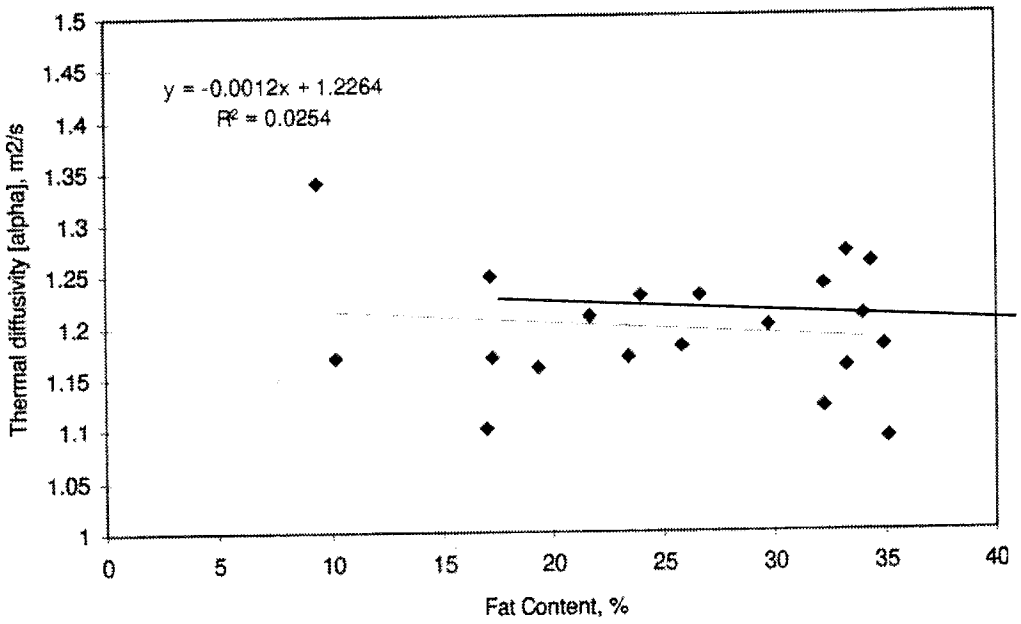
The thermal conductivity of Cheddar cheese ranged from 0.354 - 0.481 W/m°C. The thermal conductivity increased with moisture and protein content and decreased with fat content. Figure 1. illustrates the strong linear relationship between k and cheese moisture content.

Figure 1. Thermal conductivity vs. moisture content of Cheddar cheese



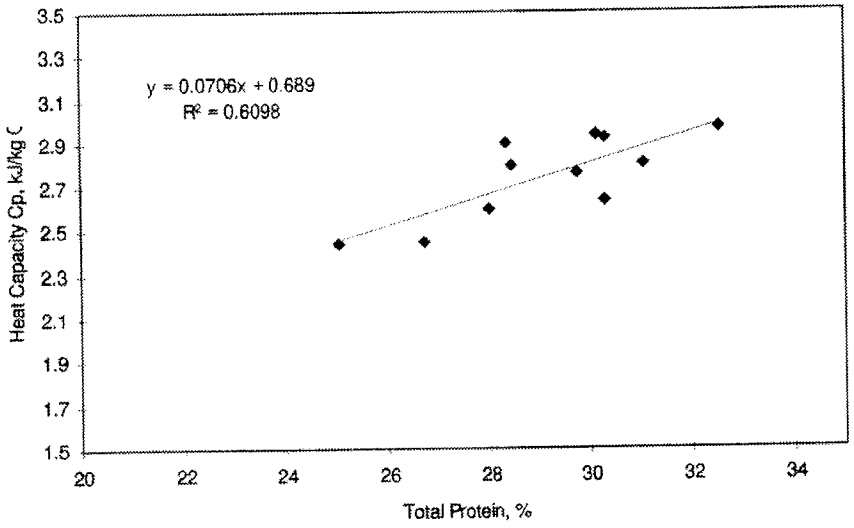
The thermal diffusivity of Cheddar cheese ranged from 1.07×10^{-7} - 1.53×10^{-7} m^2/s. The experimental data of thermal diffusivity of Cheddar cheese were hampered by high measurement errors and poor statistical correlations (Figure 2).

Figure 2. Thermal diffusivity vs. fat content of Cheddar cheese



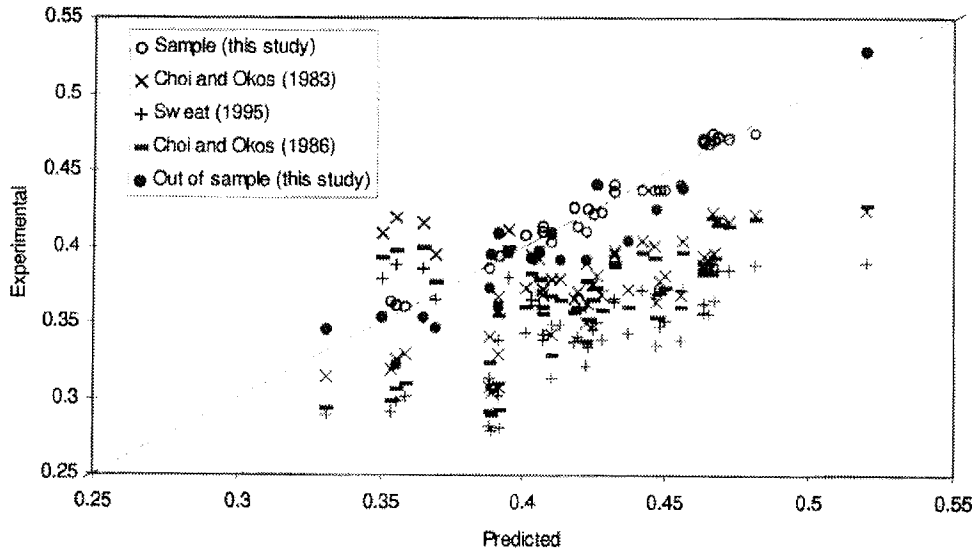
The heat capacity of Cheddar cheese ranged from 2.4 - 3.1 kJ/kg°C. Heat capacity increased with moisture and protein content but decreased with fat content. An example curve of C vs. cheese protein content is presented in Figure 3.

Figure 3. Heat capacity vs. protein content of Cheddar cheese



No statistically significant effect (at the 10% level) of age on thermal properties could be detected. The models we have developed were better than the currently available models for predicting thermal properties of Cheddar cheeses. Figure 4 depicts the experimental and predicted values of several models including the one we have developed for thermal conductivity.

Figure 4. Comparison of experimental and predicted values of thermal conductivity of Cheddar cheese using a number of models available in the literature and from this study.



Dielectric Properties:

The dielectric properties, dielectric constant (ε') and loss factor (ε") 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.

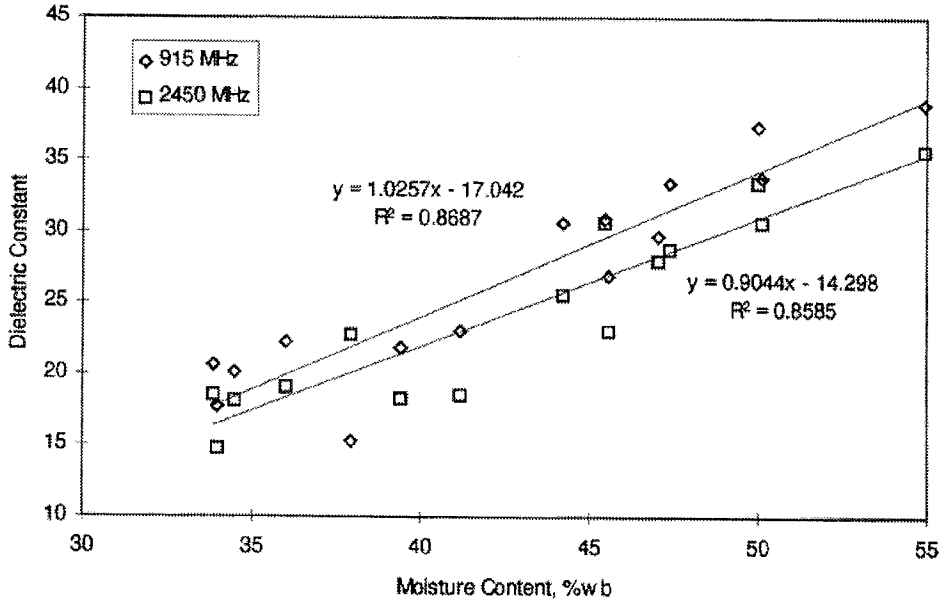


Figure 5. Dielectric constant vs. moisture content of Cheddar cheese

The major findings were:

Dielectric constant and dielectric loss of Cheddar cheese increased as moisture increased (Figure 5).

- The presence of salt slightly increased the dielectric constant and the dielectric loss of Cheddar cheese. The result indicated binding and complexing processes of salt with other food constituents.
- Both dielectric constant and dielectric loss of Cheddar cheese decreased as the fat content increased due to low dielectric activity of fat (Figure 6).
- A positive correlation between total protein content and dielectric constant was found (Figure 7).
- The effect of age on dielectric properties of Cheddar cheese was not clear.
- The composition-based prediction models available in the literature could not be used for the dielectric properties of Cheddar cheese because they were only valid for narrow compositional ranges different from that of Cheddar cheese used in this study.

The results of this study provides first of its kind data on thermal and dielectric properties of Cheddar cheeses. The functional relationships (models) we have developed between the properties and cheese compositional factors will be of significant importance in developing cheeses and/or methods to prepare cheese-containing foods.

Figure 6. Dielectric constant vs. fat content of Cheddar cheese

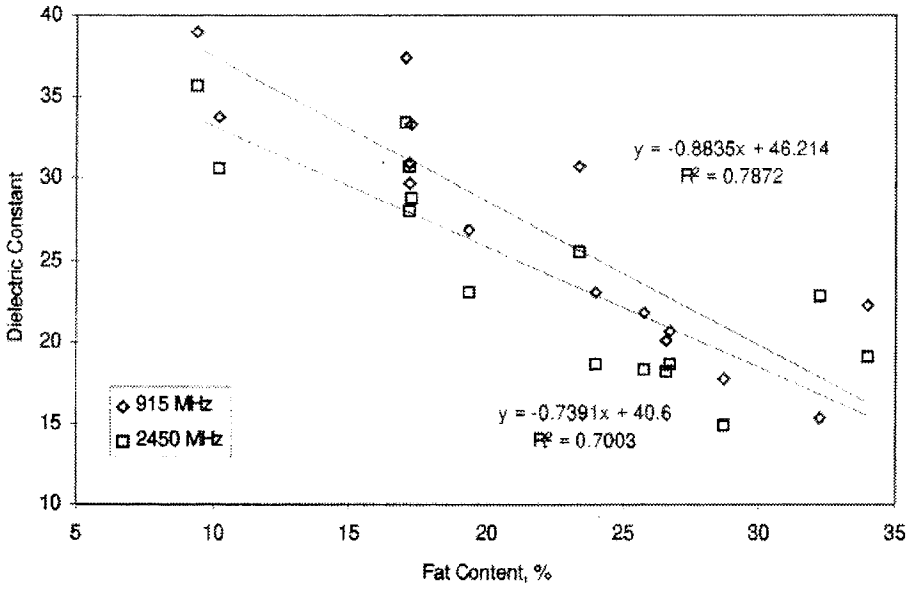
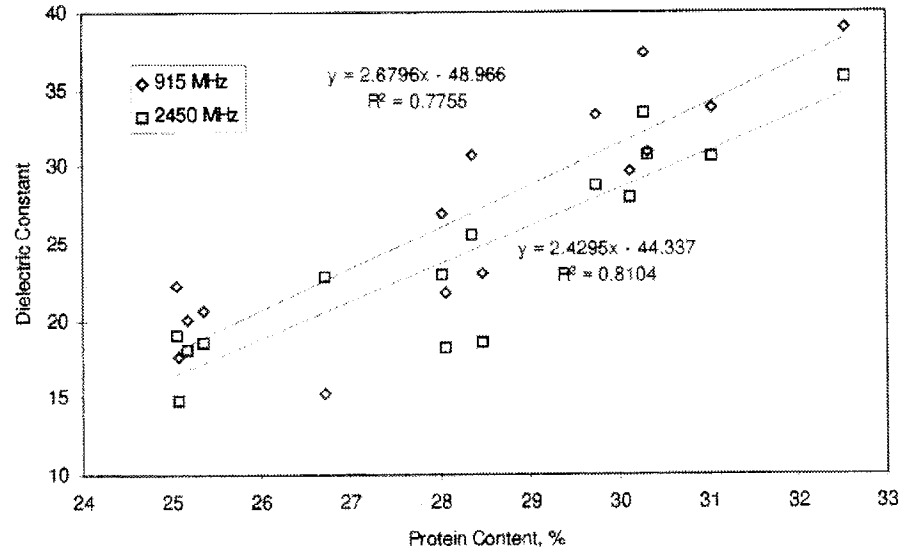


Figure 7. Dielectric constant vs. protein content of Cheddar cheese



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, professor, Biological Systems Engineering. M-I. Kuo, 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

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

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

3. 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.

4. 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 of post manufacture) the Mozzarella cheese exudes free moisture at the block and freshly cut surfaces, making it unsuitable for shredding and melting. Freezing of the block and/or shredded Mozzarella cheese soon after its manufacture is also preferred since it improves 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 concomitant changes in cheese properties will enable us to formulate methods to optimize the desirable end-use properties of the cheese. Such an investigation is also needed on the "pizza cheese" newly developed at the University of Wisconsin. This is a non-stretched, non-brined, stirred curd cheese which is comparable to the LMPS (low-moisture, part-skim) Mozzarella cheese in composition, melt and stretch properties. During maturation, apparently the free moisture is bound to the cheese structure and makes the cheese suitable for shredding and melting. However, the exact nature of cheese matrix-water interactions has not been investigated.

We investigated the water distribution and mobility in LMPS (low-moisture, part-skim) Mozzarella cheese during early stages of maturation using the NMR (nuclear magnetic resonance) techniques.

LMPS Mozzarella cheese was manufactured at the dairy plant of the Food Science Department at UW-Madison and used. The NMR experiments were conducted in the Biochemistry Department at UW-Madison using a Bruker Instruments DMX-400 Advance console, 9.4 T wide-bore magnet spectrometer. Tests were carried out at 5^0C on 2, 4, 6, 8 and 10 days post manufacture. The inverse-recovery pulse sequence was employed to determine T_1. The Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences were used to measure T_{2s} of different ranges. The two-component model was used to analyze the spin-spin relaxation data. This resulted in two amplitudes (A_{21} and A_{22}) with two spin-spin relaxation time constants (T_{21} and T_{22}).

The T_{22} increased slightly with increasing maturation time, suggesting water in this T_2 range became more mobile. No significant change in T_1 and T_{21} was ob-

served. An increase in A_{21} (and decrease in A_{22}) suggested a shift of moisture from region of high mobility to region of lower mobility. These results indicate a redistribution of amplitude and mobility between two water fractions. The quantification of nature and extent of water mobility and redistribution would help control cheese quality factors relevant for further processing.

INTERIM REPORT

Investigating reasons for hardening of reduced fat cheddar cheese during heating

Personnel: S. Gunasekaran, 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 1999

Objectives

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

2. Evaluate the 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.

3. Evaluate the usefulness of certain emulsifying agents (KCl and Na-Citrate) and a surfactant (Tween 20) to reduce hardening of lower fat cheeses.

4. Evaluate if the experimental approaches proposed in this project will help to alleviate the skin formation which occurs when very low fat cheeses are heated.

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

Summary

Earlier work in our laboratory showed that the viscosity of melted reduced fat Cheddar cheeses increased when the softened/melted cheese was held at high temperatures before it was 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. It is known that hydrolysis of α_{s1}-CN influences cheese softening and hydrolysis of β CN affects its meltability. Therefore, selective and relative hydrolysis of these two major casein fractions in cheese may enable independent control of cheese softening and melting. We investigated the use of combination of enzyme from *C. parasitica* and chymosin to regulate the relative hydrolysis of α_{s1}-CN and β-CN and determined the resulting physical and sensory properties of cheeses.

Four Cheddar cheeses (labeled A, B, C, and D) were manufactured using chymosin and the enzyme from C. *parasitica* in four ratios: Cheese A=1:0; Cheese B=0:1; Cheese C=2:1; and Cheese D=1:2 and. The cheeses were aged 12 wks, allowing for hydrolysis of casein fractions to occur. The following properties of cheeses were measured: hardness (by uniaxial compression); thermal meltability, and flavor, body, and texture attributes (by descriptive sensory panel analysis).

Cheeses A and C were weak in acid and bitter flavors. Cheese C was not different from A in bitterness, but showed a significant increase in hardness compared to Cheeses A and D. However, the meltability of Cheeses A and C was not significantly different. Cheese B melted the best and was firm (along with C) but, as expected, Cheese B was the most bitter. Therefore, a ratio of two coagulants in between those used for Cheeses B and C is recommend to increase both meltability and hardness and without any significant level of cheese bitterness.

Therefore, the enzyme from C. *parasitica*, which is now used for manufacturing Swiss cheese, can be used to manufacture Cheddar cheese, for better control of its softening and melting properties.

FINAL REPORT

Identification of potential gas-forming bacteria in cheese

Personnel: Mark Johnson, senior scientist, Kristen Houck, research specialist, Center for Dairy Research, John Luchansky, associate professor, Food Research Institute, Jeff Christenson, research assistant, Dept. of Food Science

Funding

Dairy Management Inc.

Dates

July 1996 — June 1997

Objectives

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

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

Summary

Classical microbiological plating techniques have been used to differentiate or at least enumerate bacteria when the causative organism was the dominant bacteria in a cheese. However, if the culprit organism was not the dominant bacteria, or not viable, the problem of isolation became much more difficult, if not unlikely. Another method, PCR amplification, can be used to identify and verify bacteria not found by classical plating methods. In this study, DNA sequences for 16s rRNA were obtained for target species. The target species were a variety of bacteria commonly found to produce gas in cheese. Downloaded sequences were grouped by species and compiled 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 were used to search DNA sequence databases to determine the uniqueness of the proposed primers.

Experimental determination of primer specificity was performed in duplicate with the targeted bacterial species. Chromosomal DNA was extracted from pure cultures and screened using PCR with all species specific primer sets. The DNA products of the PCR (if any) were electrophoresed, sized and quantitated. The results of these screens were tabulated and they indicated that the primers were specific for the targeted bacterial species. Though some cross-specificity of primers occurred, band patterns and size allowed unambiguous identification of each isolate screened.

Extraction of bacterial DNA from cheese (composed of a complex matrix of high fat and protein levels, which can inhibit PCR reactions) was accomplished by a two step procedure. First, the cheese slurry was concentrated through centrifugation. This step separated the fat layer from the concentrated cheese slurry pellet. During centrifugation, cells remained in the pellet (established microscopically). Chemical extraction of DNA was then performed on the remaining pellet. In order to estimate detection sensitivity, cheeses were homogenized with serial dilutions of various cultures. Using species specific primers, it was possible to detect DNA from as low as 104 CFU/g of the added bacteria. DNA from starter bacteria (at 108 CFU/g) did not interfere with the detection of DNA from added bacteria. Sensitivity was enhanced to correctly identify DNA from 102 CFU/g added bacteria by a Qiagen DNA affinity column (Westburg b.v., Leusden NL) purification step after chemical extraction of DNA. This demonstrates that DNA from as few as 100 bacteria per gram cheese could be extracted from cheese and detected using PCR and that this DNA could be detected in the presence of DNA extracted from as many as a billion other bacteria. If a selective agar has been developed for a specific bacteria, numbers of that bacteria can be determined in cheese even if the numbers are less than 10 per gram. If there were not a selective agar for a particular bacterium, it would be nearly impossible to detect it if other bacteria outnumbered it by as little as 100 fold. This may be the case with gas forming bacteria in cheese. They may be greatly outnumbered by other bacteria and there may or may not be a selective agar media for their detection. Traditional methods for the detection of heterofermentative *Lactobacillus* sp. from cheese is to first plate the cheese on a selective media that allows

the growth of most species of *Lactobacilli.* Colonies are then picked from the plate and placed into a tube of broth for growth and gas detection. If a gas producer is present but in low numbers compared to the other *Lactobacilli sp.* a colony of it may not be picked from the agar plate (indeed there may not be any representative colonies of it on a plate, i.e. it was diluted out). The PCR technique would not be able to enumerate the gas-producing bacteria but it would be able to detect the presence of it. This would be useful for companies to prescreen cheeses for use as a food ingredient. If the food contained sugar, even a low level of heterofermentative bacteria could pose a problem. In addition, since specific primers can be used to detect specific bacteria, it may be possible to determine the ecological niche or source of a specific bacteria.

Both PCR and conventional plating techniques were used to detect the bacteria responsible for the gas in a cheese with a visible gas defect. The agar used was selective for heterofermentative Lactobacilli. After incubation, representative colonies were picked from plates and placed in broth tubes. Extraction of DNA was performed on both those tubes containing gas and the gassy cheese. PCR was conducted with several primers for heterofermentative, homofermentative and facultative *Lactobacilli sp.* Primers detected the same species in both the cheese matrix and broth tubes. The PCR tests on gas producing isolated strains indicated the presence of facultative *Lactobacilli (Lb. plantarum).* This was surprising since this organism has not been implicated as the causative bacteria for the type of gas defect (internal gas holes) observed in the cheese. Obligate heterofermentative organisms such as *Lb. buchneri* and *Lb. fermentum* were not detected by PCR. This showed that conventional techniques for the detection of obligate heterofermentative Lactobacilli may give ambiguous results (non-heterofermentative species would grow on the agar), and that facultative Lactobacilli are capable of producing a sufficient quantity of gas to show visible defects in cheese.

In conclusion, PCR can be a useful tool for identifying low levels of potential gas formers within a mixed bacterial population. This is not always possible with conventional plating techniques especially if there is not a specific selective medium for the gas-producing bacteria. PCR can also be used to verify the efficacy of traditional isolation techniques. PCR is less time consuming than conventional plating- preliminary results are ready within two days. This is especially true where classical microbiological methods are unable to provide answers after several days or, at best, give ambiguous results. A data bank of specific primers would give testing laboratories a means of identifying the presence of any known gas-producing bacteria in cheese without having to first isolate the bacteria on agar. This technique would be useful as a screening tool for cheese to be used as a food ingredient. It could also be useful to determine the appropriate agar and growth conditions to enumerate the gas-producing bacteria if that is necessary.

INTERIM REPORT

Growth of nonstarter lactic acid bacteria in reduced fat Cheddar cheese

Personnel: Mark E. Johnson, senior scientist, Kristen Houck, research specialist, Wisconsin Center for Dairy Research, James Steele, associate professor, Bilal Dosti, graduate research assistant, Department of Food Science, University of Wisconsin, Jeff Broadbent, Rebekah Allen, research specialist, Food Science Department, Utah State University, Logan, Utah.

Funding

Dairy Management Inc. CW11

Dates

July 1997 – December 1999

Objectives

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

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

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

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 nonstarter bacteria. We will then be able to follow the individual growth and death of specific bacteria in cheese. It has been confirmed (Jeff Broadbent) that we can differentiate between individual strains of *Lactococcus lactis, Lactobacillus casei,* and *Lactobacillus helveticus.* DNA can be isolated from cheese and used as a template for amplification of 16S rRNA genes that will allow us to speciate bacteria that we can culture directly from cheese as well as determine the presence of bacteria that we may not be able to culture in the laboratory.

Experiments are being conducted on *Lactobacillus casei* strains to establish specific enzyme activity (L and D-lactate dehydrogenases), racemase activity, and citrate utilization. This strain (s) will be used in later cheese making experiments. Preliminary results indicate that a separate racemase enzyme (not a lactate dehydrogenase enzyme) is responsible for the conversion of L-lactate to D- lactate. Work is now in progress to confirm this finding. This will eventually enable us to determine the necessity of the racemase activity for growth in cheese.

The first cheese making trials were completed in November, 1998. The first sensory testing will be completed in February 1999 (3-months). Prior to cheese making, the dairy plant, equipment, and raw and pasteurized milk were sampled to obtain a representative number of bacteria. These will later be analyzed (through PCR analysis) to determine whether these bacteria are also found in the cheese. 50% reduced fat Cheddar cheeses were made with or without a water rinse and with or without a Lactobacillus adjunct. Bacteria are being isolated from each cheese at various intervals and frozen for future PCR analysis. The Lactobacillus adjunct was added to determine whether an initially dominant Lactobacillus sp. would continue to dominate the cheese microflora throughout ripening. This adjunct is the same Lactobacillus sp. being studied for racemace activity.

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

APPLICATIONS PROGRAM REPORT

CDR specialty cheese applications program

Personnel: Jim Path, outreach specialist, John Jaeggi, assistant researcher, Joanne Gauthier, communications specialist

Funding

Wisconsin Milk Marketing Board, UWA9703

Dates

January 1998—December 1998

Objectives

1. Continue deveoping the Artisan workshops, a module of the Wisconsin Master Cheesemaker® Program.

2. Provide technical support to cheesemakers, including workshops, consulting, and on site manufacturing trials.

3. Manage the Wisconsin Master Cheesemaker® Program.

4. Develop a cheese database.

Summary

Artisan workshops
On Feb 24-25, 1998 the third Wisconsin Process Cheese Course was held as part of the Wisconsin Master Cheesemakers curriculum. The course filled again. It is still the only course of it's type in the USA.

Norhern European Seminar was held May 19-20, 1998. Attendance: 22.

Trappist Cheeses Seminar and Cheddar Flavor Workshop was held November 17-19, 1998. Attendance: 20.

Plant visits
We made approximately 30 plant visits in 1998.

Master Cheese Maker program
Four new Wisconsin Master Cheesemakers (class of 1998) were certified by the Master Cheesemaker Board on December 3, 1997. They were officially recognized in April of 1998, at the CDR/WCMA ceremony in Madison, Wisconsin. The eight cheesemakers in the class of 1999 have completed a second round of cheese sample testing during the apprenticeship phase of their program. The next class, class of 2000, have completed the oral exam and plant visit phase of the apprenticeship.

Computer library
The work on the computer library of cheese names, descriptions and manufacturing procedures is continuing. We are in the process of entering descriptions and manufacturing procedures to the cheeses.

FINAL REPORT

Contribution of endopeptidases from *Lactobacillus helveticus* CNRZ32 to cheese flavor development

Personnel: James L. Steele, associate professor, Kurt M. Fenster, research assistant, Yo-Shen Chen, research assistant, Kirk L. Parkin, associate professor, Dept. of Food Science, Mark E. Johnson, senior scientist, Center for Dairy Research

Funding

Dairy Management Inc. SPJ95

Dates

July 1994—June 1997

Objectives

1. Characterization of two genes.

2. Construction of CNRZ32 derivatives with altered endopeptidase activities.

3. Evaluation of the contribution of individual endopetidases to degradation of casein-derived peptides.

4. Purification and characterization of selected endopeptidase(s).

5. Determination of the role of selected endopeptidase(s) in CNRZ32's ability to reduce bitterness and accelerate cheese flavor development.

Summary

A genomic library of *Lactobacillus helveticus* CNRZ32 previously constructed in *Escherichia coli* DH5a was screened for endopeptidase activities using the substrates N-Bz-Phe-Val-Arg-NHPhNO_2, N-Bz-Pro-Phe-Arg-NHPhNO_2, and N-Bz-Val-Gly-Arg-NHPhNO_2. Two isolates, which had qualitatively different endopeptidase activities, were identified from this screening. One clone hydrolysed N-Bz-Phe-Val-Arg-NHPhNO_2 and N-Bz-Pro-Phe-Arg-NHPhNO_2, but did not hydrolyse N-Bz-Val-Gly-Arg-NHPhNO_2. The gene encoding this endopeptidase activity was sequenced and found to have identity with thiol-dependent general aminopeptidases (PepC and PepG) from a variety of lactic acid bacteria. The endopeptidase activity encoded by this gene was designated PepE. The second clone hydrolysed N-Bz-Val-Gly-Arg-NHPhNO_2, but not N-Bz-Phe-Val-Arg-NHPhNO_2 and N-Bz-Pro-Phe-Arg-NHPhNO_2. The gene encoding this endopeptidase activity was sequenced and found to have identity with the metal-dependent endopeptidase (PepO) from *Lactococcus lactis*. The activity encoded by this gene was designated PepO.

Nucleotide sequencing of *pep*E revealed a 1,314 bp ORF, which could encode a protein of 52.1 kDa. Putative -10 and -35 transcriptional promoters were identified, which indicates that *pep*E may be transcribed from its own promoter. Also, a putative *rho*- independent transcriptional terminator (DG=-25.4 kcal/mol) was observed in the 3'-noncoding region. The presence of these putative transcriptional promoter and terminator sequences suggest that *pep*E is transcribed monocistronically. PepE is probably located intracellularly, because no signal sequence was detected at the N-terminus of the amino acid sequence deduced from *pep*E.

Protein sequence homology searches revealed that PepE had a high amino acid sequence identity with PepC from *Lactobacillus delbrueckii* DSM7290, *Lb. helveticus* CNRZ32, *Streptococcus thermophilus* CNRZ302, and *Lactococcus lactis* AM2 as well as PepG from *Lb. delbrueckii* DSM7290. The amino acid sequence identities of PepE with the PepC proteins from these bacteria were 41.7%, 40.8%, 39.1% and 37.4%, respectively. The amino acid sequence identities between PepE and PepG was 72.3%. A search of the PROSITE Dictionary of Protein Sites and Patterns with the deduced *pep*E amino acid sequence identified two highly conserved domains involved in substrate binding and catalysis that are characteristic of proteinases from the cysteine proteinase family. The amino acid residues instrumental in substrate binding and catalysis by cysteine proteinases of procaryotic and eucaryotic origin were found to be conserved in PepE (Gln-64, Cys-70, His-362, Asn-383, and Trp-385).

Phe-Val-Arg-pNA and N-benzoyl-Val-Gly-Arg-pNA, respectively. The introduction of the CNRZ32 *pepO* into *Lc. lactis* LM0230 on the low copy number (6-9 copies/cell) vector pTRKL2 did not result in a significant increase in endopeptidase activity.

Two transcripts were detected in CNRZ32 throughout the growth phase. One transcript had a calculated size of 2.2 kb which corresponds to the ORF of *pepO*. The other transcript had a calculated size of 1.5 kb. Similar analysis with a CNRZ32 *pepO*$^-$ mutant detected transcripts of 1.8 kb and 1.5 kb.

In summary, data which suggest that the CNRZ32 *pepO* gene is monocistronic include i) the putative promoter region and the putative terminator, and ii) results from Northern hybridization. High level of protein sequence homology to PepO from *Lc. lactis* P8-2-47 suggests an ancestral association between these two enzymes. While a metalloprotease motif (His-Glu-Xxx-Xxx-His) identified from the deduced PepO sequence was also present in the P8-2-47. In contrast to the lactococcal *pepO*, nucleic acid sequence analysis of 2.82 kb upstream and 0.55 kb downstream of the CNRZ32 *pepO* gene, suggests that this gene is not associated with oligopeptide transport genes. A second copy of *pepO* in lactococcal strains, designated *pepO2*, has been recently reported (M.A. Hellendoorn, I. Mierau, M. van der Horst, G. Venema and J. Kok. Abstr. FEMS 5th Symp. on LAB. 1996, K13). The presence of single bands in Southern hybridizations using *pepO* as the probe in both CNRZ32 and its *pepO*$^-$ derivatives indicate that there is only one copy of *pepO* in CNRZ32. Additionally, the >94% reduction in hydrolysis of N-ben-Val-Gly-Arg-pNA by the *pepO*$^-$ mutant suggests that there is a single copy of *pepO* in CNRZ32 and that this substrate could function to selectively quantify PepO activity. To evaluate the physiological role of PepO, studies were conducted to compare the growth and acidification rates of CNRZ32 wild type, *pepO*$^-$, *pepO*$^-$ *pepX*$^-$, and *pepX*$^-$ mutants in both amino acid defined media and skim milk. The results revealed that the *pepO*$^-$ and *pepO*$^-$ *pepX*$^-$ strains did not differ significantly in their growth or acidification rates from wild type and *pepX*$^-$, respectively. This is similar to the results reported by Mierau et al. for PepO in lactococci. These results suggest one or more of the following: (i) that PepO is not involved in the hydrolysis of milk-derived peptides, (ii) that other peptidases possess overlapping specificities with PepO, or (iii) that alternative milk-derived peptides can be utilized to obtain essential amino acids. Endopeptidases probably contribute to cheese flavor development. However, the contribution of PepE and PepO to cheese flavor development is not known. There is evidence for a third endopeptidase in CNRZ32 which may also contribute to cheese flavor development. PePE and PePO do not hydrolyze β-casein fragment 193-209, although the third endopeptidase activity identified appears to hydrolyze this substrate.

FINAL REPORT

Construction of exopolysaccharide-producing strains of *streptococcus thermophilus* with increased bacteriophage resistance

Personnel: James L. Steele, Associate Professor, UW-Madison Department of Food Science, Joseph M. Sturino, Research Assistant, UW-Madison Department of Bacteriology

Funding

Wisconsin Milk Marketing Board UW9603

Dates

June 1996—June 1998

Objectives

1. To evaluate the effectiveness of three *Lactococcus lactis* bacteriophage resistance determinants (AbiA, AbiC, and LlaI) as expressed by various industrial strains of *Streptococcus thermophilus.*

2. To screen industrial strains of *S. thermophilus* for native bacteriophage-resistance mechanisms or to identify loci involved in lytic bacteriophage sensitivity or proliferation.

3. To clone and characterize the genes encoding bacteriophage defense or sensitivity mechanisms and construct bacteriophage resistant stains.

Summary

Exopolysaccharide (EPS) producing cultures of *S. thermophilus* are an important tool in the manufacture of Italian cheeses and possess unique functional properties. The exopolysaccharides function in the cheeses to bind water, increase viscosity and affect mouth feel. The dairy industry is producing more low fat cheese varieties, so these functional attributes, especially water binding, are increasing in importance. As seen with other lactic acid bacteria, prolonged use of *Streptococcus thermophilus* has resulted in the emergence of lytic bacteriophages specific for industrial isolates.

Traditional methods of isolating and developing unrelated strains of *S. thermophilus* that possess increased bacteriophage resistance and consistent functional properties have not been successful. Therefore, we used the tools of biotechnology in order to construct exopolysaccharide-producing strains of *S. thermophilus* with enhanced bacteriophage resistance.

Insertion sequence *S1* (IS*S1*) mediated mutagenesis has been successfully employed in the construction of bacteriophage-insensitive derivatives of lactococci. Insertional mutagenesis allows for the random inactivation of host encoded genes involved in lytic bacteriophage adherence and maturation. We applied this approach to *S. thermophilus* utilizing the temperature sensitive integration vector pG$^+$host9::IS*S1*. This approach yielded eighteen bacteriophage insensitive mutant (BIM) derivatives of *S. thermophilus* JLS130 designated BIM1-BIM18. The mutant designated as BIM5 was excluded from further study due to its slow growth rate.

IS*S1*-specific hybridization studies were performed to determine the number of unique integrations represented by BIM1-BIM4 and BIM6-BIM18. A total of nine banding patterns were found in the seventeen mutants tested. Since pG$^+$host9::IS*S1* integration can occur into a single locus in two different orientations, it became clear that seven distinct loci involved in bacteriophage sensitivity had been identified.

Efficiency of plaquing (EOP) and bacteriophage adsorption tests were used to quantitatively assess the level of bacteriophage resistance inherent to the various BIM strains in comparison to their JLS130 parent strain. Two genetically distinct bacteriophages, jls130-j and jls130-g, capable of infecting the JLS130 parent strain were used during these tests. Bacteriophage jls130-j is a *cos*-containing phage and encodes the conserved Sfi18 module identified by Brussow. In contrast, bacteriophage jls130-g is a *pac*-containing phage and *does not* encode the conserved ΦSfi18 module.

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, Edward G. Dudley, research assistant, UW-Madison Bacteriology

Funding

Dairy Management Inc. 133BM89

Dates

July 1997 —December 1999

Objectives

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

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

3. Evaluate 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

Succinate is an organic acid known to affect the flavor of fermented foods and beverages. Nonstarter lactobacilli are primarily responsible for producing succinate in Cheddar cheese, however limited information exists concerning the pathways used.

Two strains of *L. plantarum*, twelve strains of *L. casei*, and eight strains of *Lactobacillus rhamnosus* (formerly *L. casei* subsp. *rhamnosus*) were grown to carbohydrate exhaustion in a complex medium under anaerobic conditions, and were resuspended in phosphate buffer saline pH 7.0 containing one of the following: 10mM citrate, 10mM L-lactate, 10mM citrate plus 10mM L-lactate, or 10mM aspartic acid. After 3 days incubation at 37°C, succinate production was detected under all four conditions for *L. plantarum* ATCC 14917, and under all conditions except for aspartic acid for *L. plantarum* ATCC 14431. No succinate production was detected with any *L. casei or L. rhamnosus* strains studied. Current work includes studying succinate production by *Lb. casei* and *Lb. rhamnosus* under different conditions. Whole cells of ATCC 14917 and ATCC 14431 converted approximately 44% and 15% of the citrate and 33% and 5% of the lactate to succinate, respectively. Additionally for both strains, the amount of succinate produced in the presence of both citrate and L-lactate was higher than the sum of the amounts produced by citrate and L-lactate alone. Therefore, it appears that *L. plantarum* possesses at least three distinct biochemical pathways for succinate production, and these strains are able to cometabolize citrate and L-lactate.

Currently, ^{13}C-NMR spectroscopy is being used to deduce the citrate catabolic pathways of *L. casei and L. plantarum*, and the L-lactate and aspartate catabolic pathways of *L. plantarum*. Future experiments will include repeating the above whole cell incubations at pH 5.1, and studying the biochemical reasons for the differences in citrate, L-lactate, and aspartate catabolism between *L. casei* and *L. plantarum*. The results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

APPLICATIONS PROGRAM REPORT

Whey applications research program

Personnel: Kimberlee J. Burrington, coordinator, Karen Smith, researcher

Funding

Wisconsin Milk Marketing Board, Dairy Management, Inc.

Dates

January 1998—December 1998

Objectives

Enhance the value of whey-derived ingredients by providing technical support to the whey processing industry. Provide processing and applications support for whey, permeate, lactose, whey protein concentrate, whey protein isolate, and whey protein fractions.

Conduct industry directed whey applications projects which evaluate the functional attributes of specific whey ingredients in finished food systems. Areas of food applications for whey ingredients are dairy and bakery products, beverages, soups, sauces, meats, neutraceuticals, and infant formula.

Develop a pilot plant facility which provides the ability to conduct whey processing projects with industry, to evaluate existing and new processing conditions. The pilot plant should be able to process whey from the cheese vat to the spray dried ingredient.

Summary

This year completed the first year of the whey applications program. In 1998, the whey applications program was in contact with 9 Wisconsin-based whey processors and 15 national whey processors, ingredient suppliers, and whey end-users. Many of the activities of this first year focused on setting up the program by visiting the regional whey processors to discuss their technical support needs. Both processing support and applications support were needed. Most of the regional and national processors need access to a whey processing pilot plant. Introductions to the program, as well as general whey processing and whey functionality/applications information were presented 10 times over the course of the year.

The focus of applications development was in the bakery products area. An energy bar was developed with a whey protein isolate as the primary protein source. This energy bar was shown at the CDR Open House, the American Association of Cereal Chemists meeting, and the Minnesota and Chicago IFT Suppliers Night meetings. Another project evaluated whey ingredients in cookie and cake applications with sensory, textural and chemical analysis assessed over the shelf life of the products.

Many of the needs of the whey processors and end-users have been informational needs. Typical requests are for standard methods for chemical and functional analysis, specifications, whey ingredient sources, literature searches, formulations for specific applications, and processing trouble-shooting questions.

Development of the pilot plant was initiated with ordering of parts for current equipment, investigation of needed equipment and the requests for funding for the primary components of the pilot plant. Requests for funding are in place for ion exchange and microfiltration equipment, a separator/clarifier, and spray drier.

Presentations

Whey Applications Program, Concentrated and Dried Milk and Whey Products Symposium. Kimberlee J. Burrington. March 30-31, 1998, San Francisco, CA

Whey Applications Program, CDR Open House. Kimberlee J. Burrington. April 21, 1998, Madison, WI.

CDR's New Whey Applications Program, Wisconsin Whey and Whey Utilization Short Course, Kimberlee J. Burrington, May 5-6, 1998, Madison, WI.
Whey Utilization in Dairy Products, Wisconsin Whey and Whey Utilization Short Course, Karen Smith, PhD. May 5-6, 1998, Madison, WI.

VISITING SCIENTIST

Enhancing the flavors of specialty cheeses made from cow's and sheep's milk blends

Personnel: Esther Sendra, Ph.D., Dept. of Food Science

Funding

Wisconsin Milk Marketing Board

Dates

Summer 1997

Objectives

1. To survey sheep's milk for the concentration of free and bound branched-chain fatty acids related to distinctive sheep flavors, including 4-methyloctanoic acid, 4-methylnonanoic and 4-ethyloctanoic acid, 2-ethylphenol, 3- and 4 -ethylphenol, p- and m- cresols.

2. To determine influences of stage of lactation as well as dry-lot and pasture diets on concentrations sheep flavor compounds.

3. To determine the differences in concentrations of key sheep flavor compounds between authentic Manchego, imported Manchego-style, and domestic and imported Manchego-style cheeses manufactured from cow's and sheep milk blends.

4. To relate results of flavor analyses of sheep's milk and cheeses to the flavor quality of domestic blended cow's and sheep's milk specialty cheese, and to recommend improvements in the distinctive sheep flavors in domestic blended cow's and sheep's milk cheeses.

Summary

Sheep's milk samples were obtained from two Wisconsin farms, UW-Sheep Research Station in Spooner,WI and Kaufman farms, Chippewa Falls,WI. The diet regime was dry lot and pasture supplemented with corn, respectively.

Cheese samples were commercial samples of imported Manchego-type cheeses (Spain, 100% sheep's milk) which had previously obtained high scores in a cheese contest in the US and Shepherd's Blend from UW Dairy Store (80% cow's-20% sheep's milk).

Methods for branched-chain fatty acids (Ha and Lindsay, 1990) and for free alkylphenols (Han, 1994) were employed to quantify key species-related, sheep flavor compounds in milks and cheeses.

Fatty acid contents

Typical n-chain fatty acid data for selected dry-lot and pasture milks are shown in Table 1. The potential flavor that can be developed from n-chain fatty acids milk in dry-lot and pasture milks appears to be similar. Both farms were feeding the same amounts of corn daily, although the UW diet was a cracked form in a concentrate and the Kaufman farm fed a whole kernel form.

Analysis of volatile branched-chain fatty acids (Tables 2, 3, 4) indicated that slightly higher amounts were present in the pasture milk. However, carbohydrate supplements are the main determinants for the formation of branched-chain fatty acid forms (Garton et al, 1972). Since both farms fed corn, the branched-chain fatty acid profiles were similar. Free 4-methyloctanoic (dairy sheepy) and 4-ethyloctanoic (goaty) acids were present in detectable concentrations in both milks; 4-Methylnonanoic, actually described as responsible for muttony flavor appears to be below its threshold value (Brennand et al, 1989).

The stage of lactation did not appear to affect the branched chain fatty acid profiles (data not shown).

Alkylphenol contents

Typical alkylphenol contents in dry-lot and pasture sheep's milks are shown in Table 5.
Contents of 2-ethylphenol , 3- and 4-ethylphenol increased when sheep were on pasture (Table 6), and these compounds have been described as responsible for sheepy flavors (Ha and Lindsay, 1991). Thus, cheese prepared from milks obtained from sheep on pasture would be expected to provide stronger, unique sheepy flavors. The stage of lactation did not appear to affect levels of alkylphenols (data not shown).

Table 1
Total fatty acids in sheep's milk (μg/g)[a]

sample	dry lot 1	dry lot 2	pasture 1	pasture 2
C4:0	2814	2346	2847	3235
C5:0	9	6	7	8
C6:0	1913	1533	1840	1894
C7:0	20	19	20	19
C8:0	1650	1394	1604	1630
C10:0	4334	3992	4644	4703
C11:0	40	38	42	44
C12:0	2426	2175	2490	2540
C14:0	3437	3685	4044	4515
C15:0	391	402	515	555
C16:0	18557	17812	19512	21595
C18:0's	25077	20257	25900	29865

[a] (± 10%)

Table 2.
Total volatile branched-chain fatty acids (ng/g milk).

	dry lot	pasture + grain
4-methyloctanoic	253	306
4-ethyloctanoic	21.6	50.1
4-methylnonanoic	6.67	8.61

Table 3.
Free volatile branched-chain fatty acids (ng/g milk).

	dry lot	pasture + grain
4-methyloctanoic	57	39
4-ethyloctanoic	3.14	3.81
4-methylnonanoic	1.35	1.43

Typical data for key sheep-flavored alkylphenol and fatty acid compounds in cheese are summarized below:

Free 3- plus 4-ethylphenol contents in cheeses:

Domestic cow's (80%) - sheep's (20%) blended milk cheese: 1.1 ng/g
Imported pure sheep's milk cheese: 6.7 ng/g (range 4.2 to 9.5)

Free 4-methyloctanoic acid contents in cheeses:

Domestic cow's (80%) - sheep's (20%) blended milk cheese: 145 ng/g

Imported pure sheep's milk cheese: 1042 ng/g (range 453 to 2270 ng/g)

These results show that the critical flavors in sheep's milk cheese which yield the unique sheep flavors are deficient in domestic blended cow's and sheep's milk cheeses. It has been reported in the literature that carbohydrate source affects branched-chain fatty acid production, and barley in the diet increases the formation of methyl-branched chain fatty acids such as 4-methyloctanoic. Barley is used extensively in Spain and this can partially explain differences in this important sheep's milk flavor compound. The most common sheep diet in Spain employs a mixed-system pasture plus concentrates. The most common sources of

Table 4
Total volatile branched chain fatty acids (μg/g milk)[a]

fatty acid	dry lot 1	dry lot 2	pasture 1	pasture 2
C4:0	2813.60	2343.21	4145.45	2846.63
2mC4	4.67	5.35	6.25	5.38
3mC4	0.38	0.45	tr	tr
2eC4	0.27	0.21	tr	tr
C5:0	9.36	5.75	2.17	1.34
3mC5	0.08	0.29	0.25	0.21
4mC5	0.20	0.40	tr	tr
2mC6	0.02	0.05	tr	tr
C6:0	1913.00	1532.61	2953.76	2385.37
2eC6	2.21	4.22	0.94	1.76
4mC6	0.08	0.06	0.06	0.66
C7:0	20.03	19.38	27.06	13.53
mC7	0.44	0.75	0.54	0.40
6mC7	0.08	0.14	0.02	0.04
eC7	0.14	0.11	0.35	0.06
C8:0	1650.39	1394.35	2437.04	2072.69
4eC7	0.02	0.09	0.01	0.03
4mC8	0.33	0.24	0.21	0.46
dmC8	0.03	0.04	0.04	0.03
C9:0	10.75	10.86	2.32	2.34
4eC8	0.03	0.01	0.04	0.06
4mC9	0.01	0.01	0.01	0.01
8mC9	0.09	0.11	0.12	0.11
dmC9	0.20	0.22	0.57	0.37
C10:0	4333.71	3992.26	5940.55	4592.46
mC10	0.45	0.22	0.25	0.23
2eC10	0.60	0.25	0.26	0.54
9decenoate	5.99	4.06	7.34	16.09
mC10	0.25	0.10	0.60	0.49
C11:0	39.74	38.46	58.72	43.06
mC11	0.01	0.01	0.15	0.08
decenoate	0.06	0.07	0.30	0.23
10undecenoate	0.03	0.05	0.15	0.18
mC11	0.06	0.06	0.35	0.32
C12:0	2425.68	2175.50	3538.07	2843.79

[a] (± 20%)
tr: trace
m:methyl
e:ethyl

carbohydrates are barley, soy flour, and by products from local food industries (citrus, brewery, sugar beet)

Based on our results, improving the unique dairy sheep flavor in blended cow's-sheep's milk cheese should be achieved by placing sheep on pasture, because it increases the content of sheepy-flavored phenolic compounds. In order to increase the amount of methyl-branched chain fatty acids, introducing barley and/or wheat in concentrates would give greater proportions of branched-chain fatty acids than corn does (Duncan et al., 1974), and hence amplify the sheep's milk flavor contribution.

Since sheep's milk presents a different kind of flavor profile than cow's milk, characterizing the key flavor compounds is necessary to develop methods to obtain a desirably flavored blended sheep's and cow's milk cheese with these unique sensory characteristics. From this study we have suggested modifications in the sheep's diet in Wisconsin which would yield more flavorful blended cheeses, and this includes grazing on pastures and using barley or wheat in concentrates. This research will serve as a basis for future efforts to improve distinctive blended cheese flavors by other means of dairy technology. Using blends of sheep's milk will expand cow's milk usage and provide more valuable, distinctive specialty cheeses.

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chapter 2 section 2

Cheese safety

INTERIM REPORT

Microbiological safety of reduced fat and fat free pasteurized process cheese products

Personnel: Eric A. Johnson, professor, Kathleen A. Glass, researcher, Food Research Institute

Funding

Dairy Management, Inc.

Dates

January 1997—December 1999

Objectives

1. Evaluate the effect of fat and fat replacers on growth of *Clostridium botulinum* in full fat, reduced fat, and fat free process cheese products.

2. Determine the efficacy of antimicrobials in full fat, reduced fat, and fat free process cheese products.

3. Identify factors that inhibit botulinal toxin production in full fat, reduced fat, and fat free process cheese products.

4. Develop the foundation to expand the FRI model to predict growth and toxin production by *Clostridium botulinum* in reduced fat and fat free process cheese products.

5. Evaluate the effect of moisture-fat-free and nonfat solids in process cheese products made with skim milk cheese, disodium phosphate, NaCl and water.

Summary

During the first year of our study we determined whether the safety of process cheese is affected by the type of natural cheese from which it is derived. Process cheese spreads were manufactured with natural cheese having one of three fat levels: full fat Cheddar cheese, 30% reduced fat Cheddar, or skim milk cheese. The cheese spreads were supplemented with ingredients to conform with the Federal Standard of Identity for pasteurized process cheese spread and the formulations were adjusted to 59% moisture, pH 5.8, and 3 or 4% total salts (sodium chloride + disodium phosphate). These formulations were chosen to permit growth and toxin production by *Clostridium botulinum*. In this way, differences in inhibition of growth and toxin production could be evaluated and statistically analyzed.

Results revealed that botulinal toxin was delayed two days in skim milk process cheese formulated with 4% salts compared with the other formulations tested. Additionally, the number of toxic samples were fewer for process cheese made with skim milk cheese compared with formulations manufactured with reducedfat or full fat cheese. The cheese base type used influenced the safety somewhat but did not appear to be overall significant to the inhibition of *C. botulinum*.

Our preliminary research evaluating growth of *Clostridium botulinum* suggested that moisture was not a valid indicator of safety in 5% fat and fat-free process cheese products. These products appeared to exhibit greater stability than full fat products with similar moisture, pH, and salts. We proposed that a more useful parameter to predict safety may be moisture-fat-free (MFF) to compensate for the different fat levels. In this reporting period we evaluated the effect of moisture-fat-free in process cheese products manufactured with skim milk cheese, disodium phosphate, sodium chloride, and water. All products were standardized to 2.8% total salts and pH 5.8. Product moistures evaluated were 70, 65, 60, or 55%. Each formulation was replicated three times. As expected, decreasing moisture delayed botulinal growth and toxin production. However, these formulations supported toxin production more rapidly than previous fat free trials formulated with added process cheese ingredients (whey products, fat replacers, flavor adjuncts, enzyme modified cheese, etc.). This observation suggests the safety of reduced fat process cheese products may rely on other parameters or the interaction of several ingredients.

Previous work also suggested that enzyme modified cheese (EMC) may inhibit growth and toxin production of *Clostridium botulinum* in media. We compared the proximate analysis and the antibotulinal activity in media of 13 types of EMC (including one EMC powder) supplied by six cheese manufacturers. The effect varied greatly among the cheeses and did not correlate with initial moisture, pH, water activity or salt. The most

APPLICATIONS REPORT

Safety/quality applications program

Personnel: Marianne Smukowski, coordinator, Kristen Houck, microbiologist

Funding

Wisconsin Milk Marketing Board, UWA9801

Dates

January 1998—December 1998

Objectives

1. Maintain and improve HACCP-based safety/quality programs used by manufacturers and producers

2. Continue strong relationships with DMI, IDFA, and FDA for implementation of HACCP-based applied technologies

3. Assist in executing national safety/quality program

4. Conduct technology development targeted at WI cheese manufacturers

5. Assist the Wisconsin Master Cheesemaker® Program

6. Participate and assist in UW and industry sponsored courses

Summary

The Safety/Quality Applications program continues to maintain and improve HACCP-based programs used by Wisconsin manufacturers. This program continues to challenge manufacturers to prove they are able to manufacture a safe dairy product with documentation, record keeping and using GMP's accordingly.

The Safety/Quality Applications program assisted many plants in Wisconsin by helping them design and adjust their current HACCP plans, thus improving the quality of their dairy products. During the past year, phone calls resulted in plant visits to address issues. During these plant visits, the issues and their resolution are reviewed with the appropriate plant personnel. These issues have included microbiological, or calcium lactate problems, HACCP implementation, GMP's, quality issues, whey collection from 640 boxes, plant environment and performance of plant audits.

In addition, the applications program assists the Wisconsin Master Cheesemaker® Program with plant visits, problem solving and sampling/grading of cheeses. The program participated in the WI Dept. of Agriculture Trade and Consumer Protection meetings for the Food Safety Task Force committee facilitating the review/ revision of the state of Wisconsin cheese and butter grading program. The task force is charged with determining if the grading standards for cheese and butter need to be updated or improved so the grades better serve industry needs.

In conclusion, safe high quality dairy products are still the goal of manufacturing plants. With this program, I am able to assist the dairy plants in improving and maintaining their GMP's and HACCP-based plans.

Publications and presentations

HACCP for the Dairy Industry US AID. Vilnius, Lithuania

WI cheese Grading short course Italian Cheese Evaluation

Intercollegiate Dairy Products Evaluation Contest Lead Butter Judge

WI CIP Workshop Plant Sanitation Audits

WI Dairy Products Assoc. Cheese and butter Evaluation Clinic Overview of Butter Grading

Portrait of a Dairy HACCP System ADPI Conference Chicago, IL

HACCP in the Cheese Industry FDA/DATCP Cheese Product Safety Workshop Chippewa Falls, Madison, Appleton, WI

Milk Quality and HACCP for Small Cheesemaking Operations American Cheese Society. Madison, WI

INTERIM REPORT

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

Personnel: Steven C. Ingham, associate professor, Dept. of Food Science

Funding
Wisconsin Milk Marketing Board UW9607

Dates
July 1996—June 1998

Objectives

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

2. 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.

3. 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 cheeses 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 several published methods for enumerating endospores of *C. 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. The concentration of endospores of lactate-fermenting, gas-producing *Clostridium* spp. never exceeded 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*. Characterization of 33 isolates obtained from the milk samples yielded five isolates that were presumptively identified as *C. tyrobutyricum*. Of several methods tested for differentiation of these isolates, gas chromatographic analysis of cell membrane fatty acids and Pulsed Field Gel Electrophoresis of genomic DNA appeared to be highly discriminatory. Qualitative gas chromatographic analysis of volatile organic acid byproducts was somewhat less discriminatory; biochemical profiling and gas chromatographic analysis of non-volatile organic acid byproducts were considerably less useful for discriminating among isolates. Following reports that mass spectrometry could be useful for differentiating bacteria, we tested this technique on several of our isolates but could not differentiate them. Of 24 *Clostridium* spp. isolates tested, all but one produced significant amounts of gas during ripening of Gouda cheese following inoculation of high numbers of endospores. From this part of the project, we concluded that endospores produced by lactate-fermenting, gas-producing *Clostridium* spp. are common in Wisconsin milk used to make cheese. Although the majority of these endospores are not *C. tyrobutyricum*, their presence in high concentrations may result in the late blowing defect. Most of these results are published (Int. J. Food Microbial. 43:173-183).

We are now focusing on the interaction of % water-phase NaCl and temperature on the growth of gas-forming *Clostridium* spp. in high-pH cheese. Ongoing experiments are being conducted using a model system composed of in-process Gouda cheese curds and phosphate buffer. Results to date suggest that % water-phase NaCl corresponding to 2.0% NaCl in a 40%-moisture Gouda cheese will inhibit endospore germination at temperatures ranging from 4°C to 15°C. Results for % water-phase NaCl corresponding to 1.0 and 1.5%

chapter 3

Fluid milk

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

Funding

Dairy Management Inc. FF18

Dates

June 1997—December 1999

Objectives

1. To screen strains of *Lactobacillus helveticus* for the type and level of bioactive peptides/bioactive peptide precursors which accumulate from the organisms growth in milk.

2. Determine which components of the proteolytic systems of the selected strains of *Lactobacillus helveticus* are essential for the accumulation of bioactive peptides/bioactive peptide precursors from milk.

3. Construct strains of *Lactobacillus helveticus* which accumulate elevated levels of bioactive peptides/ bioactive peptide precursors.

Summary

Our progress towards objective one involved developing 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 a result of the organisms growth in milk.

Research towards objective two has focused on the cell-envelope proteinase specificity of strains of *Lactobacillus helveticus*. To date, the proteinase specificity of eight strains has been examined using the $_{s1}$-(f1-23) as a substrate. These eight strains can be grouped into specificity classes. However, *Lactobacillus helveticus* CNRZ32 has a unique cell surface proteinase specificity. By using primers designed from the nucleotide sequence of the *Lactobacillus delbrueckii* subsp. bulgaricus proteinase gene, we have been able to amplify by PCR portions of the *Lactobacillus helveticus* CNRZ32 proteinase gene. The entire *Lactobacillus helveticus* CNRZ32 proteinase gene (called *prtH*) has been cloned and sequenced. A *prtH*-negative CNRZ32 strain has been constructed via gene replacement. This will allow us to construct isogenic derivatives of CNRZ32 which differ only in cell surface proteinase specificity. These derivatives will allow us to determine if proteinase specificity has an essential role in the accumulation of bioactive peptides/bioactive peptide precursors from casein during growth of *Lactobacillus 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.

standardized formulation. We have begun testing the efficacy of nisin in infant formulas against our 3-strain *B. cereus* cocktail and strain HRM44. Preliminary results show that 0.05% nisin (w/v)can inhibit the growth of HRM44 spores inoculated at 10^3 cfu/ml in formula at 8, 12, and 25°C for up to 10 days. Nisin also inhibits growth of the 3-strain cocktail at these temperatures, although these spores are less sensitive, and 0.10% nisin may be needed to provide inhibition.

We also have begun producing processed cheese spread containing 10^3 spores/gram of the 3-strain *B. cereus* cocktail or strain HRM44, and will evaluate the ability of nisin to prevent spore outgrowth in this product for up to 6 months of storage at 8, 12, and 25°C.

chapter 4

Communications

Center for Dairy Research Communications Program

Personnnel: Joanne Gauthier, communication specialist, Tim Hogensen, program asst., Karen Paulus, editor, Mary Thompson, outreach specialist

Funding

Wisconsin Milk Marketing Board

Dates

January 1998—December 1998

Outreach Events, conferences and workshops

Process Cheese Short Course
February 1998

Cheese Technology Short Course
February 1998

Producer Value Showcases
March 28 - Madison
March 30 - Green Bay

Dairy Microbiology Course
April 8 - 9, 1998

CDR Open House
April 21, 1998

American Cheese Society Annual Conference
August, 1998

World Dairy Expo
September, 1998

Impact of Codex on Cheesemaking
Using Cheese as a Food Ingredient
October 1998

Surveyed the dairy industry to evaluate the use of CDR services and products

CDR On-Line

CDR's web site has a new look in 1998. You can read past and present issues of the Dairy Pipeline, send e-mail to CDR staff, check on current events, and view proceedings from CDR conferences.