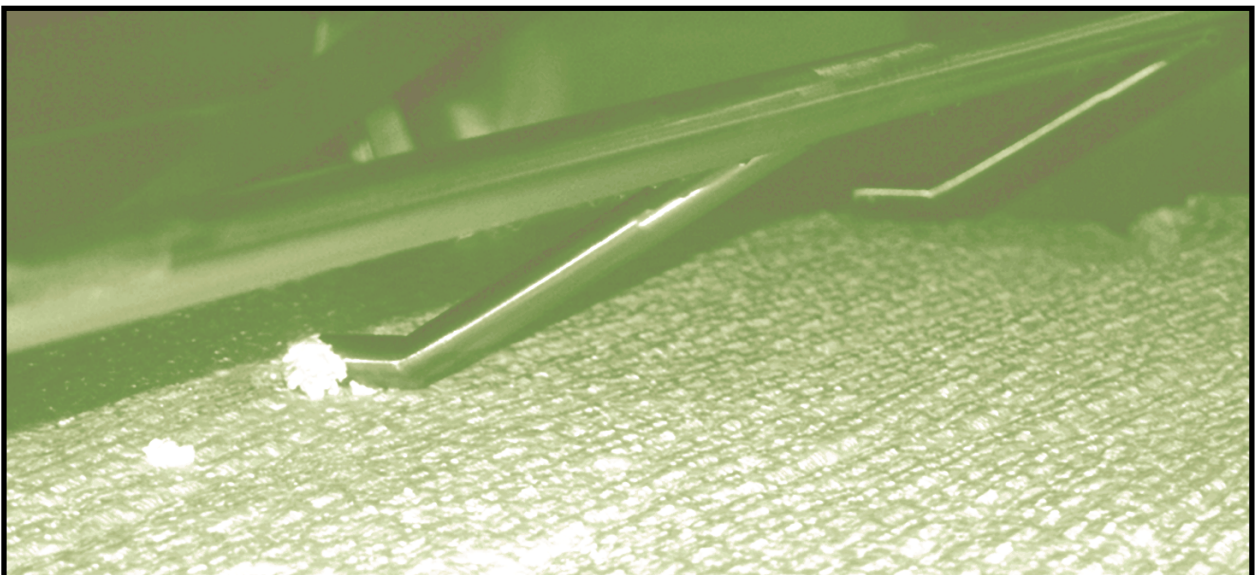
Wisconsin
Center for Dairy Research

Annual Report
94-95



FISCAL YEAR 1995
JULY 1, 1994 TO JUNE 30, 1995

Wisconsin Center for Dairy Research

Cover photos

Top Whey tanks (Nonfat Solids)
Middle left Dipper (Quality and Safety)
Middle right Milkfat (Milkfat) Courtesy of Wisconsin Milk Marketing Board
Bottom Cheese curds (Cheese)

CDR Annual Report

Published Sept.1, 1995, 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 1995. 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.

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Dear Colleagues:

It has been said many times by many people, "If you're not moving forward, you're moving backwards." This, of course, is true especially in the world of research and technology development. In order to keep pace with this new world of an "information now" lifestyle, CDR is strategically positioning itself to better serve the industry. During recent development of a five-year strategic plan, CDR developed a new mission statement and identified five objectives. The mission statement follows:

> 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.

The five objectives are:

1) Conduct an aggressive, balanced basic and applied research program in the following areas:
   * Expand the usage of cheese and cheese products through a further understanding of all properties of these products and systematic techniques to enhance functionality.
   * Improve the value and expand the usage of milkfat.
   * Recover and/or modify nonfat solids, especially whey components, to enhance their value for food and nonfood uses.
   * Maintain and enhance consumer confidence by developing technologies that will strengthen dairy food safety and quality systems.
   * Provide dairy product marketing information, business management tools and policy analysis to improve the economic well-being of individual dairy processors and the Wisconsin dairy industry in general.
   * Undertake research and develop programs in traditional or novel areas.
2) Create a closer working relationship/partnership with industry.
3) Effectively communicate research/technical information.
4) Provide education to industry and university students.
5) Increase and diversify funding resources.

CDR will also go "On-Line" this fall with a home page and access to our projects, informational databases, etc. This will be a key industry resource given the tremendous progress being made in the areas of milkfat fractionation, cheese as a food ingredient, HACCP development, lower fat cheese production, specialty cheese devlopment, etc. Leaders who will thrive and not just survive in the 21st century will be those who successfully manage both relationships and information. The team approach of CDR and industry is the only way to fully attain our goal of improving the competitive position of the dairy industry. Thank you for your continued support.

Sincerely,

J. Russell Bishop
Director, Center for Dairy Research

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Chapter 2 Nonfat Solids

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The Center for Dairy Research

CDR was established in 1986 to:

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

CDR structure

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

CDR staff

Director — J. Russell Bishop, Ph.D.
Administrative Officer — Tom Szalkucki
Carmen Huston, financial specialist 2
Mary Skalitzky, program assistant 2

Research staff

David Bogenrief, researcher
Kurt Carlson, research specialist
Carol Chen, researcher
David Everett, graduate student, Department of Food Science
Brian Gould, senior scientist
John Jaeggi, associate researcher
Mark Johnson, senior scientist
Kerry Kaylegian, researcher
Jim Path, outreach specialist
Marianne Smukowski, research specialist
William Tricomi, assistant researcher

Communications

Linda Hewitt, program assistant 2
Karen Paulus, associate editor
Sarah Quinones, outreach program manager

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 esssential 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 1994-1995) 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 (ex-officio),WMMB
Tom Szalkucki (ex-officio), CDR
V. Edward Yaghoubian, DMI

Technical Advisory Committee

The Technical Advisory Committee (TAC) plans the CDR research program, and evaluates and approves research projects for scientific merit. Members (FY 1994-1995) include:

Bishop, J. Russell, Wisconsin Center for Dairy Research
Bremel, Robert, Department of Dairy Science
Bumbalough, John, Land O' Lakes
Burrington, David, Wisconsin Milk Marketing Board
Dobson, William D., Dept. of Agricultural Economics
Etzel, Mark, Dept. of Food Science
Geyer,James, Foremost Farms
Gould, Brian, Center for Dairy Research
Gruetzmacher, Thomas, Dean Foods
Hartel, Richard, Department of Food Science
Hill, Charles, Dept. Chemical Engineering
Johnson, Eric, Dept. of Food Microbiology & Toxicology
Johnson, Mark, Center for Dairy Research
Jorgensen , Neal , College of Agricultural & Life Sciences
Krug, David, DMI
Lindsay, Robert, Department of Food Science
Malcheski, John, DMI
Muck, George, Dean Foods
Ney, Denise, Dept. of Nutritional Sciences
Olson, Norman F., Department of Food Science
Riesterer, Brian
Rose, David, WMMB
Sellars, Robert, R. L. Sellars & Associates, Inc.

Szalkucki, Thomas, Wisconsin Center for Dairy Research
Yaghoubian, V. Edward, NDPRB

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 1994-1995):

Bishop, J. Russell, Wisconsin Center for Dairy Research
Burrington, David, Wisconsin Milk Marketing Board
Bush, Robert, Schreiber Foods, Inc.
Byrne, Rob, National Cheese Inst/Amer Butter Inst.
Cobian, Francis, Lake O' Lakes
Cords, Bruce, Klenzade/Ecolab Research Center
Crawford, Robert, Borden Foods, Inc.
Engebretson, Doug, Quality Ingredients Corp.
Geyer, Jim, Foremost Farms
Hermsen, Phil, AMPI-Morning Glory Farms
Kasten, James A., AMPI-Morning Glory Farms
Kohn, Allen, WMMB
Kozak, Jerry, Nat Cheese Inst/Amer Butter Inst
Krug, David, WMMB
Lamb, Leslie, Wisconsin Milk Marketing Board
Legreid, Bradley, Wisconsin Dairy Products Association, Inc
Lemmenes, Larry, Alto Dairy Coop
Malcheski, John A., DMI
Mathison, Matt, Sanofi Bio Industries, Inc.
Muck, George, Dean Foods
Nilsestuen, Rod, Wisconsin Federation of Coops
Rose, David, WMMB
Rank, Tom, Chr. Hansen's Laboratory
Rice, Harold, DMI
Sellars, Robert, R. L. Sellars & Associates, Inc.
Sommer, Dean, Golden Guernsey
Storhoff, Donald, Foremost Farms
Umhoefer, John, Wisconsin Cheese Makers Assoc.
Wagner, Dr. Richard, Weyauwega Milk Products
Weiss, Ronald, Marschall Products/Rhone Poulenc
Wuethrich, Dallas, Grassland Dairy Products, Inc.
Yaghoubian, Dr. V. Edward, DMI

Program Area Coodinators

Cheese Technology— Robert Lindsay, Dept. of Food Science, University of WI-Madison
Mikfat Utilization—Rich Hartel, Dept. of Food Science, University of WI-Madison
Nonfat Solids Utilization—Mark Etzel, Dept. of Food Science, University of WI-Madison
Quality and Safety—Eric Johnson, Food Research Institute, University of WI-Madison

Chapter 1

MILKFAT

Milkfat Summary

The Center's milkfat program seeks to increase the use of milkfat. Fractionating and modifying milkfat are methods that several projects are using to produce value added milkfat products. Following are some highlights of our successes.

Can you imagine the wonderful baked butter aroma of butter cookies and shortbread? Then you'll realize the significance of this new method that intensifies this delicious aroma. Lindsay and his group used their flavor chemistry expertise to follow up on their initial work with free alkylphenols. They identified a source, buttermilk solids, and a method to produce baked butter flavors of varying intensities and quantities. The food industry can use these intense baked butter flavored milkfat ingredients directly, or they can incorporate them into butter products for bakery or other applications.

Modifying milkfat also took a big step forward when Hill's group developed a method for purifying pregastric lipases. These animal lipases are fairly crude preparations, traditionally used for producing lipolyzed butter oils. They were poor candidates for an immobilized enzyme process because of their low specific activity. However, the new purification procedure leads to higher specific activity, and now Hill's group can use the immobilized enzyme process to produce lipolyzed butter oil and other enzyme modified butteroil products.

Enzymatic glycerolysis is another milkfat modification technique being studied and evaluated in the Center's research program. This ongoing project, which investigates the production of mono- and di-glycerides (emulsifiers), has progressed to evaluating the butteroil based emulsifiers in ice cream. The butteroil based emulsifiers performed well when compared to a commercially produced vegetable oil based product.

Commercializing milkfat fractionation continues to progress. WMMB's is purchasing a Tirtiaux fractionation pilot plant and several companies have joined the Milkfat Fractionation Consortia. The pilot plant and texturizer should be operating around the beginning of 1996. Fractionation is a key part of CDR's milkfat program and researchers report their progress investigating the use of fractions (baked goods, confections, butter cream icings) and fractionation technology.

INTERIM REPORT

Investigation of Baked Milkfat Flavor Development in Milkfat Ingredients for the Bakery and Food Industries

Personnel: Robert C. Lindsay, professor, Dept. of Food Science, Qiaoling Zeng, graduate research assistant, Dept. of Food Science (In collaboration with researchers at the New Zealand Dairy Research Institute)

Dates: February 1993 – January 1995

Funding: Wisconsin Milk Marketing Board #92-7

Objectives

1. To establish a sensory panel trained in the recognition and descriptive sensory anlysis of baked milkfat flavor in baked products (NZDRI).

2. To establish a model baking system for the assessment of baked milfat flavor in baked goods (NZDRI).

3. Using the model baking system , undertake and correlate chemical and sensory analyses to identify the key compounds responsible for baked milkfat flavor in baked products (UW and NZDRI).

4. To define the specific chemical reactions involved in the development of enhanced baked milkfat flavor (UW and NZDRI).

5. To formulate and prepare milfat based ingredients which provide enhanced baked milkfat flavor (UW and NZDRI).

Summary

Free alkylphenols were found to contribute key flavor characteristics to baked flavors where they are present in low ppb concentrations. They are present in both the free and conjugate bound forms in butter, but only the free forms contribute to flavors. Free alkylphenols can be isolated from dairy foods using simultaneous distillation extraction (SDE), and then can be detected using selected-ion-monitoring mass spectrometric techniques. However, this approach to analysis is extremely slow, and a single sample analysis can take 3 or 4 days to complete. In order to reasonably investigate the quantitative aspects of alkylphenol contributions to baked butter flavors, a rapid, sensitive analytical method is mandatory.

The approach that was chosen for development involved the formation of dansyl derivatives of the alkylphenols which yielded spectrofluorometrically detectable compounds. This approach provided a very selective means for measurement of the alkylphenols in the complex matrix of isolates from dairy products and foods containing them as ingredients. Both two-phase and one-phase reactions were investigated to determine which would provide the best results with baked butter flavor systems. It was found that the one-phase derivatization using acetone saturated with sodium carbonate provided superior results. The two-phase dansylation used the deprotonation of phenolic substances to anions which were extracted into an organic phase. The advantage of the two-phase system is a short reaction time at room temperature, but the sensitivity of the procedure was inferior.

After the reaction conditions were worked out, attention was turned to developing HPLC conditions which would separate a maximum number of the alkylphenol isomers. Fluorescence intensity is higher in low dielectric solvents, and therefore normal phase HPLC conditions (Lichrosorb 60 column) provided higher sensitivities than reversed-phase systems. Many solvent combinations were evaluated, including tetrahydrofuran, dioxane, benzene, methylene chloride, hexane, and 2,2,4-trimethylpentane. However, a system containing varying amounts of acetone (1-4%) in 2, 2, 4,-trimethylpentane was found to give the best results

for the compounds of interest. A concentration of 1.65% acetone in 2,2,4-trimethylpentane used as an isocratic solvent gave suitable retention times with adequate resolution for the compounds of greatest interest in this study. However, the concentration of acetone can be varied carefully to achieve separations of selected co-eluting pairs of alkylphenols if the specific information is of value. The extremely sensitive method was capable of detecting minimum concentrations of individual alkylphenols from 0.2 to 2 nanograms absolute.

Combination of the dansylation procedure with the HPLC separation method provided the basic requirements for the routine analysis of free alkylphenols that are key to baked butter flavors. The isolation of free alkylphenols from skim milk or other non-fatty foods was adapted from earlier work, and good replication and sensitivity was achieved. This method involved extraction of alkylphenols from the matrix using XAD adsorption resin, followed by release with methanol. Studies showed that most of the conjugate-bound alkylphenols (not directly contributing to flavor) were present in the aqueous phase (skim milk; serum phase) while the majority of free alkylphenols were partitioned into the milkfat phase. The total flavor potential of butter or other dairy products could be measured by acid/enzyme hydrolysis of the alkylphenol conjugates followed by HPLC analysis of dansyl derivatives.

Since the majority of the free, flavorful alkylphenols were present in the milkfat phase of butter, it was essential to devise a means of analyzing these compounds which excluded the presence of milkfat. Attention was then directed towards isolation of alkylphenols from butter and other fatty foods, including butter cookies. Several approaches were investigated regarding the isolation of free alkylphenols from fatty foods. These included simultaneous distillation extraction (SDE), alkaline liquid extraction, and alumina adsorption.

A major problem existed regarding extraction of alkylphenols in that the alkylphenol conjugates are extremely sensitive to hydrolysis during extraction by some solvents. Thus, the free and bound ratio of alkylphenols has been distorted in many literature reports of the past. To overcome the solvent-induced hydrolysis, solvents were screened and characterized for their effects on conjugate hydrolysis. Polar solvents, such as ethyl acetate and chloroform, induced hydrolysis, and could not be used. Since some polarity was needed to solubilize the alkylphenols, it was discovered that diethylether could be employed under certain conditions to permit extraction without hydrolysis or distortion of the ratio of free to bound alkylphenols. Conditions which permitted isolation of free alkylphenols from milkfat without distortion of the ratio of free to bound included; near neutral pH, short time exposure, near ambient temperature, presence of excess water, and saturation of the aqueous phase with sodium chloride.

With these isolation and analysis strategies suitably defined, a complete system was then devised which allowed complete analysis of butter ingredients or other fatty foods for free alkylphenols. This involved the primary isolation of free alkylphenols by ethyl ether extraction to separate them from the main matrix of the food, and particularly separated the free from the bound alkylphenol conjugates. However, the free alkylphenols in the ethyl ether isolates were accompanied by the fat components, and a secondary isolation involving simultaneous distillation extraction proved necessary to separate the alkylphenols from the fats. This distillate was then concentrated and reacted with dansyl chloride to yield the dansyl derivative of the alkylphenols which were then analyzed by HPLC with a normal phase solvent system composed of 1.6% acetone in 2,2,4-trimethylpentane, and a Lichrosorb 60 column. The alkylphenols found in butter that can be separated when appropriate concentrations of acetone are employed include 2,6-diisopropylphenol, 2,5-diisopropylphenol, thymol, carvracrol, 2-isopropylphenol, 3-isopropylphenol, 4-isopropylphenol, 2-ethylphenol, 0-cresol, m-cresol, p-cresol, 3-ethylphenol, 4-ethylphenol, and phenol.

Studies showed that butter contained reasonably high (still low ppb levels) of free alkylphenols, but the amount of aqueous serum phase restricted the total amount of precursor present. Surveys of butters, canned and fresh milk and cream for free alkylphenols revealed a great deal of variation between samples. In order to obtain higher amounts of alkylphenol precursors for conversion

in the baked butter ingredient manufacture, dried and concentrated dairy products were analyzed. We found that a large reservoir of alkylphenols resided in the sulfate and glucuronide conjugates present. However, evidence for phosphate bound conjugates in cow's milk systems has not been obtained. Still, the flavor character of the milk system reflects the concentrations of free alkylphenols. In these systems the flavor contribution is perceived as milky, and as the concentration increases the descriptors likely to be used include cowy and feedy.

Several approaches to the preparation of intensely baked butter flavored ingredients have been evaluated, including conjugase treating of cream, milk, butter, whey solids, nonfat dry milk solids, and buttermilk solids. All of these function to a degree, but the best system employs conjugase treating (sulfatase + glucuronidase) a solution of buttermilk solids (about 30%) for 24 hr at 21°C, and then combining this mixture with an approximately equivalent amount of anhydrous milkfat before autoclaving at 121°C for 30 minutes. The conditions can be varied quite widely with varying intensities and qualities of baked butter aromas resulting. The key compounds involved in the final flavor are alkylphenols, lactones, methyl ketones, short chain fatty acids, and Maillard browning compounds (furanones).

The resulting intensely baked butter flavored milkfat ingredients can be used directly as butteroils, or they can be refabricated into butter products for bakery or other applications. The highly unique feature of this technology is the flexibility of the milkfat ingredient used to prepare the baked butter flavor, and lower value milkfat fractions could readily be incorporated into the process. The flavor strength of the baked butter flavor ingredients depends on the ratio of buttermilk solids to milkfat that is used in the manufacture of the ingredient. Using the most efficient process, replacement of 5-20% of the milkfat or shortening ingredient with the intensely flavored butter in low-moisture bakery products yields pronounced baked butter flavors.

Intensely flavored baked butter flavor ingredients have been evaluated in a variety of products, and they are especially effective in butter cookies or shortbreads. When the moisture content of the baked goods is elevated, the intensity of the flavor is suppressed. This likely reflects a partitioning effect of the water and flavor compounds, and research is needed to determine if it will be possible to improve the performance of the ingredients in this application. Trials at comparable levels of substitution in milk chocolate and butter toffee gave equally dramatic flavor enhancements of the desirable sweet dairy flavors. It is likely that the chocolate industry and reduced fat applications will benefit from the ingredients.

Significance to the dairy industry

This project focuses on a unique and desired aspect of milkfat — flavor. Applying research methods to determine the elements and chemical reactions that produce milkfat flavor may eventually allow industry to use the enhanced milkfat flavors in more applications.

INTERIM REPORT

Use of Pregastric Lipases Immobilized in a Hollow Fiber Reactor to Produce Lipolyzed Dairy Products

Personnel: Charles G. Hill, Jr., professor of Chemical Engineering; 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

Dates: July 1994 – June 1996

Funding: Dairy Management Inc. HLL 95

Objectives

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

2. Use these kinetic data to develop both uniresponse and multiresponse mathematical models of the reaction network which can be used in process design and simulation, control, and optimization analyses. Both intrinsic kinetics (including inhibition effects) and enzyme deactivation effects will be quantified.

3. Conduct experimental studies combining two or more reactors in a series configuration, each reactor containing an immobilized lipase from a different source.

4. Assess the functional and sensory properties (softening point, flavor, etc.) of promising products.

5. Identify technical issues to implement this technology in an industrial setting (operation, cleaning, recharging, microbiology, maintenance, etc.).

6. Assess the technical and economic aspects of the proposed processes to determine commercial viability.

Summary

Our most significant results during the past year have evolved from an effort to purify crude preparations of pregastric esterases from the salivary tissues of calves, lambs, and kids (goats). These enzymes are particularly interesting because of their very high specificities for releasing short chain fatty acids from fats and oils. Our purification process gives the enzyme a much greater specific activity than that of the commercial precursors. It consists of the following steps:

1. Refrigerate a suspension of the crude tissue preparation in a phosphate extraction buffer. This facilitates dissociation of the esterases from the large hydrophobic proteins they are normally associated with in the salivary tissue.

2. Centrifugation of the suspension, followed by microfiltration through a 0.2 μm membrane to remove suspended solids.

3. Ultrafiltration of the permeate from step 2 using a membrane with a molecular weight cutoff value of 30,000 daltons.

The resultant concentrated solution of the enzyme possesses a much higher enzyme activity per unit of soluble protein than its precursors. You can use this solution (method described in previous publications) to immobilize the esterase via physical adsorption on the polypropylene hollow fibers contained within the reactor. This approach drastically improves the efficacy of fatty acid residue release. Figure 1 depicts the free fatty acid content of the effluent butteroil stream as a function of the space time (residence time) of the

butteroil in the reactor. The circles represent data obtained using an esterase purified by the protocol described above. The smooth curve represents the best fit curve through data obtained using an earlier protocol for purification of the esterase. The marked improvement in the performance of the reactor implies that if this technology is used, significantly shorter contact times would be required to achieve the concentrations of free short chain fatty acids characteristic of commercial lipolyzed butteroil products. By using this approach, you could take advantage of the economic benefits of employing immobilized enzyme technology to produce a lipolyzed butteroil rather than the free enzyme process traditionally employed by commercial manufacturers.

Note that the stabilities of the immobilized enzymes generated via our current protocol are sufficient for the proposed commercial application. In an experiment involving continuous operation at 45°C for a week, there was no significant change in the effluent composition during the course of the experiment. There was no statistically significant change in the activity of the immobilized calf pregastric esterase during the week.

In addition, we have conducted other studies intended to optimize the protocols used for extracting, purifying, and immobilizing the indicated pregastric esterases, and initiated the modelling studies indicated in Objectives 1 and 2. We expect that future efforts will focus more on characterizing the reaction kinetics and the associated reactor modelling work.

Significance to the dairy industry

Using immobilized enzyme technology to produce lipolyzed butter oils offers several advantages over the conventional batch process based on the use of soluble enzymes. From an economic standpoint, one can produce far more product per unit of enzyme. Also, the final product does not contain active enzyme so you don't have to heat inactivate free enzyme to prevent further reaction in the product. In addition, the use of immobilized lipases offers the potential for developing products with unique sensory, functional, or nutraceutical properties by using lipases with different specificities, either alone or in combination with one another.

Hydrolysis and interesterification reactions represent techniques which manipulate the chemical composition of milkfat in efforts to design foods with specified physiological functions. For example, there is a growing awareness of the role of foods in human health. You can use these reactions to produce nutraceuticals or pharmafoods which replace undesirable fatty acid residues in the triglycerides of milkfat with more desirable ones. Researchers have attributed anticarcinogenic and antioxidant properties to conjugated linoleic acids, suggesting they may have significant dietary benefits. Thus, using immobilized lipases offers the intriguing possibility of producing specially designed foods for selected segments of the population. In particular, those individuals who are at a high risk for cardiovascular problems. These products represent a very significant long term marketing opportunity for the dairy industry.

Publications/presentations

"Immobilization of Pregastric Lipases in a Hollow Fiber Reactor for Continuous Production of Lipolyzed Butteroil," by H.S. Garcia, A. Qureshi,L. Lessard, S. Ghannouchi, and C.G. Hill, Jr., *Lebensmittel-Wissenschaft und Technologie*, **28**, 253 (1995).

"Improving the Continuous Production of Lipolyzed Butteroil with Pregastric Esterases Immobilized in a Hollow Fiber Reactor," by H.S. Garcia and C.G. Hill, Jr., accepted for publication in *Biotechnology Techniques.*

"Production of Lipolyzed Butteroil by Immobilized Calf and Kid Goat Lipases," by H.S. Garcia, and C.G. Hill, Jr., presented at the 90th Annual Meeting of the American Dairy Science Association, June 25-28, 1995, Ithaca, New York.

"Production of Lipolyzed Butteroil by Immobilized Lipases," by H.S. Garcia and C.G. Hill, Jr., invited paper to be presented at the Symposium on Bioseparations, Ixtapa, Mexico in September, 1995.

"Hydrolysis of Milkfat by Lipase Immobilized in a Hollow-Fiber Membrane Reactor," presented at The University of Illinois at Chicago, Department of Chemical Engineering, April 7, 1995.

"Recent Research Results Concerning the Production of Lipolyzed Butteroils Via Immobilized Enzyme Technology" — presented to a review panel consisting of industry representatives and researchers concerned with the Milkfat Fractionation and Utilization program of the University of Wisconsin Center for Dairy Research, the "Milkfat Industry Team" (May 26, 1995).

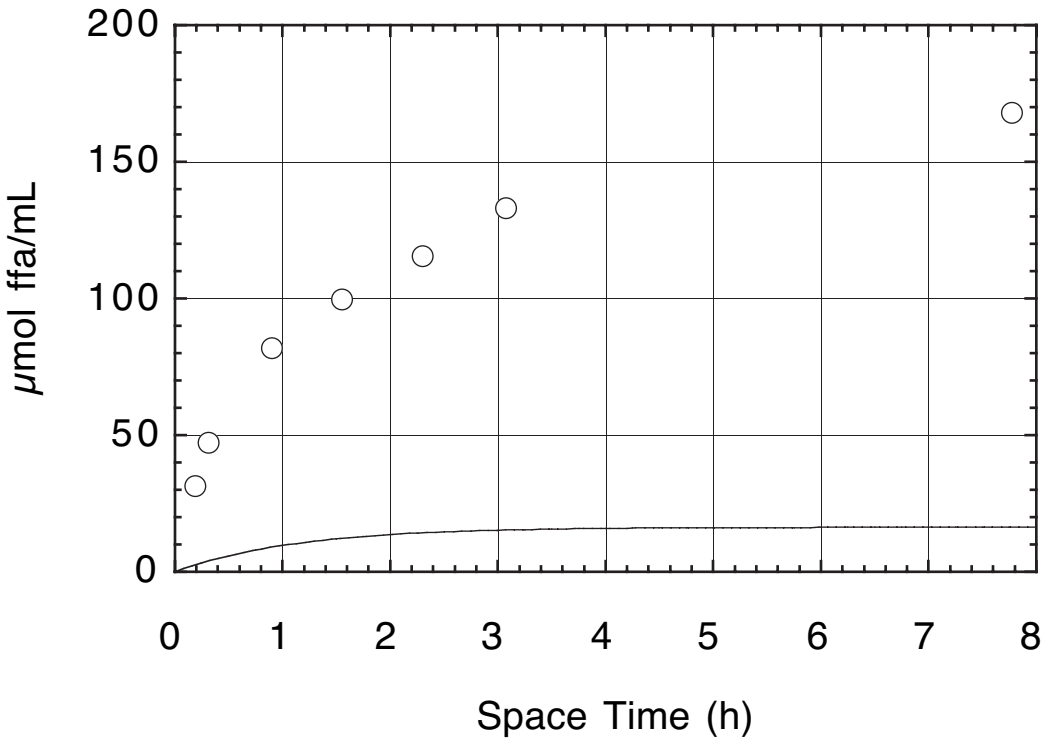

Figure 1. Comparison of reactor effluent compositions for the calf pregastric esterase purified by the new protocol (data points) and that purified solely by ultrafiltration (solid line representing the best fit model).

VISITING SCIENTIST REPORT

Improvement of the Performance of Immobilized Pregastric Esterases on a Hollow-Fiber Polypropylene Reactor for Preparation of Lipolyzed Butteroil

Personnel: Hugo S. Garcia, associate professor, Dept. of Food Technology, Centro de Graduados, Inst. Tec. de Veracruz, Veracruz, Mexico

Funding: Wisconsin Milk Marketing Board

Objectives

The work done last year yielded very encouraging results, for the first time pregastric esterases were successfully immobilized. These enzymes are the only ones that are of interest to the lipolyzed butteroil (LBO) industry.

Summary

Our prime objective was to increase the productivity of the reactor. Last year we proved that the pregastric esterases could be immobilized, but the activity of the immobilized preparations was rather low, mostly in the case of calf lipase. In the case of kid lipase, the one that displayed the highest activity, ca. 9 hours of space time were needed to release short chain (i.e. C4, C6 and C8) fatty acids in concentrations similar to those found in a commercial LBO product.

This was achieved by a series of strategies: Enzyme preparations consisting of dried ground salivary tissue from suckling animals, with no salt or nonfat dry milk added were used. Extraction from the crude powder was made possible, as the previous year by cold-storage (4 °C) of a buffer suspension of the enzyme, in order to induce the dissociation of the enzyme molecules from the large molecular weight proteins naturally associated with the enzyme. As last year, to separate the lipase from the large particles in the crude powder, the suspension was microfiltered using a Millipore™ membrane with pores of 0.2 μm (1st. Permeate). The permeate containing the enzyme was then concentrated by a second filtration step, this time using a 30,000 da ultrafiltration membrane. The volume of the first permeate was concentrated at least five times.

The set-up immobilizing the enzyme molecules on the polypropylene hollow fiber reactor was similar to what we used last year. The reactor was operated at space times from 6 minutes to 8 hours. The amountof calf lipase that we could immobilize was one order of magnitude larger than last year's preparation. For kid lipase, the new procedure produced more than twice the already high activity observed last year. It was possible to obtain fairly strong smelling butteroil effluents at space times shorter than 1 hour. Even though glyceride analysis proved that some free fatty acids are transformed into MAG (monoacylglycerides) by the enzyme, the overall hydrolysis rates obtained were high. PAGE analysis showed a decrease in intensity of most of the protein bands from the 2nd Retentate. Studies of temperature and pH dependence showed that overall, a pH of 6.0 and 40°C were adequate operating conditions. Further research work is needed to study the change in specificity of the lipases with respect of pH, as well as their stability.

Difficulties dealing with reactor re-loading were resolved by devising a cleaning procedure that uses from highly non-polar to polar organic solvents in order to eliminate a range of reaction products of different polarities.

Significance to the dairy industry

This work contributes to efforts at the CDR to find value added alternatives for milkfat. Semi-purification of the lipases using two membrane separation processes yielded immobilized preparations which were considerably more active. Reactor space times of ca. 1 hour were enough to produce LBO's with strong aroma, as compared to almost 10 hours of last year for kid lipase only. This may help industry adopt the technology easier.

INTERIM REPORT

Evaluation of Monoacylglycerols Derived from Butteroil as Emulsifiers in Foods

Personnel: Kirk L. Parkin, associate professor, Kathleen Wiederholt, research specialist, (7/1/93-10/21/94) replaced by Purwiyatno Hariyadi and Shu-Jung Kuo, research assistants, Department of Food Science

Dates: July 1993 – October 1995 (extended from original June 30, 1995 termination date)

Funding: Dairy Management Inc. PRK 94

Objectives

1. Prepare monoacylglycerol (MAG)-rich fractions from butteroil at levels suitable for functionality and applications evaluation.

2. Develop procedures to partially purify and/or fractionate MAG-rich fractions prepared from butteroil for functionality and applications testing.

3. Evaluate the physicochemical properties and related functionality of MAG-rich fractions prepared from butteroil as emulsifying agents.

4. Evaluate the effectiveness of selected MAG-rich fractions prepared from butteroil in selected food systems.

Summary

Starting with about 100 g of butteroil, a MAG-rich (>90%) fraction of about 40 g can be prepared using various lipases. The 90% pure MAG fraction was obtained from the final product mixture by facile precipitation with hexane, followed by filtration. Amano type PS-30 (Pse*udomonas cepacia*) and Novozym 435 (*Candida antarctica* B lipase produced by a cloned *Aspergillus oryzae* strain) lipases were effective in this manner. The latter shows promise as a food-grade preparation. Two food-grade lipases, Novo's Lipozyme IM and Palatase M (both from Rhi*zomucor miehei*) are also effective, but only if butteroil is first saponified and the fatty acids then combined with glycerol and catalyst. Both PS-30- and Palatase M-derived MAG preparations became enriched in myristic, palmitic and stearic acids, and diminished in shorter chain-length and unsaturated fatty acids, compared to native butteroil. Thus, preparing MAG from butteroil in this fashion also resulted in an effective fractionation of fatty acids into different pools. The shorter chain-length and unsaturated fatty acids are expected to be retained in the tri- and diacylglycerol and fatty acid fractions remaining after reaction and separation (analyses in progress will determine if this is so). We also used high- mid- and low-melting butteroil fractions as starting materials for preparing MAG fractions. The resulting MAG preparations became increasingly enriched in 4-10 carbon saturated, and unsaturated fatty acids, when progressively lower-melting butteroil fractions were used as the starting material. This illustrates another method to manipulate the fatty acid profile of MAG derived from butteroil.

Physicochemical analysis of the MAG-fractions derived from butteroil indicated these preparations reduced surface tension at an oil-water interface (at levels of addition of 0.5-1.0% of the oil phase) as effectively as a common commercial distilled MAG product. Starch-complexing activity was also comparable between the butteroil-derived MAG fractions and the commercial MAG product. Likewise, preliminary studies indicated that the butteroil-derived MAG was equally as effective as a commercial emulsifier blend when used in ice cream (this result evolved into a CDR-sponsored project to focus specifically on this application -see project by Hartel and Parkin). Work in progress is exploring the functionality of butteroil-derived MAG fractions in reduced fat spreads and selected baked goods.

Significance to the dairy industry

We plan to determine the functionality of lipase-modified butteroil components as emulsifying

agents. The means to transform butteroil into MAG and DAG has already been established. Thus, our study will extend previous work to a point where decisions can be made regarding the commercial viability of butteroil emulsifiers. The current global market for emulsifiers is estimated at $400 million. Dairy lipid components make up little, if any, of this market. If butteroil-derived emulsifers compare favorably with present emulsifiers, and commercial adaptation proves to be viable, the market for dairy lipid components could expand in the future.

INTERIM REPORT

Application of Emulsifiers From Butteroil or Milkfat Fractions in Ice Cream

Personnel: R.W. Hartel, associate professor, K.L. Parkin, associate professor, B. Liang, research associate, Dept. of Food Science

Dates: September 1994 – August 1996

Funding: Dairy Management Inc. HRP95

Objectives

1. To study the application of emulsifiers made from enzyme modified butteroil and milkfat fractions in ice cream.

2. Evaluate the cost of producing the butteroil emulsifiers and compare to commercial emulsifiers for use in ice cream.

Summary

We used an enzyme process, developed at the University of Wisconsin, for producing mono- and di-glycerides from butteroil to produce an emulsifier for ice cream. In a preliminary study, enzymic glycerolysis of butteroil, followed by chemical separation, produced relatively pure fractions of monoglyceride and diglyceride components. These purified fractions were blended together at different ratios to produce emulsifiers with monoglyceride content ranging from 30 to 87%. These emulsifiers contained only a few percent triglycerides and free fatty acids.

Ice creams were produced with these emulsifiers in a soft-serve freezer set for 50% overrun. Emulsifiers were added to the mix at 0.1%, and compared to a commercial emulsifier containing 80% mono/diglycerides and 20% polysorbate. In addition, an over emulsified ice cream was made with 1% of the butteroil emulsifier and 83% monoglycerides. Ice creams were evaluated at draw, after hardening and after storage at -15°C. Measurements included relative amount of fat destabilization (using a spectrometric technique), melt down rate (drip test), hardness (by penetrometer), ice crystal size distribution, and air cell/bubble distribution. Ice crystals were measured using a refrigerated glove box, optical microscopy technique, while air cells (or the bubbles arising from the air cells) were measured using a modified "squash" method, followed by image analysis from optical photomicrographs.

Ice creams made with these butteroil emulsifiers were virtually identical to those made with the commercial emulsifier blend, except for the melt-down rate. The commercial emulsifier had slower melt-down when compared to the butteroil emulsifiers. The ice creams made with the butteroil emulsifier began to melt down after 20 minutes at room temperature, while the commercial ice cream did not begin to melt down until 40 minutes at room temperature. All other parameters, including degree of fat emulsion destabilization, ice crystal size, air cell/bubble distribution, hardness, and appearance, were identical between emulsifiers. The over emulsified ice cream, however, showed significant differences from the other ice creams. It had slightly higher overrun, smaller air cells/bubbles, smaller ice crystals, and slower melt-down rate.

Significance to the dairy industry

Some ice cream requires emulsifiers to provide proper melt-down rates and appearance. However, emulsifiers (mono- and di-glycerides, polysorbates, etc.) listed on the ingredient label are often viewed as negative. The one major commercial ice cream with no added emulsifiers, promoted as all natural, commands the largest market share in the US. An emulsifier produced by a natural process from butteroil or milkfat fractions would potentially satisfy the requirements for an all-natural product, while still providing necessary emulsification. This would increase the consumption of both ice cream and milkfat.

INTERIM REPORT

Milkfat Applications Research Program

Personnel: Kerry E. Kaylegian, researcher, Center for Dairy Research

Dates: July 1994 – June 1995 (continuing)

Funding: Wisconsin Milk Marketing Board UW 133-Y401

Objectives

1. Provide technical support for butter and milkfat fractions to the dairy, bakery and confectionery industries

2. Work toward increasing expertise in established milkfat fractions areas, and investigate potential new applications for milkfat fractions

3. Conduct applied research in butter and milkfat fractions

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

5. Coordinate milkfat technology transfer activities in conjunction with CDR communications group

Summary

The Milkfat Applications Research Program has successfully identified and prepared to meet the needs of the dairy and food industries in the milkfat area over the next several years. The Wisconsin Milk Marketing Board approved $300,000 toward the purchase of a Tirtiaux fractionation pilot plant. The pilot plant will be installed at CDR sometime this fall. The commercialization of milkfat fractionation in the U.S. is imminent, and we have established a Milkfat Fractionation Consortium to address the needs of both potential manufacturers and users of milkfat fractions. The Consortium's focus is to promote industry input and support into the milkfat fractionation program to develop integrated research programs that address strategic and applied issues of milkfat fractionation technology and applications. The sponsors will receive many benefits, including CDR expertise and assistance, access to milkfat fractions, time on the equipment, access to product development, evaluation, and analytical support services. Industry response has been very positive and many companies are very willing to support us financially. We expect to receive at least $50,000 in industry contributions by the end of summer 1995.

An experimental pastry bakery was set up to support milkfat fraction applications research and the Milkfat Fractionation Consortium. A research project is currently using the pastry bakery to evaluate the performance of specialty pastry butters made from milkfat fractions in puff pastry. The experimental pastry butters are made from milkfat fractions that are blended to meet a specific melting profile. These butters have different emulsifier systems and different levels of total fat (i.e., 80% and 82%). The pastry butters were produced on a Gerstenberg & Agger Perfector at Texas A & M University. The butters in puff pastry are being evaluated for handling, baking, textural and sensory characteristics.

This has been a communication year for the Milkfat Applications Research Program. "The Handbook of Milkfat Fractionation Technology and Applications" has been published by the American Oil Chemists' Society and is selling well. Many talks on milkfat fractionation technology and applications have been given at research conferences, trade meetings, and for individual groups. Several articles written about the milkfat fractionation program and pilot plant equipment have been published in the CDR Pipeline, local and regional newspapers, and trade journals. The milkfat database will be addressed with other CDR computer needs in the next year.

Industry has begun to recognize, even more than before, the milkfat expertise at the Center for Dairy Research and UW, and is willing to tap into that expertise when needed. Personnel from the Milkfat

Applications Research Program have traveled to several companies for on-site technical assistance and delivered lectures on milkfat fractionation research. We continue to stay current with the research literature in this area, and attend short courses that are relevant to milkfat technology and applications.

Significance to the dairy industry

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. Fractionating milkfat to produce specialty ingredients will increase the value of milkfat and expand its use in the food industry. CDR will provide much needed research facilities, data, and technical support through the implementation of the Milkfat Fractionation Consortium and from the publication "The Handbook of Milkfat Fractionation Technology and Applications" to assist both manufacturers and users of milkfat fractions as the industry begins to commercialize milkfat fractionation in the US.

Publications/Presentations

Kaylegian, K.E., and R.C. Lindsay. 1995. Handbook of Milkfat Fractionation Technology and Applications. Amer. Oil Chem. Soc. Press, Champaign, IL.

Kaylegian, K.E. Functional Characteristics and Nontraditional Applications of Milk Lipid Components in Food and Nonfood Systems. J. Dairy Sci. (in press)

Kaylegian, K.E. Functional Characteristics and Nontraditional Applications of Milk Lipid Components in Food and Nonfood Systems. Presentation at the American Dairy Science Association annual meeting 1994

Milkfat Fractionation in the U.S.: The Potential for Commercialization. Presentation to Pennsylvania State University Food Science Department. State College, PA 12/94

Milkfat Fractions Innovative Food Ingredients. Presentation to IDFA's Ice Cream Technology Council Conference. Scottsdale, AZ 2/95

Milkfat Fractions Innovative Food Ingredients. Presentation to dairy farmers at WMMB Winter Meeting. Green Bay, WI 3/95

Milkfat Fractions Innovative Food Ingredients. Presentation to Clintonville, Rotary Club Meeting. Clintonville, WI 3/95

Milkfat Fractionation Technologies. Presentation at Wisconsin Cheese Industry Conference. Green Bay, WI 3/95

Applications of Milkfat Fractions in Foods. Presentation at Wisconsin Cheese Industry Conference. Green Bay, WI 3/95

Milkfat Fractionation in the U.S.: The Potential for Commercialization. Presentation at Wisconsin Industry Conference. Green Bay, WI 3/95

Milkfat Fractions Innovative Food Ingredients. Presentation at IBC's Fat and Cholesterol Reduced Foods Conference. New Orleans, LA 3/95

Milkfat Fractions Innovative Food Ingredients. Presentation to CDR Milkfat Industry Team Meeting. Madison, WI 5/95

FINAL REPORT

Incorporation of Milkfat Fractions in Chocolates, Phase 1

Personnel: R.W. Hartel, associate professor, J. Wood-Sciple, research assistant, S. Metin, research assistant, Department of Food Science

Dates: September 1992 – August 1994

Funding: Wisconsin Milk Marketing Board 92-11

Objectives

1. To investigate the fat crystallization behavior of mixed fat systems of interest to the chocolate industry to enhance the feasibility of using milkfat fractions as cocoa butter extenders or replacers.

2. To enhance the use of butter fat by optimizing the use of milkfat fractions in chocolate products.

3. To identify the components of milkfat fractions responsible for specific behavior in mixed fat systems such as chocolates.

Summary

In the past year, we completed a second study looking at bloom inhibition in chocolate due to the addition of milkfat fractions. This study used a different cocoa butter in the chocolate and focused on delineating the effects of specific high melting fractions. We continued to study the effects of specific milkfat fractions on cocoa butter crystallization kinetics.

Milkfat was fractionated using a batch crystallization technique, followed by vacuum filtration to generate a range of milkfat fractions with different composition and melting characteristics. Milkfat was fractionated at 32, 30 and 28°C to generate hard fractions with melting points of 48.5, 46.7 and 44.5°C, respectively. A very hard fraction was produced by a two-step process. Milkfat was initially fractionated at 27°C and filtered. The hard fraction was melted and re-crystallized at 32°C , and the slurry filtered to produce a very hard fraction with melting point of 49.2°C. Several lower melting fractions were also produced by sequential fractionation of liquid components produced from the higher melting fractions. Secondary fractionations were accomplished at 23 and 17°C, to produce a total of 7 milkfat fractions for study.

A low-fat chocolate base, manufactured with an Ivory Coast cocoa butter, was obtained from Guittard Chocolate Co.(Burlingame, CA). The chocolate bases (both dark and milk chocolate) were processed through the commercial production cycle to the point where additional cocoa butter would be added to the conching process. This chocolate base contained about 22.4% fat. Additional fat was added in a laboratory mixer to make the final chocolate up to 33% fat. Cocoa butter replacement levels of 2, 5 and 10% on a fat basis, were studied. Both milk and dark chocolates were made in this manner. Chocolates with various fat phases, based on milkfat fraction addition, were tempered in a laboratory unit, poured into molds, and cooled for further evaluation. Viscosity of the untempered chocolate and hardness (by penetration depth) of the final molded discs were measured. In addition, the rate of bloom formation of a dark chocolate was quantified by measuring change in whiteness index on a Hunter colorimeter during accelerated storage conditions. Chocolate samples were subjected to temperature cycles between 19 and 29°C every 6 hours to promote bloom formation. The pure cocoa butter control chocolate bloomed in 12 days under these conditions, whereas differences in bloom formation were found for each of the milkfat fractions used.

Our previous study on bloom inhibition in chocolate used a wider range of milkfat fractions produced by acetone fractionation, and a chocolate made with a blend of cocoa butters (from Malaysia and Brazil). Significantly different results were found between these two studies. First, the chocolate made with Ivory Coast cocoa butter had a much slower rate of bloom formation, both with

and without milkfat fractions. This cocoa butter was apparently more resistant to bloom than the blended cocoa butter used in the previous trial. A second difference between studies was the shape of the bloom curve. With the blended cocoa butter, bloom formation was essentially linear with time of cycling, whereas, with the Ivory Coast cocoa butter an S-shaped curve was found. That is, there was a distinct induction time where no bloom formation was observed, followed by a period of rapid bloom formation until some maximum level was attained. Both studies showed, however, that harder milkfat fractions greatly inibited bloom formation in chocolates and softer fractions either had no effect (as compared to the control) or actually promoted bloom formation. No differences in bloom formation were found between hard fractions with melting points between 42.2 and 49.2°C, when fractions were added to the dark chocolate at 2% of the cocoa butter level.

In addition, hardness of dark chocolate with 2% addition of milkfat fraction was not dependent on which fraction was incorporated. In fact, at this level of addition, chocolates made with milkfat or milkfat fractions were only slightly softer than the cocoa butter control. However, at higher addition levels (5 and 10%), chocolates made with milkfat or milkfat fractions were softer than the control chocolate. At 10% addition level, the harder milkfat fractions softened the chocolates to a lesser extent than the intact milkfat. Milk chocolates, since they already contained a portion of milkfat, were softened significantly by addition of milkfat fractions to replace cocoa butter. Hardness generally decreased as addition level increased from 2 to 10%. Again, harder fractions resulted in less softening than either the intact milkfat or the softer fractions.

To complement the above studies following bloom formation and to provide further information on compatability between lipids, crystallization kinetics of the mixtures of milkfat fractions with cocoa butter were performed. Differential Scanning Calorimetry (DSC) was used to follow crystallization kinetics, while phase behavior was determined using Nuclear Magnetic Resonance (NMR) to measure solid fat content (SFC). Isosolids diagrams for different combinations of cocoa butter and milkfat fractions were developed from NMR data.

Using an isothermal DSC technique, the induction time for nucleation between 10 and 25°C was measured for different mixtures of milkfat fractions and cocoa butter. Milkfat and its fractions nucleated much more rapidly than the Ivory Coast cocoa butter. Addition of milkfat or its fractions to cocoa butter significantly delayed onset of nucleation of the cocoa butter. Higher addition levels (up to 20%) generally resulted in greater inhibition, although there was very little difference among fractions. That is, different milkfat fractions with a wide range of melting points resulted in approximately the same inhibition of cocoa butter crystallization at equivalent addition levels. These results are necessary, for example, to improve control of tempering operations. It is generally accepted that milkfat alters tempering conditions, however, there is little quantitative data documenting these effects.

Using X-ray diffraction and DSC melting curves, it was found that at all conditions studied, only ß' crystals were formed. That is, none of the milkfat fractions or AMF itself altered the polymorphic structure of cocoa butter during the duration of crystallization experiments. At the highest temperature studied, 25°C, the hardest milkfat fractions crystallized separately from the cocoa butter, which did not crystallize at all at 25°C.

Significance to the dairy industry

One of the main applications for milkfat fractions is in the chocolate and confectionery industry. However, to date, milkfat fractions have met with limited success in chocolates, primarily due to the softening effect of milkfat on cocoa butter. This is true despite the enhanced bloom resistance that milkfat provides in chocolates. A better understanding of which components of milkfat are responsible for softening and bloom inhibition in chocolates will enhance the use of milkfat fractions in chocolates.

Publications

Lohman, M.H. and R.W. Hartel, Effect of Milkfat Fractions on Fat Bloom in Dark Chocolates, J. AOCS, 71(3), 267-276 (1994).

R.W. Hartel, Incorporation of Milkfat Fractions In Confectionery, J. AOCS (submitted).

INTERIM REPORT

Incorporation of Milkfat Fractions in Chocolates - Phase 2

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

Dates: September 1994 – August 1996

Funding: Wisconsin Milk Marketing Board 94-02

Objectives

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

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

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

Summary

A preliminary study to look at effects of specific components of milkfat on bloom inhibition in chocolates was carried out. A dark chocolate base, supplied by Guittard Chocolate Co., was used for this bloom study. This chocolate contained Ivory Coast cocoa butter, similar to our previous study. To this chocolate base, several different fats were added, and rates of bloom formation measured during storage under temperature cycling conditions. Chocolates were stored in a cabinet with the temperature cycled at 29°C for 8 hours and then at 19°C for 16 hours. The bloom study lasted 47 days. Bloom formation was measured using a Hunter colorimeter to calculate whiteness index.

The control chocolate, made only with cocoa butter, exhibited significant bloom over the 47 day period. Chocolates were made with 2% addition (fat basis) of pure trilaurin (C12:0) or tricaprylin (C8:0) to evaluate influence of chain length for trisaturated fats. In addition, 7% addition of a hard fraction (melting point of approximately 47°C) produced by melt (surface-layer) fractionation was included in this study for comparison purposes. As expected, the hard milkfat fraction completely inhibited bloom. Very little bloom was measured even after 47 days of this cycling. In contrast, the control chocolate showed significant bloom under these conditions. Interestingly, the sample made with trilaurin showed no difference from the control while the chocolate made with tricaprylin promoted bloom formation compared to the control.

Another interesting result from this preliminary study was the apparent cyclic nature of bloom formation. That is, whiteness index measurements increased and decreased on a daily basis. This pattern was particularly evident for the rapid blooming chocolate made with tricaprylin. Whiteness index gradually increased over the period of storage, although daily values showed considerable cycling. Further work is necessary to document changes in whiteness index during different periods of the temperature cycle, as this may improve our understanding of the mechanisms of bloom formation and inhibition.

Significance to the dairy industry

One of the main applications for milkfat fractions is in the chocolate and confectionery industry. In order to optimize the use of milkfat fractions in chocolate applications, a better understanding of the effects of components of milkfat on bloom resistance, softening of chocolate and lipid compatibility is needed. In addition, a better understanding of the kinetics of crystallization in the mixed system of cocoa butter and milkfat fractions is needed to optimize processing conditions for producing (tempering) chocolates.

FINAL REPORT

Use of Melt Crystallization Techniques for Fractionating Milkfat

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

Dates: July 1993 – June 1995

Funding: Dairy Management Inc. HL394

Objectives

1. To evaluate melt crystallization techniques which use controlled crystallization to separate milkfat into unique products for use as food ingredients.

2. To define the "ideal" separation target for milkfat under different conditions.

3. To evaluate fractions, particularly high melting fractions, from these melt crystallization technologies as an ingredient in chocolate and compound coatings.

Summary

Layer-type crystallization techniques are being used for fractionation of milkfat. Milkfat contained in a vessel is cooled through an internal cooling surface. By controlling the temperature of the cooling surface, crystallization of milkfat occurs directly on the surface. As a solid layer forms on the cooling surface, a partition between crystalline and liquid portions of milkfat occurs, depending on the temperature gradients and milkfat composition. Temperature of the solid layer is adjusted during the process to optimize formation of the solid phase. Initially, the temperature is lowered to initiate nucleation, followed by a temperature increase to promote growth. To enhance separation of the crystalline layer, a sweating procedure and/or a washing step may be used, involving a slight heating of the layer. Finally, the layer is removed from the surface by raising the temperature to melt the solid layer and the hard fraction collected in the liquid form.

In this project, we studied the effects of thermal profiling, washing steps and flow conditions in a dynamic crystallization apparatus on milkfat fractionation at 30°C. We then determined the effects of these parameters on crystalline yield and separation efficiency.

Conditions of crystallization had a significant effect on the separation of a hard milkfat fraction during crystallization on a surface layer at 30°C (defined as the temperature at the phase boundary between the surface layer and the liquid phase). Temperature of the cooled surface must be adjusted continuously to maintain the surface temperature as the crystal layer builds up. The effects of different cooling rates (internal cooling temperature) were studied. The slowest cooling rate studied (1.2°C/h) gave hard fractions with highest melting point (47-48°C), whereas higher cooling rates (up to 4.8°C) gave faster growth rates but resulted in fractions with lower melting point (about 44°C). SFC curves and fatty acid profiles documented these changes in melting point. The flow rate of milkfat along the cooling surface had only slight effect on fractionation. An increase in Reynolds Number from 1.2 to 12 caused an increase in surface layer thickness from 0.8 to 1.35 mm, and resulted in a subsequent drop in melting point of the fraction of about 0.4°C. Yield of high melting fractions under these conditions were between 21 and 25%, depending on the nature of the starting milk fat. Summer milkfat gave the lower yields while winter milkfat produced more of the harder fraction.

Post-crystallization processes were found to have significant influence on the characteristics of the hard fraction. An effective separation coefficient, keff, was calculated as the ratio of low melting compounds (C4:0 to C10:0 and C18:1 fatty acids) in the hard fraction to the original feed. For a given crystallization program, comparison of k_{eff} for n-hexane rinse, sweating and squeezing operations

with no post-treatment were made. n-Hexane was used for washing, although, in commercial practice, a portion of the liquid product would be used for washing. Sweating involved slowly heating the surface layer to allow liquid oil entrapped within the crystalline matrix to diffuse out. All post-treatments resulted in some improvement of separation, giving slightly lower k_{eff}, as compared to the control with no post-treatment. Melting point of the hard fractionation also increased with post-treatment. The best post-treatment was found to be the washing step, although further experiments using liquid fraction to wash are necessary.

When the identical milkfat was crystallized at 30°C in a suspension crystallizer for comparison purposes, slightly different results were obtained. Stirred operation resulted in hard fractions with melting point between 42 and 44°C, and yields of only 13 to 18%. Stagnant operations resulted in higher melting points (45-47°C), but lower yields (only 2-4%). Clearly, these results show the potential benefit of using surface layer crystallization for milkfat fractionation.

Due to the nature of the milkfat crystal, a significant portion of liquid is incorporated into the crystalline structure, making separation by filtration extremely difficult. This limits the nature of the separation that is obtainable with conventional technology.

Preliminary experiments were performed to identify nuclei during crystallization of milkfat under different conditions in an attempt to identify the "purest" component of milkfat crystal. In addition, acetone washing of the vacuum filter cake produced from conventional fractionation was studied to help identify the exact nature of milkfat crystals. Acetone washing, however, produced limited results since it appeared that some of the milkfat crystal was dissolved. Further work on this will be necessary to identify the truly crystalline material produced during conventional fractionation.

We found that, under well-controlled stable static conditions with spontaneous nucleation, the nature of the initial solid phase was identical. That is, the melting point, DSC curves and chemical composition were the same regardless of whether crystallization was slow or fast, or whether crystals were large or small, and were not dependent on the stage of crystallization (early or late). However, the nature of the initial crystals was dependent on the temperature of crystallization. These results demonstrate the need for further detailed study on the nature of nucleation of milkfat crystals. A better understanding of this process, whether in suspension or on a surface layer, is necessary for optimal control of fractionation technology.

In order to verify that hard fractions made using the surface layer technology behaved in the same way as those produced from suspension crystallization, these fractions were used to make chocolates. Chocolates made with the high melting fractions produced by surface layer technology were identical to those made from milkfat fractions produced by suspension crystallization technology at the same level of addition (7%). The chocolates tempered in the same manner, had identical hardness and snap, and were similarly resistant to bloom formation.

Significance to the dairy industry

Techniques that improve separation efficiency between solid and liquid phases to produce more distinct milkfat fractions are needed. Layer-type melt crystallization techniques, popular in the organic chemical industry, have the potential for providing controlled crystallization with minimal separation requirements to produce distinct fractions. In many instances, layer crystallization is more economical than suspension-type crystallization. In this project, we have demonstrated that surface-layer melt crystallization techniques can produce high melting milkfat fractions that are indistinguishable from those produced by existing technologies and at similar yields. Further work is needed to document the cost savings associated with fractionating milkfat to provide a range of fractions using this technology.

Publications

Tiedtke, M., S. Niehorster, J. Ulrich and R.W. Hartel, Separation of Milk Fats 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

Physical Chemistry of Lipid Mixtures: Dairy Based Spreads

Personnel: R. W. Hartel, associate professor, R.C. Lindsay, professor, J. Knollenberg, research assistant, B. Liang, research associate, Dept of Food Science

Dates: September 1994 – August 1996

Funding: Dairy Management Inc. HRL95

Objectives

1.To relate the specific triglyceride composition of milkfat fractions to phase and crystallization behavior of milkfat fractions.

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

Summary

A technique was developed for studying the intersolubility of hard milkfat fractions in soft fractions (liquid oils). This technique relies on measuring change in turbidity (absorbance) with increasing levels of addition of hard fraction in the liquid fraction. In this technique, the liquid oil was maintained at constant temperature (either 25 or 35°C) in a jacketed beaker and a specific amount of hard fraction added as a liquid fat. The system was allowed to equilibrate for at least 40 minutes before measuring the absorbance. Additional melted hard fat was then added and absorbance again measured. Addition levels of hard fraction (melting point of 46°C) of up to 35% in the liquid fraction (melting point of 15°C) were studied.

Using this technique, a clear solution is obtained with no change in absorbance until the point is reached where the hard fraction crystallizes separately from the liquid fraction. At this point, the absorbance goes up dramatically. The point of increased absorbance provides a measure of the intersolubility of one lipid in another. While this is not a thermodynamic equilibrium, the intersolubility measured in this way provides an estimate of how much hard fraction can be accomodated in the liquid fraction before crystallization occurs. For the fractions studied (with melting points of 46 and 15°C), the intersolubility was measured as about 16% at 35°C and about 7.8% at 25°C.

The phase behavior of mixed lipid systems can be seen in isosolids diagrams, where solid fat content is measured at different temperatures for the range of mixtures from 100% hard fraction to 100% soft fraction. These diagrams provide information about the compatibility of two lipids. When two lipids are incompatible, distinct decreases in solid fat content for the mixtures are observed. However, when two fats are miscible (compatible), the solid fat curves change linearly between the two pure fats. Using a Bruker Minispec PC-120 NMR, the solid fat curves for mixtures of several hard and soft milkfat fractions were measured. In general, mixtures of hard and soft milkfat fractions resulted in straight lines on the isosolids diagram, indicating complete compatibility between these fractions. These results were also confirmed on DSC thermograms of the same mixtures, where the peaks associated with each component changed uniformly with increasing addition of one fraction to the other.

Efforts are underway to correlate the above behavior of mixtures of milkfat fractions to the chemical composition of each fraction. Specifically, concentration of major species of triglycerides in each fraction (for example, trisaturated fats) will be correlated to phase behavior. Future efforts will correlate composition with crystallization kinetics of mixtures of milkfat fractions. Similar studies will be performed for mixtures of milkfat fractions and canola oil.

Significance to the dairy industry

Increased use of milkfat and milkfat fractions in dairy-based spreads requires an understanding of how chemical composition influences physical behavior. In this study, the focus is understanding the effects of chemical composition on phase and crystallization behavior. Through these studies, we hope to improve applications of milkfat fractions in dairy-based spreads.

INTERIM REPORT

Effects of Defined Milkfat Fractions on Postprandial Lipid Metabolism in the Rat

Personnel: Denise Ney, associate professor, Hui-Chuan Lai, graduate student, Mike Grahn, research specialist, Dept. of Nutritional Sciences

Funding: Wisconsin Milk Marketing Board 92-9

Dates: September 1992 – August 1995

Objectives

The overall objective of this research is to characterize the nutritional effects of defined milkfat fractions on lipid metabolism. Evidence suggests that cholesterol and triacylglycerol (TAG) metabolism will be altered in conjunction with changes in the fatty acid and TAG profiles of milkfat due to fractionation. Our research focuses on postprandial (i.e., after ingestion of a meal) lipid metabolism in animals fed diets containing either a low-melting point (liquid) or high-melting point (solid) milkfat fraction. A specific objective of this project is to:

Characterize the composition of lymph chylomicrons in animals fed liquid or solid milkfat fractions compared to palm oil or corn oil.

Summary

The composition of mesenteric lymph chylomicrons and the time course of lipid digestion were determined in animals adapted to eat a daily 3-hour meal containing 16% fat from liquid milkfat enriched in C 18:1fatty acids, solid milkfat enriched in C16:0 and C18:0 fatty acids, palm oil, or corn oil.

Results indicated significant differences in the composition of lymph due to ingestion of liquid versus solid milkfat fractions. The data suggest that ingestion of liquid milkfat fractions or corn oil results in production of chylomicron particles with a significantly higher TAG to phospholipid ratio, suggesting larger chylomicron particles, compared to feeding solid milkfat or palm oil. The similarity in response when comparing liquid milkfat and corn oil suggests that modification of the fatty acid and TAG profiles of milkfat improves the lipid response associated with ingestion of milkfat.

Determination of the fatty acid and TAG profiles of diet and lymph chylomicrons provided additional information about the effects of milkfat on lipid metabolism. The profile of TAG species in lymph based on total acyl carbon number, indicated that TAG species with less than 40 carbons are present in the milkfat diets, but they do not appear in lymph. Similarly, lymph from animals infused with liquid or solid milkfat fractions contained few short-chain fatty acids but the profiles of C14:0, C16:0 and C18:1 fatty acids were maintained compared to the emulsions infused.

Significance to the dairy industry

The American public currently demonstrates great interest in issues related to diet, nutrition and health. In order to market dairy products to consumers, the dairy industry needs valid information about the nutritional effects of milkfat. Our research extends and compliments work conducted at CDR by providing new information regarding the *in vivo* nutritional effects of defined milkfat fractions on lipid metabolism. This will be key knowledge for marketing future dairy products to consumers.

Publications/presentations

Lai, H. -C., Lasekanm J. B., Monsma, C. C. and Ney, D. M. 1994. Alteration of plasma lipids in the rat by fractionation of modified milkfat (butterfat). J. of Dairy Science, 78:794-803, 1995.

Lai, H. -C., Ney, D. M, Corn oil, palm oil and butterfat fractions affect postprandial lipemia and lipoprotein lipase in meal-fed rats. J. Nutr. 125:1536-1545, 1995.

CDR Technical Cheese Conference, March 22-23, 1995, Green Bay, WI.

INTERIM REPORT

Kinetics of Milkfat Crystallization

Personnel: R.W. Hartel, associate professor, Dept of Food Science, D.B. Patience, research assistant, Dept of Food Science, D. Illingworth, New Zealand Dairy Research Institute

Funding: New Zealand Dairy Board and Wisconsin Milk Marketing Board 92-8

Dates: Feb 1994 – January 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

Optimal separation by filtration of milkfat crystals from a slurry produced in a crystallizer depends on the size, shape and number of crystals. These parameters are controlled by the operating parameters in the crystallizer, which determine the kinetics rates of crystal formation and growth. The initial aim of this project is to relate the kinetics of crystallization of milkfat to the efficiency of filtration, and further, to relate these parameters to the mixing conditions in a batch crystallizer.

A preliminary experimental design has been completed to investigate techniques for measuring crystallization kinetics in a batch crystallizer, and for determining filtration efficiency in a custom-designed pressure filtration cell. Several different agitator designs and agitator speeds were tested in this initial series of experiments. Impeller (propeller-type) to tank diameter ratio, bottom impeller clearance to impeller diameter ratio, and number of impellers (1 or 2) were studied using a 1 L jacketed crystallization tank with temperature maintained at 30°C. Crystallization kinetics (induction time for nucleation, and growth rate) were determined using an image analysis technique. Milkfat fractions produced were analyzed for SFC, DSC thermal profiles and composition by GC. Filtration kinetics were evaluated at different pressures using the custom-made pressure filtration cell.

Filtration curves were obtained for each crystalline slurry by measuring the amount of material passing through the filter with time at constant pressure. These filtration curves were analyzed using a standard filtration equation. An important result was that filtration data could be linearized by plotting time of filtration divided by cumulative filtration volume vs. the cumulative filtration volume. These plots can then be used to characterize filtration characteristics (cake resistance and resistance of the filtration medium) for different slurries under different filtration conditions (pressure, filter media, etc.). Some scatter in the filtration curves was observed for replicates of crystallization conditions. Further work is underway to characterize factors that affect this filtration process.

Based on these preliminary results, a complete series of experiments is currently underway to investigate the influence of mixing parameters on crystallization kinetics, crystal size and shape, and their subsequent influence on the filtration process.

Significance to the dairy industry

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

FINAL REPORT

The Implications of Changing Dairy Product Consumption for the Demand for Farm Milk-Phase 2

Personnel: Brian W. Gould, senior scientist, Kurt A. Carlson, assistant researcher, J. H. Park, research assistant, Center for Dairy Research and Department of Agricultural Economics

Dates: June 1994 – June 1995

Funding: Wisconsin Milk Marketing Board
UWAP9401

Objectives

1. To determine if consumer acceptance of reduced fat dairy products in meeting dairy product demand has increased over the 1991/92-1993/94 period

2. To determine if dairy processors in general and cheese manufacturers in particular can improve financial viability by marketing reduced fat dairy products

3. To determine if butter demand is more price and income sensitive than other sources of food fats and oils

Summary

The U.S. dairy industry is restructuring, both at the farm and processing level. On the farm, there has been a tremendous shift in production of milk, decreasing in traditional milk production areas and increasing in the west and south central regions of the U.S. On the processing side, there has been significant consolidation of processing firms and this is expected to continue. The dairy processing industry has increased efforts to develop reduced fat dairy products in response to consumer demand based on increased health concerns about dietary fat intake.

To analyze the influence of changing dairy product consumption patterns on the demand for farm milk we focused on consumer health awareness and socio-economic status. We not only looked at household consumption of dairy products, but also choices made within the household regarding reduced fat varieties. For FY 1995 we proposed to extend an earlier study by analyzing increased demand for reduced fat dairy products during 1991-1994. In order to conduct this analysis we augmented the 1991/92 Dairy Product Consumer Panel with an updated 1993/94 Consumer Panel. From both these consumer panels we were able to identify households that supplied consistent data over both years using two criteria: 100% response rate and 85% response rate. Using the 100% criteria, 6740 households reported dairy product purchases in both years. We used the 100% sample criteria in this analysis to control for the effect of lack of response when we compared households across years.

Hypothesis 1: Consumer Acceptance of Reduced Fat Dairy Products in Meeting Dairy Product Demand has Increased Since 1991

Butter, Margarine, Butter Blends

Over the 1991/92 survey year, households in the Nielsen Dairy Product Consumer Panel spent an average of $38.72 on food fats and oils. This decreased to $33.15 in 1993/94. For consumers of butter, the average expenditure was $13.27 in 1991/92 and $11.47 in 1993/94. The percent of the households purchasing butter increased from 46.5% to 51.8%. For butter/margarine blends, the percent of households purchasing decreased slightly. Close to 90% of the sample households purchased margarine over this three year period with margarine consumers purchasing more than 2 1/2 times the amount of butter purchased by butter consumers.

We also looked at the distribution of sample households that consume butter, margarine or butter/margarine blends. Margarine accounted for

the largest number of households consuming only one commodity, with 32.1% of the households consuming only margarine in 1991/92 and 34.9% in 1993/94. This compared with 4.2% consuming only butter and 1.1% consuming only blends in 1991/92. We also found that 90.4% of households consumed margarine in 1991/92 versus 88.2% in 1993/94. For butter these percentages were 46.5% and 51.8%. For blends these percentages were 36.6% and 33.9%.

Fluid Milk

Since the mid-1980's, per capita reduced fat milk consumption has exceeded whole milk consumption. In addition to the shift in consumption away from whole milk, total per capita consumption of fluid milk has been decreasing. In response, several new industry efforts have attempted to increase fluid milk consumption. For example, the Fluid Milk Processor Education program, funded by fluid milk processors, addresses consumer misconceptions and attitudes about milk as a healthy beverage.

Table 1 shows mean purchase patterns for consuming households for a variety of fluid milks. Sample households consumed an average of 39.0 gallons of fluid milk in 1991/92 compared with a 1993/94 level of 34.4 gallons. Households in this sample show significant diversity in their purchase patterns – less than 25% of milk consuming households purchase only 1 type of milk. Also, the percentage of total milk originating from one milk source is relatively small. For example, for whole milk consuming households, whole milk accounted for less than 42% of total milk consumed. Consumers of 2% milk appear to be the most specialized.

Cheese

The introduction of reduced fat varieties of traditionally full fat cheeses is more recent than for fluid milk or butter. Table 2 shows mean annual cheese consumption levels for a variety of cheeses regardless of fat content. Approximately 99% of our sample households purchased some type of cheese over both sample years. Mean per household purchase is 27.9 pounds in 1991/92 and 25.9 in 1993/94. Associated annual expenditures are $68.08 and $64.34, respectively. The most commonly purchased cheese is processed cheese where over 85% of cheese purchasing households, purchase processed cheese. A majority of this processed cheese are American varieties. Mean Processed Cheese expenditures were $26.29 and $24.21 in 1991/92 and 1993/94, respectively.

Within the Nielsen Dairy Product Consumer Panel, we can identify whether a particular cheese is a reduced (non) fat variety. Separating cheese purchases by this identifier, Table 3 shows mean reduced fat cheese purchases over the 3 year study period. Excluding cottage cheese, for the 6740 sample households, there is a slight increase in the percentage of households purchasing reduced fat cheese (58.5% in 1991/92 and 61.6% in 1993/94) and a slight increase in the percent of total cheese purchased associated with reduced fat varieties (12.1% in 1991/92 and 15.9% in 1993/94). In 1993/94 17.8% of processed cheese purchased was of a reduced fat variety This represents a 3% increase over the 1991/92 level. For Cheddar cheese there was no change in the relative importance of reduced fat varieties. There was a 3 percentage point increase in the relative importance of reduced fat Mozzarella cheese increasing from 7.5% of total Mozzarella purchases in 1991/92 to 10.5% in 1993/94. Not surprisingly, for both years over half of the cottage cheese purchased was reduced fat.

Yogurt

Table 4 shows mean yogurt purchase patterns for 1991/92 and 1993/94. Over the 3 year study period, the percent of households purchasing non-fat yogurt increased from 43.5% in 1991/92 to 48.4% in 1993/94. There are substantial differences in mean consumption of yogurts with various fat contents for both years. In both years, consumers of full fat yogurt consumed considerably less than consumers of reduced and non-fat yogurt. For example, in 1993/94, consumers of full fat yogurt purchased an average of 6.4 pints compared to 10.0 and 11.5 pints for reduced and full fat varieties, respectively. The mean non-fat yogurt consumption level represents a 24.3% increase over the 1991/92 level. For both years the reduced and non-fat yogurt prices are more than 65% higher than full fat yogurt price.

Table 1: Mean Annual Fluid Milk Purchases, 1991/92-1993/94

Commodity	Annual Purchase Characteristics[a]											
	% Purchase	Gallon	Expend.	% Who[b] Consume1 Milk	% Total Milk	Price	% Purchasing	Gallon	Expend.	% Who[b] Consume1 Milk	% Total Milk	Price
	1991/92						1993/94					
All Milk	97.7	39.0	90.57	21.6	100	2.32	97.2	34.4	84.90	24.0	100	2.47
Whole Milk	55.5	15.2	36.91	7.5	41.1	2.43	50.3	12.9	32.57	7.8	40.8	2.53
2% Milk	71.7	21.6	47.63	8.7	52.0	2.21	69.4	19.1	44.91	9.5	52.6	2.35
1% Milk	46.0	15.0	33.28	1.7	35.3	2.22	43.8	14.1	33.23	1.9	36.3	2.36
Skim Milk	42.8	15.7	34.93	3.4	41.2	2.22	42.5	16.6	39.85	4.4	48.3	2.40
Buttermilk	25.0	2.0	6.07	0.3	8.2	3.00	23.2	2.0	6.47	0.3	9.0	3.18

Source: 1991/92 and 1993/94 Nielsen Dairy Product Consumer Panel--100% response rate criterion, 6740 observations.
Note: a = calculated for consumers of each milk type
b = percent of milk consumers who consume only this milk type

Table 2: Mean Cheese Purchases, 1991/92-1993/94

Cheese	1991/92 Cheese Purchase Characteristics					1993/94 Cheese Purchase Characteristics				
	%[a] Purchase	Pounds[b]	Expend.[b]	Price[b]	% of Total[c]	%[a] Purchase	Pounds[b]	Expend.[b]	Price[b]	% of Total[c]
All Cheese	98.6	27.9	68.08	2.44	100	98.5	25.9	64.34	2.48	100
All Except Cottage Cheese	98.1	19.9	58.27	2.92	72.9	97.0	19.3	49.33	2.55	74.6
Muenster	3.7	1.1	3.75	3.50	5.7	2.4	1.2	4.25	3.57	6.9
Mozzarella	62.1	4.0	12.84	3.22	15.6	58.7	3.9	12.39	3.18	16.5
Brick	0.5	0.8	2.17	2.65	3.1	0.2	0.9	2.58	2.84	3.2
Remaining	25.1	1.7	5.64	3.40	7.8	23.8	2.0	6.19	3.05	9.0
Colby	22.1	2.2	6.87	3.11	8.8	20.1	2.1	6.60	3.08	8.6
Cheddar	70.6	4.4	14.60	3.29	18.8	70.0	4.8	14.70	3.09	21.4
Ricotta	23.7	3.8	6.23	1.66	15.5	22.1	3.8	6.33	1.67	16.5
Grated	54.5	1.3	6.88	5.50	7.2	52.1	1.2	7.08	5.71	8.0
Processed	86.3	9.7	26.29	2.72	40.6	85.1	9.0	24.21	2.70	41.3
Swiss	20.7	1.6	7.02	4.50	7.6	16.3	1.9	7.82	4.23	9.0
Imported	18.7	1.1	6.39	5.71	6.7	16.2	1.1	6.69	5.87	6.9
Cottage	68.8	10.8	12.91	1.19	37.1	64.6	10.1	13.23	1.32	36.7
Cream Cheese	77.3	3.8	8.20	2.18	15.6	75.7	3.8	8.23	2.19	17.0

Source: 1991/92 and 1993/94 Nielsen Dairy Product Consumer Panel--100% response rate criterion, 6740 observations.
Note: a = Except for All Cheese, the percentages are calculated based on All Cheese consuming households.
b = These values are calculated for consumers of each particular cheese.
c = These percentages represent the percent of each cheese variety of Total Cheese for consumers of each variety.

Table 3. Reduced Fat Cheese Purchases Conditional on Cheese Purchases

	1991/92 Purchases				1993/94 Purchases			
Cheese Type	% Purchasing Reduced Fat Cheese	% Reduced Fat of Total Cheese	Conditional Quantity for Full-Fat Varieties	Conditional Quantity for Low-Fat Varieties	% Purchasing Reduced Fat Cheese	% Reduced Fat of Total Cheese	Conditional Quantity for Full-Fat Varieties	Conditional Quantity for Low-Fat Varieties
All Cheese	74.4	21.1	22.0	8.0	73.3	23.3	20.0	8.4
All Cheese (Ex. Cottage Cheese)	58.5	12.1	17.8	5.9	61.6	15.9	16.4	6.1
Cottage	73.5	52.5	7.9	7.4	72.6	53.0	7.4	7.3
Muenster	3.2	3.1	1.1	0.4	4.3	3.6	1.2	0.5
Mozzarella	19.8	7.5	3.8	1.3	23.4	10.5	3.7	1.5
Cheddar	25.6	11.3	4.3	1.5	23.5	11.3	4.6	1.9
Co-Jack/ Mont. Jack	12.9	8.6	1.6	0.9	12.5	8.5	2.0	1.2
Colby	14.4	10.2	2.2	1.0	12.1	9.1	2.2	1.1
Brick	---	---	0.8	---	---	---	0.9	---
Processed	38.8	14.8	8.9	3.0	38.0	17.8	8.2	3.6
Ricotta	---	---	3.8	---	---	---	3.8	---
Grated	---	---	1.3	---	---	---	1.2	---
Swiss	20.5	14.0	1.5	0.9	9.6	6.4	1.8	1.0
Imported	---	---	1.1	---	---	---	1.1	---

Source: 1991/92 and 1993/94 Nielsen Dairy Product Consumer Panel--100% response rate criterion, 6740 observations.

Table 4. Average Yogurt Purchases: 1991/92 and 1993/94

Year	Type	All Yogurt Consumers			
		Obs.	Pints	Expend	Price
1992	Full Fat	1265	6.5	6.02	1.04
	Reduced	3452	9.9	12.76	1.80
	Non-Fat	2934	8.7	11.68	1.72
1994	Full Fat	1030	6.4	6.05	1.02
	Reduced	3427	10.0	13.20	1.79
	Non-Fat	3259	11.5	15.64	1.70

Source: 1991/92 and 1993/94 Nielsen Dairy Product Consumer Panel --100% response rate criterion, 6740 observations.

Hypothesis 2: Dairy Processors in general and cheese manufacturers in particular can improve financial viability by marketing reduced fat dairy products.

Within the Nielsen Dairy Product Consumer Panel are data listing price paid, fat content of product, product form (for example with cheese, we know whether it is bulk, sliced, etc), use of coupon and value of coupon. To examine this hypothesis we will focus on the cheese industry but will also provide some comments with respect to food fats and oils and yogurt.

Cheese

Table 5 describes the amount, expenditure and price of full fat and reduced fat varieties of several cheeses. There is some variation in the differences in the prices across cheese type. These differences may be reflecting differences in yields, market maturity and product form. We found that purchases of reduced fat cheeses occurred via the use of coupons. For 19 of the 25 cheese/type combinations, the percent of reduced fat cheese consuming households using coupons exceeded the percent of full fat cheese consuming households. This is surprising, given the above result of the net price of reduced fat cheese being greater than the net price of full fat varieties.

Hypothesis 3: Butter Demand is More Price and Income Sensitive than Other Sources of Food Fats and Oils

To better understand the relationship between household income, commodity price and fats and oils demand, we estimated econometric demand models for butter, butter/margarine blends, corn oil, olive oil, vegetable shortenings and other oils. Except for margarine, there is a large proportion of the sample that does not purchase a particular fat and oil during the survey year. We hypothesize a two-stage purchase process where a household decides whether to purchase a commodity, then conditional on that decision, determines the quantity to purchase. We hypothesize that household characteristics influence both stages of the purchase process.

Substantial progress has been made in our analysis of the purchasing of alternative dairy products, specifically reduced fat varieties of cheese, butter blends, and other traditional products. Our analysis, though descriptive, forms the foundation for future work. We are currently developing parametric econometric methodologies for examining the statistical inter-relationships between the consumption of dairy products where substantial numbers of the population are non-consumers. Using traditional demand system models in this environment are not acceptable as such methodologies can lead to biased parameter estimates. Besides our activities with respect to econometric based demand analyses, we are also initiating research in the area of non-parametric demand system estimation. This new methodology has the potential for allowing us to examine demand inter-relationships for a great number of disaggregated dairy products. Using parametric methods, problems with estimation occur very quickly with few disaggregated dairy products.

As can be seen by this analysis, we grouped all household purchases into annual totals and averages. This helps us understand general purchase trends but hides the panel nature of the Nielsen Dairy Product Consumer data set. During FY 1996 we will be using the true panel nature of this data set to examine the actual adoption process by which households adopt new dairy products into their market basket. On a weekly basis we will: (i) examine what dairy products consumers are using, (ii) how commodity promotion affects adoption of new products, (iii) what happens after such promotion (if it does impact adoption) is stopped, and (iv) what is the impact of the adoption of new dairy products on the consumption of previously purchased dairy products, that is do these new products add to the dairy budget or "crowd out" traditional dairy products?

Significance to the dairy industry

This project will provide valuable information regarding the current consumers of full and reduced fat varieties of cheese, fluid milk, yogurt and other dairy products. We will also analyze consumption of butter, butter-based spreads, other food fats and oils and the development of these dairy markets.This project addresses many of the issues associated with reduced fat dairy products (and cheeses in particular) and the effects on demand for farm milk.

Table 5. Average Annual Cheese Purchases by Type (For Consuming Households)

Cheese	Fat Content	1991/92				1993/94			
		Pounds	Expenditure	Price	% Price Difference	Pounds	Expenditure	Price	% Price Difference
Cheddar	Full Fat	4.3	13.65	3.20	32.5	4.6	13.54	2.98	38.3
	Reduced	1.5	6.49	4.24		1.9	7.83	4.12	
Processed	Full Fat	8.9	23.58	2.64	23.9	8.2	21.10	2.58	28.6
	Reduced	3.0	9.74	3.27		3.6	11.89	3.32	
Cottage	Full Fat	7.9	9.02	1.14	9.6	7.4	9.35	1.27	7.1
	Reduced	7.4	9.25	1.25		7.4	9.98	1.36	
Mozzarella	Full Fat	3.8	12.14	3.17	24.9	3.7	11.59	3.12	23.7
	Reduced	1.3	5.11	3.96		1.5	5.64	3.86	
Colby	Full Fat	2.2	6.82	3.06	31.6	2.2	6.57	3.04	23.0
	Reduced	1.0	4.03	4.03		1.1	3.93	3.74	
Swiss	Full Fat	1.5	6.72	4.45	9.2	1.8	7.69	4.18	27.8
	Reduced	0.9	4.42	4.86		1.0	5.18	5.34	
Cream	Full Fat	3.1	6.38	2.06	19.5	3.0	6.30	2.09	29.2
	Reduced	1.4	3.64	2.56		1.7	4.49	2.70	

INTERIM REPORT

Characterization of Creaming and Aeration Functionalities of Milkfat Fractions for Cakes, Cookies, and Butter Cream Icings

Personnel: Robert C. Lindsay, professor, Dept. of Food Science; Kerry Kaylegian, associate researcher, CDR; Johana Setiabudhi, graduate research assistant, Dept. of Food Science

Dates: July 1993 – December 1995

Funding: Dairy Management Inc. LS394

Objectives

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

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

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

Summary

Studies have focused on the functionality of milkfat and milkfat fractions in a selected butter cake formulation. Functionality has been measured by various methods, including specific gravity of the batter and the volume of the baked cake as determined by the rapeseed displacement procedure. The specific gravity of a batter is its density, determined by dividing the weight of a given volume of batter by the weight of an equal volume of water. In this usage, the greater the density of the batter, the smaller its volume will be per given weight.

Anhydrous milkfat, milkfat fractions, butter, and hydrogenated vegetable oil shortenings were investigated for their functionalities in batter and resulting butter cakes. The mixing temperature is an important variable in the determination of fat functionalities in baking. Mixing temperatures ranging from 55° to 80°F were investigated in relation to their effect on specific gravity and volume. We found that higher mixing temperatures diminish the functionalities of milkfat to a greater extent than hydrogenated vegetable oil shortening. Thus, improved functionality of milkfat and fractions can be obtained by suitably controlling the temperature of mixing at the optimum temperature.

Investigations have been started to determine the effects of a wide range of emulsifiers on the functionality of milkfat and fractions in butter cakes. Optimum types and levels of emulsifier usage in butter cakes are determined using loaf volume, ratio of cake volume/weight of batter, viscosity of batter, and scanning electron microscopic examinations of batters and finished cakes. These data will permit the recommendation of specific emulsifiers for use with anhydrous milkfat and milkfat fractions. Research will continue on these aspects, and investigations will be extended to cookies and butter-cream icings.

Significance to the dairy industry

Developing fundamental data by investigating milkfat properties in cakes, cookies, and icings will help to improve the functionality of milkfat in baked goods and expand the opportunities for using both milkfat and milkfat fractions in the baking industry.

Nonfat Solids Summary

Chapter 2

NONFAT SOLIDS

Milk proteins and lactose are the valuable components left in whey after making cheese. CDR's nonfat solids research program explores technologies that enhance the value of these components in food and nonfood uses.

After refining his whey protein fractionation procedure, Etzel demonstrated the feasibility of using ion-exchange membranes for purifying glycomacropeptide (GMP) from whey. Purified GMP is a potential protein source for treating people with metabolic disorders such as phenylketonuria, or PKU, who cannot metabolize aromatic amino acids. Once he achieved the separation, Etzel moved to the next phase of the project: purifying Lactoferrin (LF) and Lactoperoxidase (LP) from whey. The concentration of LF in human milk is 20X greater than in bovine milk so supplementing bovine-derived infant formula with LF makes formula more like human milk. Although significant quantities of LF and LP were purified from whey, refining the process is still necessary to improve recovery.

With the emphasis and value on the protein portion of whey, methods for "value added" use of the remaining permeate are also being investigated. Doug Cameron and Mark Etzel are examining the production of propylene glycol through the fermentation of whey permeate. The fermentation process yields an optically pure R-propylene glycol, which has a smaller but higher value market. To date, they have adapted an organism to use lactose while still producing propylene glycol. Also, initial tests confirmed the ability to remove the lactate and acetate produced during the propylene glycol fermentation. This is an essential part of an economically viable process. Optimization of the system will continue.

INTERIM REPORT

Fractionation of Whey Proteins Using Ion-exchange Membranes

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

Dates: November 1993 – November 1996

Funding: Wisconsin Milk Marketing Board 93-9

Objectives

The overall objective of this research is to develop an economical large-scale technology for producing pure individual whey proteins (PIWP) that will allow existing whey protein concentrate manufacturers to convert to making PIWP without having to invest in a new plant. This new technology is needed to exploit the unique nutritional and functional properties of PIWP not found with other proteins, such as soy and egg proteins. The specific objectives are to:

1. Show that glycomacropeptide (GMP), lactoferrin (LF) and lactoperoxidase (LP) can be fractionated from whey using an ion-exchange (IEX) membrane cartridge.

2. Show that beta-lactoglobulin (BLG), alpha-lactalbumin (ALA) and immunoglobulin (IgG) can be fractionated into pure products using multicomponent adsorption behavior of the IEX membrane.

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

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

Summary

Initial efforts centered on fractionating GMP from whey. GMP is the moiety cleaved from kappa-casein by chymosin during cheese making. It occurs at a concentration of 1.2 to 1.5 g/L in sweet whey, comprising 15 to 20% of the total protein. The aromatic amino acids phenylalanine, tryptophan and tyrosine are absent in GMP as are the amino acids histidine, arginine and cysteine. GMP can be used as a protein source for treatment of diseases such as phenylketonuria (PKU), a hereditary disorder in which aromatic amino acids cannot be metabolized. GMP is an example of a purified individual whey protein which has unique medical or health benefits not found with other proteins such as soy or egg proteins. Developing a technology for fractionation of GMP from whey will allow production of a high-value nutraceutical product from whey.

GMP was fractionated from whey using a quaternary methyl amine (QMA) IEX membrane cartridge (model PSC10-QM, BPS Separations Ltd., Spennymoor, County Durham, U.K.) with a 10-mL bed volume (BV). Purity and recovery were measured at different pH, flow rate, loading volume, and eluant composition. Optimal conditions were found to be a whey pH of 5.5-6.0, loading volume of 10-15 BV, flow rate greater than 1.5 BV/min, and 0.3 M NaCl for elution. Elution peaks were concentrated by ultrafiltration and analyzed using size exclusion HPLC, spectroscopy, total nitrogen, and amino acid analysis. Purity was essentially 100% as determined by HPLC and the ratio of absorbance at 214 and 280 nm. Amino acid analysis revealed minor amounts of aromatic amino acids. The process was scaled-up by a factor of four using the optimal conditions. Ten cycles were successfully conducted, yielding 3.2 g GMP from 4.8 L of whey.

In the next phase of the project, LF and LP were fractionated from whey using a sulfopropyl (SP) IEX membrane cartridge (model PSC10-SP, BPS Separations Ltd.) with a 10-mL BV. Optimal conditions were found to be a whey pH of 5.0-5.5, elution using 0.3 M NaCl for LP, and elution using

0.9 M NaCl for LF. Decreasing the flow rate increased recovery of LP. Flow rates less than 0.1 BV/min were required for 80% recovery as measured by enzymatic activity of LP. The origin of this flow rate restriction was attributed to slow transport of proteins to the IEX moieties as a result of the large pore size of the membrane (50-300 μm) and the large size of the proteins (77 kDa). Future work will explore eliminating the flow rate limitation by using an IEX membrane with a 5 μm pore size (model S100X, Sartorius Corp., Edgewood, NY).

Significance to the dairy industry

Although the U.S. market for high value, purified whey proteins is small at present, several factors indicate it will grow. Other major dairying countries are developing markets for these purified individual whey protein products. In the future, the food industry will demand proteins with higher nutritional and functional properties because of the trend towards foods with enhanced health benefits, lower fat content and lower lactose content.

The proposed IEX membrane process offers numerous advantages. Evaluating and optimizing the process should provide the stimulus for the U.S. dairy industry to expand into the new domestic and international markets for whey protein products with enhanced nutritional and functional properties.

Publications/Presentations

Etzel, M. R. 1995. Whey protein isolation and fractionation using ion exchangers. In *Bioseparation Processes in Foods*, R.K. Singh and S.S.H. Rizvi (Ed.), p. 389, Marcel Dekker, Inc., New York.

INTERIM REPORT

Conversion of Whey Permeate to Propylene Glycol for Food and Non-Food Uses

Personnel: Douglas C. Cameron, associate professor, Dept. of Chemical Engineering; Mark R. Etzel, associate professor, Dept. of Food Science; Nedim E. Altaras, graduate student, Dept. of Chemical Engineering; Afandi B. Suhenda, student, Dept. of Chemical Engineering

Dates: July 1994 –June 1996

Funding: Dairy Management Inc. CME95

Objectives

The overall objective of this project is to develop a fermentation process for the conversion of lactose in whey permeate to propylene glycol, a large volume commodity chemical. The fermentation process yields an optically pure product, R-propylene glycol, with added value over synthetically produced propylene glycol. The specific objectives are to:

1. Screen and select microorganisms capable of fermenting lactose to propylene glycol.

2. Optimize the medium and environmental conditions for propylene glycol production from whey permeate by the organism(s) identified in Objective 1. The effects of carbon to nitrogen ratio (C/N), trace nutrients, and medium pH will be investigated.

3. Develop technology for the removal of inhibitory acetate and lactate from whey permeate-based medium during fermentation so as to achieve increased propylene glycol concentration and productivity.

Summary

The initial objective of this project was to screen microorganisms for the ability to ferment lactose to propylene glycol. The first organism tested, *Clostridium thermosaccharolyticum* HG-8 (ATCC 31960), showed significant promise, and therefore was the focus of all studies to this point. It was grown in 15 mL culture tubes under anaerobic conditions at 60°C. Serial culture was used to adapt the strain for growth on lactose. The adapted strain was also grown on galactose.

After adaptation on lactose, the strain was grown in 300 mL anaerobic flasks on a medium containing 30 g/L of either lactose, galactose, or a mixture of glucose and galactose as the carbon source. Propylene glycol was not produced from lactose or galactose. However, a total of 4.5 g/L of propylene glycol was produced from the glucose-galactose medium. The strain was then grown on spray dried whey permeate reconstituted to 50 g/L of lactose. Two different preparations of whey medium were examined: unmodified whey permeate and whey permeate supplemented with 5 g/L of autoclaved yeast extract. No propylene glycol was produced on unmodified whey permeate, but permeate modified with yeast extract gave 3.2 g/L. Since we could not have obtained this much propylene glycol from fermentation of the yeast extract alone, our experiment demonstrates that lactose is fermented to propylene glycol. Future experiments will also investigate the use of hydrolyzed whey permeate.

As part of Objective 3, we have explored the use of anion exchange resins for the removal of acetate and lactate from a simulated fermentation broth. Lactate behaves like acetate in ion exchange, but it is easier to analyze for lactate. Therefore, a column packed with Amberlite IRA-400 anion-exchange resin was used to remove lactate from aqueous solution. Extraction was carried out on 1 L fermentation broth to which lactate was added with a syringe pump in order to simulate the 18 mM/h target productivity rate of propylene glycol in the fermentation. The solution was pumped out of fermentor at a rate of 0.33 L/h, and into a column containing 50 g of resin. The effluent of the column

was returned to the fermentor to form a recycle system. Lactate concentration in the fermentor was measured vs. time. These conditions were successful in maintaining the lactate concentration in the fermentor at the target level of 5 g/L. However, in order to reach the target fermentation time of 14 h, the resin amount will have to be increased to 100-150g. The column was successfully regenerated with 1 M NaOH solution. Future work will include performing the extraction under fermentation conditions.

Significance to the dairy industry

This research will provide the basis for the industrial production of a new fermentation chemical, propylene glycol, from whey permeate. Propylene glycol is a major organic chemical with extensive applications in the food industry, both as an ingredient and as an antifreeze and heat transfer fluid. Currently propylene glycol is produced entirely from propylene, a petrochemical. This synthetic propylene glycol is a racemic mixture, a mixture of left and right handed forms of propylene glycol. The fermentation process provides a unique route to enantiomerically pure R-propylene glycol.

FINAL REPORT

Freeze Concentration of Fluid Dairy Products

Personnel: Richard W. Hartel, associate professor, Y-H Chang, research assistant, Z. Zhang, research assistant, Dept. of Food Science

Dates: September 1991– August 1994

Funding: Wisconsin Milk Marketing Board 91-17

Objectives

1. To develop a continuous freeze concentration process based on control of heat transfer conditions to maintain optimal ice crystal growth.

2. To quantify the effects of heat transfer conditions on ice crystallization during freeze concentration of fluid dairy products.

3. To quantify the effects of nucleation conditions on growth rates and ice crystal morphology during freeze concentration in fluid dairy products.

4. Evaluate the economic potential of this new freeze concentration concept based on the results of the above studies.

Summary

The primary objective of this phase of research on freeze concentration involved developing a new continuous crystallizer based on the concept of controlling heat transfer conditions to optimize ice crystal growth. The goal is to produce large, uniform, spherical crystals for efficient separation. We organized this project into three subprojects. The first involved developing a continuous freeze concentration unit and evaluating the operating conditions. Alongside these experiments, we performed a study on the effects of high concentrations of milk solids on ice crystal growth. Finally, we compared viscosity of freeze concentrated milk (both high concentration and reconstituted) to thermal evaporated skim milk.

A continuous crystallizer comprised of six individual chambers (150 mL) mounted on top of each other as a column was designed, constructed and evaluated. Each chamber had an individual jacket for refrigerant so temperature profile and heat transfer rates along the column could be controlled. In this system, refrigerant was fed to the top chamber and sequentially passed through to the bottom of the column. This allowed us to maintain a temperature profile keeping the coldest temperatures for growth at the top of the column. Ice crystal seeds moved from the bottom of the column crystallizer and flowed upwards by stirring and buoyant forces. Skim milk was fed to the top of the column and traveled downwards as it became more concentrated. Ice crystals for separation were removed from the top while product concentrate was removed from the bottom. Counter-current movement of ice and concentrate allowed increased efficiency in crystal growth as compared to normal batch operation. Agitation in each chamber was accomplished using multiple stirrer blades mounted on a single stirrer shaft. Nucleation of ice crystals occurred in the bottom chamber, maintained at low temperature by an separate, external refrigerant. The crystallizer was operated within a refrigerated glove box to minimize heat losses, until steady state operation was attained.

Three series of experiments were conducted to evaluate the effects of operating parameters (temperature in growth section, temperature in nucleation section, and stirring speed) on ice crystallization in skim milk. For all experiments, final concentration of exit product was set at 17% for an initial feed concentration of 10%. Total residence time of skim milk within the column was about 2-3 hours, in which time ice crystals greater than 500 mm were removed at the top. These ice crystals were quite uniform in size and shape, and were easily separated from concentrate by simple washing. We obtained greater rates of ice crystal growth and production with lower initial refrigerant temperatures in growth (-2.5 to -1.5°C) and nucleation (-4 or -4.5°C) chambers. However, smaller crystals were obtained with lower nucleation temperature as more ice crystals were formed. Agitation speed only had a slight effect,

with only small increases in ice crystallization rates with increasing agitator speed. The primary factor affecting ice crystallization was the temperature of the growth chambers.

A separate project evaluating ice crystal growth kinetics at high milk solids concentrations was undertaken. Ice crystal growth rates were determined at milk solids content up to 40% in a fed-batch crystallizer. Growth rates were determined by evaluating change in mean size of the size distribution with time during growth. As expected, growth rates decreased as concentration increased at all subcooling temperatures evaluated. However, at high subcoolings (low temperature operation), nucleation began to occur, particularly at higher concentrations (> 25%). This was attributed to increased viscosity and decreased heat transfer rates in the crystallizer, and crystals forming on the inside wall (cooling surface) of the crystallizer. Nucleation in a freeze concentrator must be controlled to maintain a population of large, easily filtered ice crystals. Thus, certain conditions (reduced viscosity and concentration, and sufficient agitation) must be maintained in a freeze concentration crystallizer in order to avoid undesired nucleation.

It has been claimed that skim milk produced by freeze concentration (up to 40%) and then reconstituted to 10% solids has a creamier texture as compared to the original skim milk. This claim was tested by measuring viscosity of a range of concentrated and reconstituted skim milk products. Viscosity of freeze concentrated skim milk (from 10 to 40%) was measured at temperatures from the freezing point (which varied for each concentration) to 20°C. Above 25% solids, freeze concentrated skim milk began to show slight non-Newtonian (pseudoplastic or shear-thinning) behavior, where apparent viscosity was dependent on shear rate used to measure viscosity. The behavior of freeze concentrated skim milk followed the power law model of rheology (with no yield stress), where the exponent (n) in the model fell to about 0.9 for the highest concentrations. Thermally evaporated skim milk showed exactly the same trends and no significant difference in viscosity between freeze concentrated and thermally evaporated skim milk was measured. When reconstituted to 10% solids, both freeze concentrated and thermally evaporated skim milk had slightly higher viscosity than the original skim milk. However, these differences were small.

Significance to the dairy industry

Freeze concentration has the potential for producing high quality milk or whey concentrates with functional and flavor advantages. However, current technology is expensive and applications not fully developed. Development of a more efficient, and less expensive freeze concentration technology may allow wider application of this new process in the dairy industry. In this project, a new freeze concentration technology was developed that has potential for improving the cost efficiency. However, further work must be done on a wider range of operating conditions in order to verify the economic status of this technology.

Publications

Hartel, R.W. and M.S. Chung, Contact Nucleation of Ice Crystals in Fluid Dairy Products, J. Food Eng., 18(3), 281-296 (1993).

Espinel, L. and R.W. Hartel, Freeze Concentration in Skim Milk, J. Food Eng.,20, 101-120 (1993).

Zhang, Z and R.W. Hartel, J. Food Eng. (accepted).

Chang, Y-H. and R.W. Hartel, J. Food Eng. (submitted).

Chapter 3

CHEESE

Cheese Summary

Cheese technology is an important research program for CDR since over 80% of Wisconsin milk ends up in a cheese vat. Our overall objective is to increase the use of cheese and cheese products. One way to do that is to explore the properties of cheese products and then develop techniques to regulate the properties.

Whether we like them or not, computers are the technology tools of the present and the future. CDR researchers are demonstrating how computers can help solve problems in the dairy industry. Gunasekaran et al. used a computer to develop a neural network, and showed that their system could predict the functional properties of cheeses more accurately than standard statistical methods. This will be useful for determining production techniques to produce cheeses with specific characteristics for use as food ingredients. Other researchers are using computer image processing to analyze images of cheese microstructure. A 3-D reconstruction algorithm was developed to reconstruct sequential 2-D image layers from a confocal laser scanning microscope into a 3-D network. This permits visualization and analysis of fat globule size, shape, and distribution within the cheese matrix. Analysis of full fat and reduced fat cheeses should provide information about the differing microstructure of these cheeses and the parameters that could be altered to make reduced fat more like full fat cheese.

In a more "traditional" use of computing power, Gould developed a group of software programs that can simplify the economic analysis of dairy product production. Using individualized information from cheese plants; CHEESE-AGE provides analysis of the economics of cheese aging, CHYIELD helps cheesemakers understand the factors that affect cheese yields, and CHEESE-ECO analyzes the economics of the entire cheese plant production. One of Gould's current projects evaluates the economics of specialty cheese production in small to medium size cheese plants in Wisconsin.

Path and his group continue to work closely with WMMB and cheesemakers to expand the Wisconsin specialty cheese industry. The Wisconsin Master Cheese Maker program is now in place and seminars for producing specific types of cheeses continue. New products have already shown up in the marketplace as this technology base continues to be built.

The flavor and functional characteristics of reduced fat cheeses are key to consumer acceptance. Chen, Johnson, and Jaeggi have been studying specific aspects of the cheesemaking process to produce reduced fat cheese with the desired attributes. Their trials varying

continued next page

Cheese Summary continued

the pH at the time of coagulant addition establish a means for controlling the functionality of reduced fat Mozzarella cheese, even without using a fat substitute. They have also developed a method for producing a reduced fat functional "pizza" cheese without using a mixer molder, their work in this area is continuing since the process needs refinement.

There are several projects in the Center's Cheese Technology program looking at flavor development of reduced and full fat cheeses. The researchers are investigating mechanisms of flavor development from the starter, adjunct, and contaminant "non-starter" cultures to the specific enzymes' systems present in these organisms. Microorganisms and their enzymes are responsible for the degradation and reaction of lipids and proteins to form highly desirable cheese and Cheddar flavors. Understanding and controlling these reactions are necessary to consistently produce high quality flavorful cheese.

FINAL REPORT

Relating Cheese Quality Parameters to Composition and Processing Conditions Using Neural Networks

Personnel: S. Gunasekaran, associate professor, Agricultural Engineering, N. F. Olson, professor, Dept. of Food Science, J. P. Norback, professor, Dept. of Food Science, H. Ni, research assistant, Agricultural Engineering

Funding: Dairy Management Inc. GS 294

Dates: July 1993 – June 1995

Objectives

1. To develop a generalized system based on neural networks to relate cheese quality parameters with composition and processing conditions.

2. To verify advantages of the neural network model over statistical methods in terms of accuracy and ease of use.

Summary

Neural network models with one or two hidden layers were built. Convergence of estimation error was used as the criterion for selecting the number of hidden layers. A series of tests was conducted with a different number of hidden layers, hidden nodes and different network parameters. The results indicated that different structures should be used for different sets of training data.

Experimental data on cheese composition, quality and process parameters obtained as a part of another CDR project ("Development of a systematic approach for producing cheese as a food ingredient") were used for training and testing the neural net model. There was a total of six inputs and 11 outputs. The input and output parameters are listed in Table 1. From 200 sets of experimental data, 160 data sets were randomly chosen to train the model, and the remaining 40 were used to test the model predictions. The training was performed by iteratively, or repetitively, feeding the training data until the expected and actual output parameter values were within a preset error limit. Typically, the training required about 60,000 iterations (about 5-8 minutes on a 486 computer). After the model was trained, it was evaluated with the test data set. This constitutes comparing the actual experimental values and the neural net predicted values of the same output parameters. Multiple regression analyses were also performed on selected output parameters to obtain statistical model predictions. The prediction accuracies of the neural net and statistical models are compared in Table 2. The neural net predictions using the test data set were, in general, better than the corresponding statistical model predictions. In some cases, the improvement over the statistical model was not very significant. This is because the statistical models were developed using all the data sets. Further, the statistical model prediction accuracy was tested with the same data sets used for model development. In order to have a fair comparison, the neural net model was tested using the training data set (much the same as what statistical models do). Results of this comparison show an improvement in the neural net prediction accuracy. Thus, we conclude that the neural network predictions are better than statistical model predictions. Moreover, neural net offers an added advantage of improving its prediction accuracy as more data sets are collected and presented to the model for additional training.

Significance to the dairy industry

Cheese and foods containing cheese will enjoy greater marketability and consumer acceptance if the physical and sensory properties of such products are carefully controlled to meet consumer expectations. Due to the complex nature of interrelationships between cheese quality parameters and the cheese composition and processing, however, it is currently very difficult to "design" a cheese with the desired attributes. The neural network model may help to fill this gap. Since the neural net "learns" from the data, it gets better every time new information is processed. This offers a predictive

method that gets better with use. Also, new research can build on past efforts instead of starting from scratch.

Other models can describe the physical properties of a cheese of known composition. It may also be possible for the model to predict the required cheese composition and processing parameter data, given the desired set of quality attributes. This will be very beneficial to cheese manufacturers.

Publications

Ni, H. and S. Gunasekaran. 1994. Predicting cheese quality parameters using neural network. Paper No. 943560, Presented at the 1994 ASAE International Winter Meeting, Atlanta, GA, December 13-16.

Table 1. Input and Output Parameters of the Neural Network Model

Input Parameters	Output Parameters
MNFP (moisture-in-non-fat-portion)	Schreiber Test —Microwave meltability
FDM (fat-in-dry-matter)	Tube test — 5" thermal meltability
Drain pH	Tube test — 12" thermal meltability
90-day pH	Strain at fracture
Calcium	Stress at 20% compression
TCA (trichloroacetic acid)	Stress at 80% compression
	Stress at fracture
	Flavor preference
	Body preference
	Sliceability
	Cohesiveness

Table 2. Comparison of Prediction Accuracies (%) of Statistical and Neural Net Models

Output Parameters	Statistical	Neural Net Test Data	Neural Net Training Data
Schreiber Test — microwave meltability	96	96	96.3
Tube test — 5" thermal meltability	76	78	83
Tube test — 12" thermal meltability	83	88	90
Strain at fracture	n/a	80	n/a
Stress at 20% compression	75	76	78
Stress at 80% compression	76	84	86
Stress at fracture	76	83	84
Flavor preference	n/a	84	n/a
Body preference	n/a	90	n/a
Sliceability	n/a	83	n/a
Cohesiveness	98	98	98.1

INTERIM REPORT

Evaluating Microstructure of Reduced Fat Cheese with Computer Image Processing

Personnel: S. Gunasekaran, associate professor, Agricultural Engineering, N. F. Olson, Professor, Dept. of Food Science, M. E. Johnson, senior scientist, Center for Dairy Research, K. Ding, research assistant, Agricultural Engineering, K. Muthukumarappan, associate researcher, Agricultural Engineering

Funding: Dairy Management Inc. GK394

Dates: July 1993 – June 1997

Objectives

1. To develop a system which can quantitatively characterize microstructural features of reduced-fat cheeses.

2. To study the effect of composition, manufacturing process parameters and age on structure development.

Summary

We have developed a computer image processing system to analyze microscopic images of cheese microstructure. A significant part of our efforts during the past year was to develop a 3-D image reconstruction algorithm. This was used to reconstruct sequential 2-D image layers obtained from a confocal laser scanning microscope (CLSM) into a 3-D network. Using this we were able to visualize and quantitatively analyze the *in situ* fat globule size, shape and distribution in cheese samples.

The microstructure of two-day old, low fat Cheddar cheese (13.9% fat) was studied using the CLSM and the 3-D algorithm. The average number of fat globules found in the samples studied is presented in Table 1. The boundary-chopped globules represent those fat globules that were not totally contained within the sample boundaries. The distribution of the globules by size is presented in Fig. 1. It shows that there were a large number of very small globules – almost 50% of all globules were about 1 μm in diameter. One possible reason for such a preponderance of small globules is that the milk used for cheese making was centrifuged, preferentially removing large diameter globules. However, the very small diameter globules represent only a volume fraction of the total fat in the cheese (Fig. 2). In addition, the sphericity – closeness of the fat globules to a sphere — was also calculated (Fig. 3). Small globules were more spherical (sphericity value for a perfect sphere is zero) than the large globules. Thus, we have established a mechanism for quantitatively evaluating various physical attributes of fat globules in cheese.

Our work is continuing; we are investigating the effects of composition (fat content) and cheese making parameters (e.g., stirred vs. milled curd) and cheese age.

Significance to the dairy industry

This project provides an objective method of analyzing fat globule characteristics in cheese samples. It allows us to quantify interrelationships among various parameters of cheese microstructure development. This will help us to understand the effect of microstructural features on textural and physical properties of cheese. This information will be useful in modifying and/or controlling cheese properties.

Table 1. Number and Volume of Fat Globules in 2-day Old, 13.9%-fat Cheddar Cheese Sample (Sample dimensions: 77 x 77 x 40 μm)

Globule Type	Number	%	Volume (μm^3)	%
Fully-contained	381	76	12348	41
Boundary-chopped	135	24	14902	59
Total	516	100	27250	100

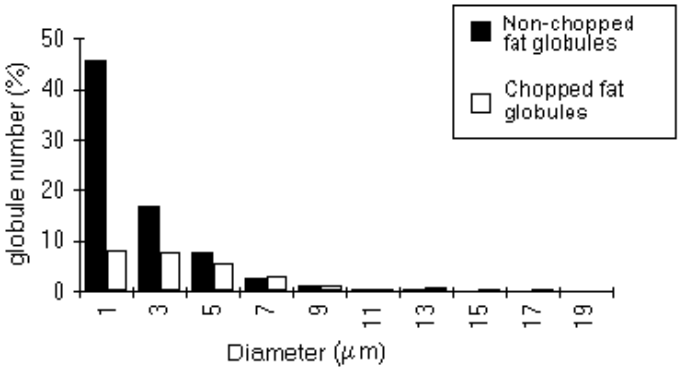

Figure 1. Distribution of number of fat globules of different diameters (treating the fat globules as spheres).

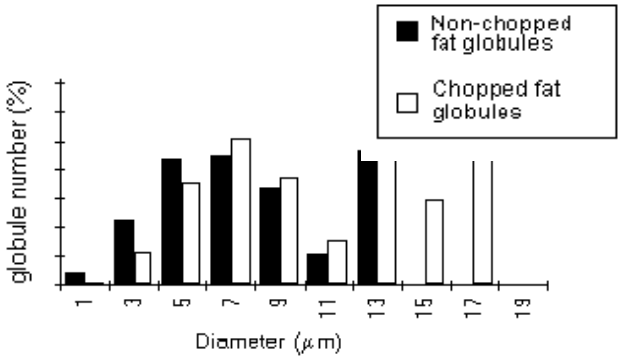

Figure 2. Distribution of volume of fat contained in fat globules of different diameters

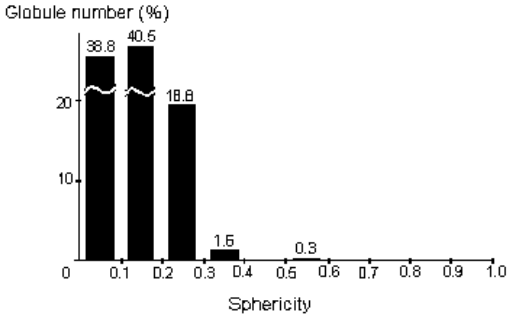

Figure 3. Distribution of number of fat globules according to their sphericity (treating sphericity of a perfect sphere to be zero).

INTERIM REPORT

A Comparative Analysis of Cheese Plant Operations

Personnel: Brian W. Gould, senior scientist, Center for Dairy Research and Department of Agricultural Economics, Kurt A. Carlson, assistant researcher, Center for Dairy Research and Department of Agricultural Economics

Dates: September 1994– August 1995

Funding: Wisconsin Milk Marketing Board UW9501

Objectives

1. Examine the economics of producing traditional commodity cheeses in Wisconsin versus smaller volume specialty cheeses in small/medium sized plants

2. Compare the economics of cheese production in Wisconsin with production in other regions of the U.S.

3. Conduct case studies of several Wisconsin cheese plants to provide benchmark data of actual plant operations

4. Analyze the impact of regional differences in milk quality and characteristics on the economics of cheese production.

Summary

Examining the economics of producing specialty cheeses will assist WMMB to answer questions regarding the economic impact when a small/ medium Wisconsin cheese plant producing Cheddar or Mozzarella changes to smaller volume varieties. The second objective will assist WMMB to answer questions regarding the existence of direct cost advantages for a small/medium cheese plant to produce cheese in Wisconsin. In order to understand the characteristics of Wisconsin cheese plant operations, we will do several cheese plant case studies. The third objective will be achieved by developing and sending a plant survey to interested small/medium (less than 15 million pounds of annual cheese production) cheese plant operators. Members of WMMB and the Wisconsin Specialty Cheese Institute identified cheese plants to examine. The final objective focuses on a more detailed analysis of one of the factors affecting costs of cheese manufacture: milk quality/characteristics. To achieve this objective, we will collect market order administration data and Dairy Herd Improvement Association data to examine regional differences in milk quality and characteristics.

We are developing economic engineering models of small and medium sized specialty cheese plants in order to accomplish our first two objectives. A significant portion of the base data to develop the plant cost simulations has been collected, including regional utility, labor, building and other cost data. We have obtained from Cornell University all the data collected by Mead and Hunt, Inc., used in a previous WMMB funded project analyzing the costs of producing large scale Cheddar and Cheddar/specialty cheese plants.

We conducted the *1995 Wisconsin Small Cheese Plant Survey* (WSCPS), an in-plant cost of production survey targeted for cheese plants producing less than 20 million pounds of cheese annually. This survey is an extension of a previous WMMB funded plant survey which also was targeted to large scale Cheddar cheese plants. The Wisconsin Specialty Cheese Institute and the Wisconsin Cheese Makers Association assisted in identifying targeted small/medium size cheese plants.

During December, 1994 we pre-tested our cheese plant survey on two cheese plants, one located in south-central and one in northeastern Wisconsin. After incorporating suggested changes, we started cheese plant interviews. By the end of February we had contacted over 30 cheese plants and conducted interviews with 24 plants located throughout the state. The results of the cheese plant survey have been entered into a variety of spreadsheets which were used for plant specific cost analyses and for overall sample mean calculations. Plant specific cost and resource utilization analyses were mailed to each participating plant manager.

A contract has been awarded to an engineering firm to conduct cost simulations for smaller sized specialty cheese plants in Wisconsin, California, Texas, New Mexico, Idaho, and Utah. This phase of the project should be completed by the end of July. We will also be using this base data to simulate a medium size specialty cheese plant for these same states.

Significance to the dairy industry

The growth in raw milk production and processing capacity outside Wisconsin during the last decade is well documented. The Wisconsin cheese industry has undergone a dramatic restructuring, the amount of U.S. cheese produced in Wisconsin decreased from 43 to 32% from 1970 to 1991. Responses to this loss of market share include increasing smaller volume, or specialty cheeses and consolidating operations into larger plants. This project will provide information about cheese production in Wisconsin that can be used for economic analysis.

Cheese Research Applications Program

Personnel: Jim Path, outreach specialist, John Jaeggi, assistant researcher, Denise Stanley, LTE, Dave Swenson, LTE, Leesa Stefano, LTE, Kristin Haug, LTE, Inger Eidskvem, LTE, Center for Dairy Research

Dates: July 1994 – June 1995

Funding: WIsconsin Milk Marketing Board 113-Y402

Objectives

1. Laboratory and field research to develop traditional and new specialty cheeses

2. Transfer CDR cheese research results to industry

3. Develop links between CDR, WMMB, and Wisconsin cheese manufacturers by helping to solve technical problems

4. Continue to work on Wisconsin Master Cheese Maker Seminar Series

Summary

Developing cheeses and defining make schedules

We have started a computer library of cheese names, descriptions and manufacturing procedures, entering over 1,400 names of cheeses. We are continuing the process of entering descriptions and manufacturing procedures to match these names and plan to have the project operating by February of 1996.

We now have English translations of the Spanish books, *Quesos Espanoles* and *101 Quesos Magistrales.* These valuable books provide information on the types of Spanish cheeses. We also translated portions of *Formaggi Italiani* and *Tecnica Casearia* from Italian to English. *Formaggi* is an excellent resource since it has very good technical information on Italian Cheeses. Denise Stanley skillfully translated all the Spanish and Italian texts and her efforts are deeply appreciated.

Four "Special - Oste" cheese books have been translated from Danish to English by Inger Eidskvem and Kristin Haug, two visiting students from Norway. These books, complied by students at Dalum Tekniske Skole in Dalum, Denmark, contain manufacturing procedures for over 150 European specialty cheeses.

CDR worked with Grass Lake cheese factory to develop a "Fol Epi" eyed type cheese. The Grass Lake factory submitted a cheese from an early trial to the Schawano County Fair – this cheese took the Grand Champion prize.

Informational Seminars

The Wisconsin Master Cheese Maker program has started using CDR personnel for technical and training advice. This program was formally announced at the CDR-WCMA program on March 23, 1995 in Green Bay, WI. The Board held its first meeting on June 1, 1995. Over 20 people, representing approximately 15% of industry companies, have requested information and application forms.

On Feb. 30, 1995 a Wisconsin Process Cheese Course was held as part of the Wisconsin Master Cheese Makers Curriculum. This course, currently the only process cheese course in the US, filled to capacity with 42 persons. We are planning a similar course, which will include a lab, for next year. We also developed three process cheese video tape lectures to use for teaching this subject. They include: *Introduction to process cheese, Theory of process cheese* and *Process cheese defects*.

Another course in the Wisconsin Master Cheese Makers curriculum, a Dutch Cheese Seminar, was held on September 20-22 in Madison. The primary instructors were from the Van Hall Instituut in Bolsward, Holland and the Center for Dairy Research in Madison. They covered the following topics, Overviews of the Dutch Dairy Industry and Dutch Cheeses, Cheese defects, Small Business Secrets to Success (UW Small Business School),

Plasticoating and waxing of cheese and a "hands on" lab on the manufacture of Maasdamer and Gouda cheese.

We also held a Mexican Cheese Seminar on April 25-27, 1995. This course is also part of the Wisconsin Master Cheese Makers curriculum. The rapidly growing Hispanic cheese market influenced the high degree of interest in this area. Instructors from the Mexican Dairy Industry, University of Wisconsin Extension, The Wisconsin Milk Marketing Board and the Center for Dairy Research presented information regarding manufacture and use of Hispanic and Mexican types of cheese. We also covered safety issues regarding the manufacture of high moisture and high pH cheese.

Significance to the dairy industry

The specialty cheese program has helped to promote the image of Wisconsin Specialty Cheese Institute (WSCI), improve the educational process for industry personnel (Master Cheese Maker), and increase practical cheese making knowledge (Cheese Artisan Seminar Series). The program has also worked directly with cheese makers to develop specialty types of cheese.

Presentations

Participated in the UW Cheese Makers Short Course. Lecture involves three courses, Automation and Mechanization of Cheese Making, Cheese Yield and Specialty cheese.
Course is given two times per year.

Wisconsin Cheese Makers / Center for Dairy Research - Cheese Makers Conference
Green Bay - March 23,1995

Great Lakes Dairy Sheep Symposium - Madison - March 30, 1995

Other outreach activities

Dairy Expo October 8th 1994 Madison
Wisconsin Specialty Cheese Institute activity

FINAL REPORT

Manufacturing Lower Fat Mozzarella Cheese by Adding Milk Coagulant at Different pH Values

Personnel: Carol M. Chen, researcher, Mark E. Johnson, senior scientist, John J. Jaeggi, associate researcher, CDR

Dates: January 1994 – December 1994

Funding: CDR Multiple Donor Account U886

Objective

The objective of this experiment was to add milk coagulant at different times to vary pH at addition. This allowed us to evaluate the effect of pH at the time of addition on the composition, functional and sensory qualities of lower fat Mozzarella cheese. Regulating and optimizing cheese moisture by altering pH at the addition of milk coagulant could lead to improved functional characteristics (melt and stretch) and sensory attributes (less chewy after melting on pizza pies.)

Summary

Lower fat Mozzarella cheese (6.5 - 8.0% fat) was manufactured using a lactic acid starter culture to ripen the milk. Different lengths of time were used to achieve the desired pH of 6.45, 6.20, 6.05 and 5.90 after addition of milk coagulant. The cheeses were milled at pH (5.10-5.25) and stretched in a pilot size Mixer Molder (Stainless Steel Fabricating, Model 640) in 190°F brine. These 4 treatments were replicated 2 times for a total of 8 vats of cheese. Cheeses were evaluated for composition, pH, thermal and microwave meltability, texture profile analysis (Instron™), microbial plate count and sensory qualities at room temperature and melted on a pizza pie.

Completing cheesemaking processes such as draining, milling and stretching at the desired pH was difficult in treatments where the acidity at addition of the coagulant developed to 6.05 and 5.90. Lower pH at addition of milk coagulant resulted in cheese with significantly higher moisture content and greater curd pliability through the mixer molder (Table 1, 2). When the milk coagulant was added at pH 6.45 (a typical value for LMPS Mozzarella cheese), the curd run through the mixer molder was stiff and not pliable and this fractured the casein strands. As the pH at coagulant addition decreased, the curd became softer and more pliable through the mixer molder. However, when the milk was coagulated at a pH of 5.90, the curd was too soft and lacked cohesiveness through the mixer molder. This treatment also had the greatest fat losses during processing and the lowest % FDM (Fat in the Dry Matter) in the cheese.

Cheese moisture increased and the pH decreased as the pH at coagulant addition was decreased. The increase in cheese moisture can be attributed to the development of a finer protein matrix at cutting, which greatly retarded syneresis from the curd. Both thermal and microwave melt increased as pH at coagulation decreased. Greater meltability can be attributed to higher cheese moisture and decreased cheese pH. There were no significant differences in microbial plate counts among the different treatments.

A summary of sensory attributes can be found in Table 3. Sensory attributes were evaluated at 4, 8 and 12 weeks of aging. For room temperature evaluations, the cheeses coagulated at 6.20 (55% moisture) were most preferred at all ages. These cheeses were described as neither soft nor firm, and generally had the best Mozzarella flavor quality and the least off flavor intensity. Cheeses made from milk coagulated at pH 6.45 were too firm in body and bland in flavor. Cheeses made from milk coagulated at lower pH's were too soft in body. There was little difference in preference between cheeses coagulated at 6.20, 6.05 and 5.90 when they were melted on the pizza pie. The cheeses made from milk coagulated at 6.45 were significantly chewier, stretched less and less preferred. Chewiness correlated with moisture content and pH of the cheeses, as cheese moisture increased

and pH decreased, cheeses were less chewy. Overall preference scores were the highest at 8 weeks. By 12 weeks some of the cheeses had developed unclean off flavors and the body had become too soft.

Significance to the dairy industry

These cheesemaking trials demonstrated how altering manufacturing protocol can enhance the quality of lower fat Mozzarella cheese. Cheese quality, expressed by both improved functional and sensory characteristics of lower fat Mozzarella is enhanced by increased moisture and lower pH (as compared to low-moisture part-skim Mozzarella). A good target moisture for 50% reduced-fat Mozzarella is between 55-57% moisture. These cheeses had acceptable sensory attributes, both when tasted at room temperature and melted on a pizza pie. This technique may minimize the need for fat mimetics in lower fat Mozzarella cheese.

Presentation/Publication

"Update: CDR Mozzarella Research" Mark E. Johnson. Wisconsin Cheese Industry Conference, March 1995. Green Bay, WI.

Chen, C. M., J. J. Jaeggi, and M. E. Johnson. (in preparation) Evaluation of pH at milk coagulation addition in the manufacture of lower fat Mozzarella cheese. J. Dairy Sci.

Table 1. Evaluation of Mozzarella exiting the mixer molder.

Milk pH at coagulation	Approximate fat losses into mixer molder (kg fat)	Comment
6.45	.026	very stiff curd a lot of fracturing
6.20	.022	curd pliable, no fracturing
6.05	.029	curd pliable, no fracturing smoother, softer than pH 6.20
5.90	.035	curd pliable, no fracturing slightly grainy texture

Table 2. Cheesemaking and compositional information for lower fat Mozzarella cheese manufactured with different pH at addition of milk coagulants.

Milk pH at Coagulation	Length of Ripening(min)	Total Make Time (min)	% Cheese Moisture	Cheese pH(4 weeks)
6.45	30	175	51.19	5.25
6.20	115	185	55.24	5.15
6.05	145	190	56.86	5.10
5.90	155	200	58.91	5.00

Table 3. Summary of sensory evaluation lower fat Mozzarella cheese evaluated at room temperature and melted on a pizza pie.

Milk pH at Coagulation	Room Temperature	Melted on Pizza Pie
6.45	Cheeses bland in flavor Cheeses too firm	Melted cheese too chewy Poor stretch (4-5 inches)
6.20	Least off flavor intensity Best Mozzarella flavor quality Neither too soft nor too firm Most preferred	Melted cheese not too chewy Good stretch (10-20 inches) Mozzarella flavor quality good Overall preference - liked
6.05	Cheese body too soft	Melted cheese not too chewy Good stretch (10-20 inches) Mozzarella flavor quality good Overall preference - liked
5.90	Cheese body too soft	Melted cheese slightly fluid Good stretch (10-20 inches) Mozzarella flavor quality good Overall preference - liked

INTERIM REPORT

Manufacture of a New Reduced Fat Cheese for Use on Pizza Pies

Personnel: Carol M. Chen, researcher, Mark E. Johnson, senior scientist, John J. Jaeggi, associate researcher, CDR

Funding: Dairy Management Inc. CH295

Dates: July 1994 – June 1995

Objectives

1. To determine a manufacturing procedure for high moisture, 25% and 75% reduced fat pizza cheese which is not run through a mixer molder. These cheeses will have functional qualities (melt, stretch and browning) similar to Mozzarella cheese.

2. To evaluate the functional and sensory characteristics of a 25% reduced fat pizza cheese compared to low-moisture part-skim Mozzarella cheese and a 75% reduced fat pizza cheese to 50% reduced fat Mozzarella cheese.

Summary

Two different manufacturing approaches were taken to produce a high moisture, reduced fat pizza cheese. A manufacturing protocol similar to that of lower fat Mozzarella (2) or 50% reduced fat Cheddar cheese (1) was completed. In both cases, a portion of whey was removed after cutting and water added back to decrease lactose concentration in the curd and to help achieve a final pH similar to that of Mozzarella cheese.

Comments on reduced fat pizza cheese manufactured using two different pH levels at addition of the milk coagulant are included in Table 1. This type of approach to attain high moisture levels was effective in the manufacture of a high moisture lower fat Mozzarella cheese (moisture contents ranged from 55 to 59%). However, due to different starter culture acid production and total manufacturing times, resulting cheeses were too low in moisture. In addition, the whey dilution step during cheesemaking was inadequate and final cheese pH values after 1 month were too low. These cheeses were tough and dry when evaluated at room temperature and lacked appropriate stretch and melt characteristics on the pizza pies. Taste panelists also noted a high degree of oiling off on the 25% reduced fat cheeses. This was attributed a to high starter culture proteolytic activity.

The second manufacturing approach was based on the 50% reduced fat Cheddar manufacturing schedule developed at CDR. This manufacturing technique in combination with a 50% predraw/30% water addition to the whey and homogenization of part skim milk prior to pasteurization are summarized in Table 2. For the 75% reduced fat pizza cheese a cold water curd rinse was done prior to salting. Resulting cheese moisture contents were lower than targeted. In addition, the 75% reduced fat pizza cheese was too bland in flavor, had a plastic appearance after melting, and the cheese strands fractured too readily during stretching. The 25% reduced fat pizza cheese was compared directly to low moisture part skim (LMPS) Mozzarella cheese of equal age, with no significant difference in the overall preference being noted. Other observations from this series of experiments included no browning on pizza pies, a good cheese salt content, very little or no oiling off and an acceptable degree of stretching for all cheese (stretch ranged from 5 to 24 inches).

The main focus of a subsequent cheesemaking trial was to increase the final cheese moisture content by a finer milk coagulum at cutting and shorter manufacturing times. However, results are preliminary. The work on this experiment will be completed in the summer of 1995.

Significance to the dairy industry

The results of this study will benefit cheesemakers in two ways. First, it will allow manufacturers of Cheddar/Colby or Brick cheese to expand into the

growing pizza cheese market with a minimal purchase of equipment. This will give the smaller cheese manufacturer the capability of manufacturing a new variety of cheese with the same functional characteristics as low-moisture part-skim Mozzarella except a lack of browning when heated. The new manufacturing procedure will not require the traditional mixer molder and brine systems of a Mozzarella manufacturer, thus it's production has economic advantages. Since this new style of cheese is not run through the mixer molder, fat recovery in the cheese increases from about 85 to 95%, giving cheese manufacturers higher cheese yields. This cheese will not be called Mozzarella, but it is a natural cheese (if fat mimetics are not used) and can be labeled as "Cheese" (part skim milk, dairy cultures, salt and enzymes.) If you use a fat mimetic that is not dairy derived, the product will need to be labeled as Cheese Food.

References

1. Chen, C. M., J. J. Jaeggi and M. E. Johnson. 1994. Effect of coagulum firmness at cutting on quality and yield for 50% reduced fat Cheddar cheese. J. Dairy Sci. 77, Suppl. 1:13.
2. Johnson, M. E. March, 1995. Update: CDR Mozzarella Research. Wisconsin Cheese Industry Conference. Green Bay, Wisconsin.

Table 1. Manufacture of reduced fat pizza cheese[1] using a lower pH at addition of milk coagulant.

pH at addition of coagulant	pH at draining	Cheese moisture	Cheese pH at 1 month	Comments (Pertain to all cheeses)
25% reduced fat pizza cheese[2]				1. too low in moisture 2. too low in pH 3. cheese tough & dry 4. cheeses lacked appropriate stretch & melt characteristics 5. cheeses too high in salt 6. 25% reduced fat pizza cheese too much oiling off
6.20	5.70	40%	4.95	
6.05	5.35	41%	4.90	
75% reduced fat pizza cheese[3]				
6.20	5.75	46%	4.90	
6.05	5.40	48%	4.90	

[1] 20% predraw/10% water added back to the whey
[2] Cheese Fat = 26%, FDM = 44.6%
[3] Cheese Fat = 9.5%, FDM = 18.0%

Table 2. Manufacture of a reduced fat pizza cheese[1] using a manufacturing protocol similar to that of 50% reduced fat Cheddar.

Homogenization[2] of milk	Cheese moisture	Cheese pH at 1 week	Cheese pH at 1 month	Comments (pertain to all cheeses)
25% reduced fat pizza cheese[3]				1. too low in moisture 2. no browning on pizzas 3. all cheeses had acceptable stretch and shreddability 4. less meltable than LMPS Mozzarella 5. 25% reduced fat pizza cheese vs LMPS Mozzarella, no difference in preference
no	42.5 %	5.21	5.37	
yes	44.5 %	5.17	5.30	
75% reduced fat pizza cheese[4]				
no	51.0 %	5.20	5.47	
yes	50.0%	5.24	5.50	

[1] 50% predraw/30% water added back to the whey
[2] Homogenization of part-skim milk prior to pasteurization = 500/500 psi
[3] Cheese Fat = 23.5%, FDM = 41%
[4] Cheese Fat = 8%, FDM = 17%
Includes a 10 minute soak before salting where curd/whey temperature = 70˚F

FINAL REPORT

Manufacture of a 25% and 50% Reduced Fat Cheddar Cheese by Blending Low and High Fat Curd

Personnel: Carol M. Chen, researcher, Mark E. Johnson, senior scientist, John J. Jaeggi, associate researcher, Center for Dairy Research

Funding: Dairy Management Inc. CHN94

Date: July 1993 – September 1994

Objectives

1. To determine the feasibility of mixing high fat Cheddar cheese curd with lower fat, high moisture Cheddar curd resulting in a cheese that is 25% or 50% reduced fat Cheddar.
Specifically evaluating:
- non-starter microorganisms
- cohesiveness of the body
- flavor
- acceptability of body and flavor

2. To determine the appropriate composition and ratio of curd to cheese blend.

Summary

We used two different blend protocols. In the first protocol, shredded high fat, aged Cheddar cheese was blended with fresh lower fat Cheddar curd. In a separate cheesemaking trial, full fat and skim milk Cheddar cheese curd were made simultaneously and blended after milling to achieve a 25% or 50% reduced fat Cheddar cheese.

Aged Cheddar was shredded and blended (5% or 10%) with freshly prepared lower fat, higher moisture Cheddar curd. The fat in the aged full fat Cheddar melted and coated the warmer lower fat Cheddar curd. This resulted in more moisture and salt retention. The resulting 25% and 50% reduced fat Cheddar cheese was significantly higher in Cheddar flavor, but the cheeses were non-cohesive (crumbly) and grainy. If this manufacturing protocol were followed, the salt addition would have to be decreased by 40% to achieve a final salt content 1.6% in the cheese.

To improve the cohesiveness of the blended cheeses, the freshly prepared curd and aged Cheddar cheese were ground together in a meat grinder before pressing. This increased the surface area of the warmer lower fat curd. The grinding process did not result in excess moisture being trapped in the cheese or fat lost in the press whey. The levels of non-starter microorganisms of the blended cheeses were at least 1000 times greater than the control cheeses at 1 day, 1 week and 1 month. At 1 month, the blended cheeses had as many as 10^8 CFU/g cheese. By 2 months, and through the duration of aging, there was no significant difference in non-starter microorganism populations. Sensory analysis was performed at 1, 2 and 6 months. At both fat reduction levels and all ages, the blended cheeses had greater Cheddar flavor intensity, with the largest difference being noted at 1 month. Bitterness was not a problem in the blended cheeses, however blended cheese tended to develop more unclean and sulfur type flavor notes 2 through 6 months of aging. At 1 month, flavor preference scores were the highest. The cheese blending process tended to result in reduced fat cheeses which were softer in body (also slightly higher in moisture) and mealy in texture. This generally resulted in lower body and texture scores for the blended cheeses versus control cheeses. Taste panel results also indicated that a 5% blend (vs 10% blend) of aged Cheddar cheese with fresh Cheddar curd is enough to accelerate flavor development in the curd.

In the second protocol, full fat and skim milk Cheddar curds were made simultaneously and then blended, milled and salted. They were either pressed, or ground in the meat grinder and then pressed. In this cheesemaking trial to obtain a 25% reduced fat Cheddar, 65% full fat curd and 35% skim milk curd were blended; and for a 50%

reduced fat Cheddar cheese 40% full fat curd and 60% skim milk curd were blended. Sensory attributes were evaluated at 1, 2 and 5 months of age. At both fat reduction levels the pressed curd cheeses had slightly more Cheddar flavor intensity than the ground/pressed cheeses. However, only in the 1 month evaluations were the pressed curd cheeses preferred in overall flavor. By 5 months, cheeses were considered to have mild to medium Cheddar flavor intensity (a typical full fat Cheddar cheese would have medium to aged flavor by 5 months). Many differences were noted in the body and texture of the 4 treatments. For the 25% reduced fat Cheddar, the pressed curd cheeses fused together well. The cheeses scored the highest in body and texture preference. The 25% reduced fat Cheddar that was ground/pressed tended to be more curdy and mealy through 5 months of aging. It is important to note that several of the experienced cheese tasters did not like the "marbling" effect from blending the high fat and skim milk cheeses. These individuals described the skim milk curd as 'hard bits' embedded in a smooth cheese matrix. For the 50% reduced fat Cheddar blended cheese, over half of the cheese was skim milk curd. The overall body and preference scores of both the pressed and ground/pressed cheeses were 'slight dislike', due to the marbling effect of the full fat and skim milk cheeses. However, the pressed curd cheeses were, generally, firmer and smoother than the ground/pressed curd cheeses.

Significance to the dairy industry

The blending of 5% aged Cheddar curd with fresh high moisture lower fat Cheddar curd resulted in greater Cheddar flavor intensities for 25% and 50% reduced fat Cheddar cheese. However, extra manufacturing steps and equipment are needed to increase the surface area of the curd and ensure that the curd properly knits together.

The blending of curd of two fat levels to achieve a reduced fat Cheddar cheese does not enhance the Cheddar flavor development and received a mix of comments from the group of experienced cheese tasters. While some thought it was a positive, novel idea, others objected to the wide variation in curd textures.

Publications

Chen, C. M., J. J. Jaeggi, and M. E. Johnson. Manufacture of a 25% and 50% reduced fat Cheddar cheese by the blending of low and high fat curd. Cultured Dairy Products Journal (in preparation).

FINAL REPORT

Effect of Casein:Fat Ratio on Stirred Curd Cheddar Cheese Yield

Personnel: Carol M. Chen, researcher, David D. Bogenrief, researcher, Mark E. Johnson, senior scientist, CDR

Dates: July 1994 – June 1995

Funding: Wisconsin Milk Marketing Board, Cheese Research Institute, Kraft Foods

Objective

To evaluate the effect of milk casein to fat (C:F) ratio on cheese yield performance by directly comparing six C:F ratios of milk on no-wash stirred curd Cheddar cheese. We used cheese composition, nitrogen, fat and solids-non-fat recovery in the cheese to evaluate cheese yield.

Summary

Cheesemaking was conducted using a no-wash protocol. Six casein-to-fat (C:F) ratios ranging from full fat to skim milk Cheddar cheese were evaluated: .74, .96, 1.39, 2.00, 3.38 and 20.1 (corresponding to full fat, 25%, 33%, 50%, and 75% reduced-fat, and skim milk Cheddar). We used several different techniques to help increase cheese moisture in the lower fat cheeses. They included: a firmer milk coagulum at cutting, lower cook temperatures, shorter stir out times, and a higher curd pH at draining and salting. Cheesemaking was replicated 5 times for a total of 30 vats of cheese. Cheese yield performance was evaluated by cheese composition, nitrogen, fat and solids non-fat recovery in the cheese.

See Table 1 for a summary of compositional changes in the cheese. As the milk C:F ratio increased, the percentage of fat in the cheese decreased, and the percentage of moisture, protein and solids-non fat/non-protein increased. Fat only contributes its own weight to the yield of cheese, where as casein (the major cheese protein in cheese) plays a much more important role. Casein forms the structural matrix trapping moisture and fat and it has the ability to contribute absorbed water to cheese yield in addition to its own weight. C:F ratios of .74 (full fat), 2.00 (50% reduced-fat), and 20.1 (skim milk) corresponded to 24.4, 32.1, and 37.4% protein; and 1.62, 1.46, and 1.39% moisture to protein, respectively. It has been suggested that the moisture to protein ratio remains constant in cheese, however in this study the ratio decreases. The decrease in the moisture to protein ratio corresponds to a denser protein matrix in the cheese (less moisture or fat to separate casein strands). This makes a firmer, harder cheese.

Total nitrogen balance was used to evaluate protein accountability in the cheese system because of the difficulties in measuring protein in cheese (not all of the nitrogen in cheese originates from casein). Differences in percentage of nitrogen recovered in the cheese, whey and total system were not significant (data not shown). The percentage of nitrogen recovered in the cheese for the six different C:F ratios ranged from 72.9 to 73.8%. Typically, differences in protein recovery are not observed unless there are extreme differences in milk composition or abnormal milk processing conditions (4). This data is expressed in percentage nitrogen recovery, the actual protein contents of the cheese did vary (Table 1). The higher the C:F ratio of the initial milk, the more protein in the system and the higher the protein content of the cheese.

Differences in the percentage of fat recovered in the total system, cheese, and whey were significantly different among the six different C:F ratios (Table 2). The total percentage of fat recovered for the skim milk cheeses was 111.8%. For the skim milk cheeses, there is very little fat in the system, thus any errors in sampling or analytical testing are misleadingly amplified. For example, in full fat Cheddar cheese 98.8% of the fat was recovered or 3 oz of fat was missing. In the skim milk cheese, 111.8% of the fat was recovered, or an extra 0.5 oz of fat in this system.

The fat balance clearly shows the trend of a lower percentage of fat recovered in the cheese and a higher percentage of fat recovered in the whey as the C:F ratio of the initial milk increases. Scanning electron microscopy shows that lower fat cheeses have a denser protein matrix, fewer fat globules, and the existing fat globules are smaller in size (2). Smaller fat globules in the cheese matrix can be shed more readily from the curd during manufacturing. Why the smaller fat globules in lower fat Cheddar? In this cheesemaking trial, the different initial milk fat levels were achieved by blending whole and skim milk. Thus, variations in the fat globule sizes are more widely distributed than if the whole milk was skimmed in milk preparations. You would expect less fat loss if milk were skimmed to the desired C:F.

The solids-non-fat of cheese or R value may be calculated from the Van Slyke cheese yield equation (4) and the Fat in the Dry Matter (FDM) yield equation (1). The R value represents salts, organic acids (lactic acid, citric acid), sugars (lactose, glucose, galactose), minerals, whey proteins and casein hydrolysis products. As the milk fat content of the initial milk decreased, the resulting R values for the cheeses increased (Table 3). Noted previously, as the C:F ratio increased, the moisture content of the cheese increased (Table 1). This increased cheese moisture, allows for more soluble components such as salt (NaCl), lactose and lactic acid . The ratio of soluble components (salt, lactose, lactic acid) to cheese moisture remains constant for the six C:F ratios.

As the C:F ratio of the milk used in cheesemaking increases, or as there are further reductions of fat in the cheese, the percentage of nitrogen recovered remains constant, percentage of fat recovered decreases, solids-non-fat increases and overall cheese yield decreases.

Significance to the dairy industry

One of the many problems that plague the manufacturer of lower fat cheese is lower cheese yield. It is widely recognized that reducing the fat content in milk leads to lower cheese yields since fewer milk solids available for cheesemaking. This study details the cheesemaking recovery of milk solids components in cheese.

Presentation/Publication

Effect of C:F Ratio on Stirred Curd Cheddar Cheese Yield. Carol M. Chen. Wisconsin Cheese Industry Conference. Green Bay, Wisconsin.

Cheese yield and economic evaluation of six different initial milkfat levels on stirred curd Cheddar cheese manufacture. (In preparation) Chen, C. M., D. D. Bogenrief, B. Gould, and M. E. Johnson. J. Dairy Sci.

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1. Kerrigan, G. L. and M. E. Johnson. 1985. Computer based decision-support program for the calculation and economic evaluation of standardizing milk for cheesemaking. Walter V. Price Cheese Research Institute. Madison, WI
2. Mistry, V. V. and D. L. Anderson. 1993. Composition and microstructure of commercial full-fat and low-fat cheeses. Food Structure. 12:259-266.
3. Phelan, J. A. 1981. Standardisation of milk for cheesemaking at factory level. J. Soc. Dairy Technol. 34(4):152-156.

Table 1. Cheese Composition

Description	C:F[1]	% Moisture	% Fat	% Protein	Moisture: Protein
Full fat	.74	39.6	30.9	24.4	1.62
25% RF[2]	.96	41.6	26.1	27.1	1.54
33% RF	1.39	43.9	20.1	30.5	1.44
50% RF	2.00	46.3	14.7	32.1	1.44
75% RF	3.38	49.3	8.9	34.3	1.44
Skim milk	20.13	52.4	1.5	37.4	1.40

[1] Casein to Fat Ratio [2] Reduced Fat Cheddar

4. Van Slyke, L. L. and W. V. Price. 1979. Cheese.
Ridgeview Publ. Co., Atascadera, CA

Table 2. Mean fat recovery in stirred curd Cheddar cheese.

C:F Ratio	Cheese	Whey	Total
.74	89.55	9.28	98.83
.96	87.68	10.65	98.33
1.39	87.45	11.79	99.24
2.00	86.00	13.81	99.81
3.38	81.87	17.03	98.90
20.13	65.78	46.04	111.82

Table 3. Solids-non-fat (SNF) component of stirred curd Cheddar cheese.

C:F Ratio	R value	% Salt	% Lactose	% Lactic acid	SNF: Moisture
.74	1.092	1.73	.92	.77	8.65
.96	1.096	1.82	.85	.97	8.76
1.39	1.109	1.94	.83	.96	8.50
2.00	1.132	2.01	1.01	1.07	8.85
3.38	1.162	2.17	.95	1.18	8.74
20.13	1.142	2.29	1.09	1.29	8.92

R=R value represents the total solids in the cheese that is not fat and not casein. It is taken from the Van Slyke cheese yield formula:

$$\%\ \text{yield} = \frac{\left[\left(\begin{array}{c}\%\ \text{recovery of}\\ \text{fat in milk in}\\ \text{cheese}\end{array} \times \%\ \text{fat in milk}\right) + \left(\begin{array}{c}\%\ \text{recovery of}\\ \text{casein in milk}\\ \text{in cheese}\end{array} \times \%\ \text{casein in milk}\right)\right] \times R}{\frac{100 - \%\ \text{moisture}}{100}}$$

INTERIM REPORT

Identification of Microbial Enzymes and Metabolites Involved in the Development of Lower Fat Cheddar Cheese Flavor

Personnel: James L. Steele, associate professor, Dept. of Food Science, Mark E. Johnson, senior scientist, Center for Dairy Research, Jeff Broadbent, assistant professor, Utah State Univ, Bart Weimer, assistant professor, Utah State Univ, Deogh-Hwan Oh, post-doctoral researcher, Dept. of Food Science Kristen Houck, research specialist, Dept. of Food Science, Song Gao, research assistant, Dept. of Food Science

Dates: June 1994 – January 1996

Funding: Wisconsin Milk Marketing Board UW 9401

Objectives

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

Phase 1

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

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

3. Detailed sensory and chemical analysis of the lower fat cheese manufactured for objective 2.

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

Phase 2: (These are the most likely targets for the second stage; however, these targets will be altered in response to the results obtained in phase 1.)

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

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

7. To characterize amino acid degradation pathways in starter cultures and flavor adjuncts and how they influence cheese flavor development.

8. To characterize the influence of α-dicarbonyl production by starter cultures and flavor adjuncts on cheese flavor development.

Summary

We began by identifying the starter cultures and flavor adjuncts to use in the cheese trials, characterizing them by their intracellular general aminopeptidase activities and intracellular esterase/lipase activities. For both of these activities, we observed significant variation among the strains. We then determined the proteinase specificity for the three starter cultures; *Lactococcus lactis* D11, SK11, and SL156 were found to have PI, PIII, and PI/PIII specificity, respectively. Previous investigators have suggested that cultures with PI specificity were more likely to produce bitter cheese.

We manufactured forty-two vats of lower fat Cheddar cheese, comparing three different starter cultures and six different adjunct cultures (two strains each of *Lactobacillus helveticus, Lactobacillus casei,* and *Brevibacterium linens*). Sensory analysis (both a small expert panel and a large consumer panel) of these cheeses indicated that both the starter culture and the flavor adjunct had a

significant impact on the quality of the final product. Both strains of *Lb. helveticus* and *B. linens* increased the "Cheddar flavor intensity" and reduced bitterness and off-flavors. Cheeses made with *Lb. casei* were more bitter and had elevated levels of off-flavors. Cheeses made with *Lc. lactis* SL156 were consistently very bitter.

The composition data for the cheeses indicate that differences observed in flavor between these cheeses are not the result of differences in gross composition. Microbial analysis determined that the adjunct cultures were added between 2×10^4 and 2×10^5 per ml. of milk. The starter cultures *Lc. lactis* D11, SK11, and SL156 reached final cell densities of 2×10^8, 1×10^8, and 4×10^9, respectively. These results indicate that the bitterness observed with SL156 was most likely due to the ability of this culture to reach high cell density in the cheese matrix. The general aminopeptidase activity increased for all cheeses during the six month ripening period. A strong positive correlation (R^2=.913) was observed between aminopeptidase activity in the curd and cheese flavor after six months of aging; this correlation was weaker at earlier times during ripening (2 months R^2=.498; 4 months R^2=.652). Capillary electrophoresis was used to follow peptide patterns and aromatic compounds in the cheeses. We found that the starter culture had the greatest impact on the electrophoragrams. The effect of adjuncts are more subtle than between the different starters, however significant changes can be observed. Efforts to identify peaks have been started and the identity of a number of peaks is now known. Additional efforts will be made during the second year of this project to identify the compounds responsible for a number of these peaks.

We developed a model system to evaluate the role of primary proteolysis in cheese flavor. This model required the purification of a lactococcal (*Lc. lactis* HP) proteinase. The purified enzyme was used in conjunction with chymosin and plasmin with either α_{s1}- or ß-casein to produce hydrolysates which should reflect the peptides formed in cheese. The reactions with chymosin or plasmin were conducted at pH 5.5 and incubated at 30°C for 2 hours. These enzymes were inactivated by adjusting the pH to 9.0 and held at room temperature for 30 minutes. Subsequently the pH was lowered to pH 5.2 and NaCl (final concentration 4%) and $CaCl_2$ (final concentration 5 mM) were added. Next we added the purified HP proteinase and incubated it at room temperature for seven days. This enzyme was inactivated by heating the solution to 75°C and holding it at that temperature for twenty minutes. The bitterness of the resulting hydrolysates was determined by sensory analysis (n=8) using a quinine sulfate solution in 4% NaCl which yielded a moderate level of bitterness as the standard. The hydrolysates of ß-casein with the lactococcal proteinase displayed the highest level of bitterness.

The catabolism of aromatic amino acids is believed to play a significant role in the development of unclean flavors in cheese. Therefore we set out to determine what catabolic pathways, if any, exist in lactococcal strains. Methods for determining amino acid transaminase, deaminase and decarboxylase activities for aromatic amino acids were developed. Additionally, a method for determining tryptophan side-chain oxidase was developed. A broad specificity aromatic amino acid transaminase was detected in all eight lactococcal strains examined. The variation between strains for the level of this activity was at least ten fold. These results suggest that lactococcal strains contribute to the catabolism of aromatic amino acids and hence unclean flavors in cheese. The ability to initiate the catabolism of aromatic amino acids varies significantly between strains.

Significance to the dairy industry

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

high quality, lower fat cheese. Lower fat cheese with the organoleptic qualities of full fat varieties will both increase consumer acceptance of lower fat dairy products and increase the demand for these goods.

Publications

B. Weimer, C. Brennand, J. Broadbent, J. Jaegi, M. Johnson, F. Milani, B. Mistry, G. Reineccius, J. Steele, and M. Strickland. Chemical and sensory attributes of 50% reduced fat Cheddar made with various adjunct bacteria. International Dairy Lactic Acid Bacteria Conference. Palmerston North, New Zealand. February 19-23, 1995. Abstract #S 3.4.

J. Steele, M. Johnson, J. Broadbent, and B. Weimer. Update: WCDR/Utah State lower fat cheese research. Wisconsin Cheese Industry Conference. Green Bay, Wisconsin. March 22-23, 1995. Paper #12.

INTERIM REPORT

Mechanisms for Production of Cheese Flavor Compounds

Personnel: Robert C. Lindsay, professor, Christine Nowakowski, research assistant, Dept. of Food Science

Dates: July 1993 – July 1996

Funding: Dairy Management Inc. LD294

Objectives

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

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

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

Summary

Research was started to accomplish our objectives, including further characterizing alpha-dicarbonyl production by lactic acid bacteria, developing model cheese systems for studying flavor compound formation upon introduction of ripening microbes and flavor precursor compounds, and improving analytical methods to measure alpha-dicarbonyl compound production in cultures and cheese. Some isolates capable of producing alpha-dicarbonyls from earlier studies and additional isolates from a range of Cheddar cheese samples were further evaluated for production of alpha-dicarbonyl compounds. Although the concentrations produced are variable, the ability to produce these compounds is quite widespread in the lactic acid bacterial flora of Cheddar cheese.

Since it would be desirable to control levels of precursors and the microbial population in model cheese system studies, we investigated the possibility of eliminating active microbe populations in aging cheeses using gamma-irradiation at dry ice temperatures. This approach employing sufficient exposures effectively eliminates microbes, but some radiation-induced flavor compounds are simultaneously formed, limiting some of the applications. Currently, high-pressure sterilizations carried out by a service laboratory are under consideration as an alternate means to avoid some of the off-flavors encountered in irradiation treatments. Additionally, model Cheddar cheese slurry systems containing introduced alpha-dicarbonyls have been developed, and these systems have shown enhanced formation of cheese flavor compounds.

Slow Maillard-type reactions initiated by the combination of alpha-dicarbonyls and free amino acids or peptides are responsible for the ultimate formation of important cheese flavor compounds. In research prior to this project, methods were developed to quantitatively measure concentrations of the free alpha-dicarbonyls present in cultures and cheeses. However, because of the very reactive nature of the alpha-dicarbonyl compounds (glyoxal, methylglyoxal, and diacetyl) with amino compounds in media and cheese, only surplus concentrations, and not total concentrations, have been measured. Using bisulfite as a competitive binding compound for amino acids, it has been possible to add sufficient levels of this compound to tie-up alpha-dicarbonyls and still permit growth of lactic acid ripening organisms. In this manner, it has been possible to study rates of actual production of alpha-dicarbonyl compounds by lactic organisms. In combination with this work, studies have been carried out on the rate of reactions between selected amino acids (i.e., lysine and glycine) and alpha-dicarbonyls, thus demonstrating their high degree of reactivity. Research

underway extends model system studies on the formation of cheese flavor compounds, and will initiate introduction of cultures and compounds into actual Cheddar cheese to study effects on the formation of flavor compounds.

Significance to the dairy industry

Understanding the mechanisms involved in forming flavor compounds will make it possible to devise culturing strategies yielding desirable and consistent cheese flavors. Improving cheese flavors will lead to an increase in cheese sales and consumption.

INTERIM REPORT

Process Modification of Starter Cultures for Flavor Enhancement in Lower Fat Cheese

Personnel: Mark. R. Etzel, associate professor, Chi Shung Brian To, graduate student, Dept. of Food Science

Dates: July 1994 – June 1996

Funding: Dairy Management Inc. ETZ 95

Objectives

The overall objective of this research is to develop more flavorful lower fat Cheddar cheese by using process-modified starter culture adjuncts that accelerate the ripening process. Processing conditions will be developed which reliably modify the culture characteristics to permit flavor enhancement in lower fat cheese. This will allow the manufacture of more consistent and flavorful lower fat cheeses, thus increasing the demand for cheese and milk. The specific objectives are to:

1. Establish methods for small scale production of cell pastes of candidate cultures.

2. Freeze, freeze dry and spray dry the cell paste solutions using processing conditions which range from attenuating to preserving of metabolic activity.

3. Analyze the cell pastes, solutions and cell powders for metabolic activity, specifically cell survival, lactic acid production, and ß-galactosidase and aminopeptidase activity.

4. Select the cultures and processing conditions which are best for flavor enhancement in lower fat cheese, and then produce large amounts of adjunct needed for cheesemaking.

Summary

The culture *Brevibacterium linens* ATCC 9174 was selected as the first candidate for investigation. *B. linens* is responsible for the ripening of surface-ripening cheeses such as Limburger cheese. Incorporating this obligate aerobe into Cheddar cheese, as an adjunct to the normal starter culture, has been shown to enhance the rate and extent of flavor development. Inexpensive methods for producing, preserving, and distributing these adjuncts cultures must be developed before widespread use in the dairy industry is economical. For this reason, the effects of processing (freezing, freeze drying and spray drying) on the characteristics of *B. linens* were measured in this research.

The culture was grown at room temperature in aerobic shake flasks containing Tryptic Soy Broth (TSB). The orange cell paste was dispersed in condensed skim milk to achieve a final total solids content of 25%. Aliquots of the cell suspension were frozen, freeze dried and spray dried. The spray drying conditions used were an inlet air temperature of 220°C, and outlet air temperatures of 70°C, 75°C, 80°C and 90°C. The freeze dried and spray dried powder samples were stored in sealed polyethylene sample bags in a refrigerator.

The viability of *B. linens* was not affected by shear in the atomizer, heating in the atomizer, freezing of the cell suspension, or freeze drying. Dehydration during freeze drying did cause sublethal injury to the cells, but eventually the initial cell viability was completely recovered. Heat was the sole mechanism decreasing cell survival during spray drying. Cell survival was halved for every 5°C increase in the outlet air temperature. Survival was 20% at an outlet air temperature of 70°C. By extrapolation, 100% cell survival would occur at an outlet air temperature of 57°C. A new spray drying chamber was built and is now being tested to achieve this temperature.

Rehydrated powder samples and cell free extracts (CFE) were assayed for aminopeptidase (AP) and lipase activity by Prof. B. Weimer of Utah State

University. Rehydrated freeze dried powder samples had the highest AP and lipase activities. Spray dried powder samples had intermediate activity, and increasing the outlet air temperature increased the AP and lipase activities. The frozen sample was the lowest in enzyme activity.

Intracellular AP and lipase activities were highest in the CFE from the spray dried samples. Increasing the outlet air temperature increased enzyme activity. The CFE from the freeze dried samples had the lowest enzyme activity. No CFE was prepared for the frozen sample.

The viability of the dried cultures was unstable during prolonged storage in a refrigerator. After storage for 50-71 days, 99% of the cells in the spray dried and freeze dried samples were inactivated. However, storage stability was improved significantly when a small amount of glycerol was added to the feed solution prior to drying, and when samples were stored in an oxygen-free atmosphere.

Significance to the dairy industry

The results of this research will help advance the cheese industry. The demand for lower fat cheese is growing. This new technology will help in developing lower fat cheeses with a flavor and texture as desirable as in full fat cheeses. These lower fat cheeses will be popular for producing spray-dried powders for use in lower fat convenience foods such as cheese nachos and frozen dinners, and will appeal to consumers on lower fat diets who may have reduced their consumption of traditional full fat cheeses. This new technology will help to accelerate the already increasing demand for cheese, which will directly result in an increased demand for milk.

In addition, the results of this research will help to advance the starter culture industry, most of which is based in Wisconsin. According to Dr. Doug Willrett, Technical Director at Marschall Products, "Much of the basic research on culture preservation is lacking and is not generally performed by the starter culture industry. Most of our resources are directed towards the direct servicing of our customers and assisting them with their applications. We depend on academic institutions such as the UW-Madison to conduct the type of basic studies you have proposed for our continued renewal. Without them, it would be difficult for our industry to advance."

INTERIM REPORT

Contribution of Endopeptidases from *Lactobacillus helveticus* CNRZ32 to Cheese Flavor Development

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

Dates: July 1994 – June 1997

Funding: Dairy Management Inc. SPJ95

Objectives

1. Characterization of the three endopeptidase genes.

2. Construction of CNRZ32 derivatives with altered endopeptidase activities.

3. Evaluation of the contribution of individual endopeptidases 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

An endopeptidase clone, designated EPII, was previously identified in a *Lb. helveticus* CNRZ32 genomic library using the endopeptidase substrates, N-benzoyl-Phe-Val-Arg-pNA and N-benzoyl-Pro-Phe-Arg-pNA. Restriction analysis of this clone revealed that the clone contained a 2.5 kbp insert. Tn1000 mutagenesis was used to localize the EPII gene to a 1.3 kbp region of the insert. The insert was completely sequenced and the open reading frame encoding EPII was found to be 1,317 bp in length. A homology search with EPII revealed that EPII shared 40% identity with the CNRZ32 aminopeptidase C and was a member of the cysteine proteinase family. Analysis of the EPII gene strongly indicates that it is an intracellular endopeptidase due to the lack of a signal sequence. Analysis of the EPII gene also suggested that this gene was transcribed monocistronically due to the presence of a putative promoter region as well as a strong terminator. Northern analysis is currently in progress to confirm that EPII is transcribed monocistronically.

CNRZ32 mutants lacking EPII have been constructed using a gene replacement technique. Preliminary characterization of these mutants in MRS broth, milk, and defined medium is in progress to determine how EPII contributes to growth of CNRZ32 in these media.

The purification of EPII is currently in progress using a protein fusion purification kit supplied by New England Biolabs, Inc. Preliminary steps, such as the construction of the fusion protein necessary for the purification, have been completed.

To determine the role of EPII in casein hydrolysis by Lb. helve*ticus* CNRZ32, isogenic strains differing in only their EPII activity will be evaluated, as well as, the specificity of the purified EPII on casein-derived fragments.

A second endopeptidase clone, designated EPIII, was previously identified in a L*b. helveticus* CNRZ32 genomic library using the endopeptidase substrates, N-benzoyl-Pro-Phe-Arg-pNA and N-benzoyl-Val-Gly-Arg-pNA. Restriction analysis of this clone revealed that the clone contained a 6.5 kbp insert. This insert was subcloned and partially sequenced. An open reading frame encoding EPIII was found to be approximately 2.0 kbp in length. A homology search with EPIII revealed that EPIII shared 47% identity with the La*ctococcus lactis* PepO endopeptidase, which has been purified and sequenced from *Lc. lactis*. The *Lc.lactis* PepO gene is

part of an operon containing the ATP-driven oligopeptide transport system. Identification of an open reading frame putatively encoding a protein associated with the ATP binding cassette of the oligopeptide transport system in CNRZ32 has been made upstream from PepO. Research is currently in progress to confirm the presence of the oligopeptide transport system and to characterize the relationship between PepO and this transport system.

Construction of a PepO mutant in CNRZ32 is in progress. The construct used to create a PepO mutant in CNRZ32 via gene replacement has been made. Once PepO mutants have been obtained, preliminary characterization of these mutants will be made in MRS broth, milk, and defined medium.

To determine the role of PepO in casein hydrolysis by *Lb. helveticus,* isogenic strains differing in only their PepO will be evaluated in cheese. PepO will also have to be purified to identify the specificity of PepO on casein-derived fragments.

Significance to the dairy industry

The construction of derivatives of *Lb. helveticus* CNRZ32 which differ only in the activity of an individual endopeptidase, will allow us to demonstrate the role of that enzyme in flavor development. If an endopeptidse is determined to be the rate-limiting enzyme for the degradation of bitter peptides and/or the formation of free amino acids or peptides with beneficial flavor attributes, then strains which overproduce this enzyme will be constructed. These strains could then be used to produce both traditional and lower fat cheese varieties; these cheeses should display less bitterness and develop cheese flavor more rapidly.

INTERIM REPORT

Influence of Lipolytic Reactions in Cheese on Flavor and Texture Development

Personnel: Sithian Pandian, adjunct professor, Universite Laval, Quebec, Canada; Mark E. Johnson, senior scientist; Carol Chen, researcher; John Jaeggi, associate researcher; Bill Tricomi, assistant researcher; Marianne Smukowski, research specialist, CDR

Funding: Wisconsin Milk Marketing Board 92-14

Dates: March 1993 – February 1995

Objectives

1. To screen a number of Lactobacillus strains for their esterase activity and identify the strains with high enzyme activity.

2. To purify the lipase from suitable source *Lactobacilli*.

3. To characterize the biochemistry of these purified enzymes, in vitro, in terms of their optimal pH and temperature, activators and inhibitors.

4. To study the stability of lipase action in cheeses that have been prepared with different manufacturing conditions, varying moisture content, fat concentration, presence or absence of fat substitutes. To determine how much activity is retained during maturation and how long it lasts.

5. To prepare pilot lab-scale cheeses by adding either purified lipase and/or lipase deficient lactobacilli mutants and to follow the development of the organoleptic properties in the absence of lipase or in the presence of added purified enzymes.

Summary (past reports covered points 1-4)

Full fat and 50% reduced fat Cheddar cheeses were made in November, 1994. The variables tested were control (no added *Lactobacilli*), freeze-shocked (2% addition) or direct culture addition (.002%) of either a *Lactobacilli* with a high esterase activity (wild strain) or its mutant with a low esterase activity. Three vats of each variable were made at each fat level. Two types of taste panels were conducted. Initially a difference test was completed to ascertain whether or not panelists could differentiate between control and experimental cheeses. If a significant difference was detected (the odd cheese was correctly identified) a descriptive test was completed to determine what attributes of the experimental cheese made it stand out as different from the control. The first taste panels were done at 6 weeks.

Descriptive taste panels on the full fat Cheddar cheeses showed that the experimental cheeses had distinctly more Cheddar cheese flavor intensity than the control cheeses. Also, there was a trend that the cheeses made with frozen cells had more Cheddar flavor intensity than cheeses made with direct culture addition. There were no differences between cheese made with wild or mutant cells. No rancidity was noted in any cheese.

In the 50% reduced fat Cheddar cheeses the experimental cheeses had more Cheddar flavor intensity, but a slight rancid flavor (this trend continued through 6 months), and more atypical flavors than the control cheeses. However, there were no differences in overall flavor preference between any of the cheeses.

Similar results occurred in taste panels conducted at 3 and six months. However the Cheddar flavor intensity difference between controls and all experimental cheeses was greater in full fat Cheddar than in the reduced fat cheese. Currently, analytical work is in progress to determine if there is a correlation with the adjunct, free fatty acid level, composition and sensory quality.

Significance to the dairy industry

The lack of well-balanced, distinctive Cheddar flavor in reduced fat cheese is a major drawback to consumer acceptance. The direct addition of enzymes (both lipases and peptidases) has not been successful in producing the desired cheese. The most promising avenue of research has been in the use of culture adjuncts. This project is part of the overall effort of the CDR to study adjuncts and to better understand the flavor chemistry of Cheddar cheese. The desired outcome will be a more flavorful cheese and a cheese with stable flavor and long lasting quality.

INTERIM REPORT

Studies of the Influence of Milkfat on the Formation of Flavor Compounds in Cheddar Cheese

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

Dates: October 1993 – June 1996

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

Objectives

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

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

Summary

We have started the systematic investigation of the effects of a range of fat contents (less than 1.7% to 32%) on the flavor formation in stirred curd Cheddar cheese. Currently, there isn't a chemical description of Cheddar flavor. We have proceeded to develop the analytical protocols that will enable us to identify and subsequently describe the compounds comprising the Cheddar flavor and the effect of milkfat on the flavor profile.

Several groups of important cheese flavor compounds, including volatile sulfur compounds, alpha-dicarbonyls, fatty acids, and amino acid metabolites, were measured during curing at 40°F and at varying intervals of up to nine months. The flavor compound measurements are supported by systematic flavor evaluations by experienced cheese flavor judges.

The research team is tasting the cheeses at 1, 2, 3, 6, and 9 months. The first two trials with the new culture had an initial pH and salt levels slightly higher than desired; dominant flavor in full and 25% reduced fats were heated, cooked cream flavors, some cooked egg/sulfur flavor. As fat was reduced there was an increase in whey taint flavors. In 75% reduced fat and skim milk cheeses the dominant flavors were umami (protein-like), sweet/salty. The umami was already definite at only 2 months of age. These flavors increased in intensity throughout aging, and typical Cheddar flavors did not develop.

Subsequent trials were closer to target pH, however flavors were very similar and even more intense. Full and 25% reduced fat had a cooked cream flavor, possibly methane thiol. These flavors turned to eggy, unclean, yeasty, rosy by 6 and 9 months of age.

The lower fat cheese had stronger umami flavor earlier in aging than did the first 2 trials, as well as more unclean flavors. Intensity of umami flavor increased with decreasing fat level. Skim milk cheeses were the only cheeses to exhibit significant bitterness, and were also rosy and unclean. Fat reduction greater than 50% typically yielded a "meaty" cheese.

Results of our initial trials will provide the direction for selecting cultures and other manufacturing parameters in subsequent cheesemaking trials.

Significance to the dairy industry

This project addresses one of the major problems that occur when developing manufacturing procedures for full-flavored low fat Cheddar cheese — defining the role of milkfat in the formation of typical full fat Cheddar cheese flavors. Removing milkfat and selecting starter cultures by trial and error has provided a partially acceptable generation of reduced-fat cheeses. However, the flavor quality of this cheese is quite different compared to full fat Cheddar cheese, and consumers don't like it.

Increasing numbers of consumers are interested in low fat food and unless tasty low fat Cheddar cheeses are provided, consumers will find alternative low fat foods. Thus, it is important to shift from a trial and error approach to low fat cheesemaking and begin basic research to produce low fat cheese that satisfies current consumers.

FINAL REPORT

Using Biopreservatives to Control Spoilage Bacteria Associated with Cheddar Cheese

Personnel: Mark E. Johnson, senior scientist, Center for Dairy Research; John B. Luchansky, associate professor, Food Research Institute; Heidi E. Uljas, research assistant, Food Research Institute

Funding: Dairy Management Inc. JLMJ94

Dates: July 1993 – June 1995

Objectives

1. Validate the antimicrobial activity of bacteriocinogenic lactic cultures and associated bacteriocins (i.e., biopreservatives) towards racemase-positive lactobacilli in Cheddar cheese.

2. Incorporate currently available bacteriocins and/or lactic acid bacteria alone and in combination, at various intervals during cheese manufacture to identify the most suitable step for adding bioactive agents.

Summary

Although lactic acid bacteria (LAB) are often desired in Cheddar cheese and contribute to its flavor and texture, LAB may also constitute the dominant spoilage flora in Cheddar cheese. Since LAB are destroyed by pasteurization, their presence in the final product is largely due to post-process contamination from the dairy environment. As one example, racemase-positive LAB present a major economic problem to cheese producers by transforming soluble L(+) lactate into the less soluble D(-) form resulting in the formation of calcium lactate crystals on the surface of Cheddar cheese. To decrease the likelihood of crystal formation it is crucial to lower the number of racemase-positive LAB in the cheese. Good manufacturing practices and strict attention to proper sanitation can lower the counts of LAB in cheese, but nonstarter-LAB are common in the dairy environment and eventually become the dominant microflora of cheese.

Several recent studies have established the potential of bacteriocins to provide an additional barrier against the growth of undesirable bacteria in foods. As such, the research efforts of this study were directed to identify and characterize bacteriocins with activity against calcium lactate crystal-forming bacteria and evaluate the efficacy of said bacteriocin(s) against crystal-forming bacteria in Cheddar cheese.

The antimicrobial activities of the cell-free, pH-neutralized supernatants from six strains (Lactobacillus isolates JBL1496, JBL1497, JBL1498, JBL1499, JBL2109, and JBL2110) displaying the greatest and most consistent antimicrobial activity against crystal-forming LAB were sensitive to several proteolytic enzymes, resistant to heat (121°C, 20 min) and stable over a wide pH range (pH 4.0 to 7.0). These data indicated that the antagonistic agents were bacteriocins. In subsequent experiments, the behavior of a crystal-forming strain, *Lb. casei* JBL1166, was monitored in microbiological media and in Cheddar cheese curd in the presence of crudely purified lactacin 99, a bacteriocin produced by *Lb. plantarum* JBL1499. Increasing the concentration of lactacin 99 from 0 to 0.08% in MRS media extended the lag phase of JBL1166 from 4 to 34 h. More important, the addition of 0.8 to 4.0% lactacin 99 to Cheddar cheese curd containing JBL1166 (7 $\log_{10}$ CFU/g) reduced numbers of the crystal former 1.1 to 4.1 $\log_{10}$ units after 28 d at 4°C compared to control treatments.

In other experiments, lactacin 99-producing cells (strain JBL1499) were evaluated as a starter adjunct to control the growth of crystal-forming bacteria during ripening of Cheddar cheese. Preliminary studies focused on the potential of JBL1499 to produce lactacin 99 in milk and inhibit the growth of a crystal former. When live cells were added to milk, in the absence of bacteriocin-producing JBL2132, numbers of the crystal former (initial inoculum 5.7 $\log_{10}$ CFU/ml) increased 1.2 $\log_{10}$

CFU/ml in 24 h. In the presence of 7.5 and 8.5 $\log_{10}$ CFU/ml of the lactacin 99 producer JBL2132, total numbers of the crystal former were 3.9 and 6.1 $\log_{10}$ CFU/ml lower than in the control, respectively, within 24 h. Similarly, when milk pre-fermented by JBL1499 was added at levels of 10 and 20% to cheese milk counts of the crystal former (initial inoculum 5 $\log_{10}$ CFU/ml) were reduced by 1.3 and 3.6 $\log_{10}$ units, respectively, in 10 h during storage at 30°C. In control treatments without pre-fermented milk, numbers of crystal-forming bacteria increased 0.5 $\log_{10}$ CFU/ml.

As a final objective, Cheddar cheese was made using the lactacin 99-producing strain as a starter adjunct in a pilot scale cheese making. Along with the starter culture *Lactococcus lactis* ssp. *cremoris* CC19, freshly pasteurized milk (227 kg) was inoculated with crystal-forming *Lb. casei* JBL2126 (low inoculum level, 0 to 1 $\log_{10}$ CFU/ml; high inoculum level, 2 to 3 $\log_{10}$ CFU/ml) and lactacin 99-producing adjunct *Lb. plantarum* JBL2132 (8 $\log_{10}$ CFU/ml). Control cheeses were prepared without lactacin 99-producing JBL2132. At appropriate intervals during storage at 7.2 °C cheese was sampled for viable cells of the starter, crystal former, and starter adjunct cultures. In control cheese, numbers of the crystal former were ca. 5.0 (low inocula) and 8.0 (high inocula) $\log_{10}$ CFU/g after ripening for 3 and 6 mo, respectively. In the presence of the lactacin 99-producing strain, numbers of the crystal former were ca. 1.9 (low inoculum) and 1.8 (high inoculum) $\log_{10}$ CFU/g units lower compared to control treatments after 3 mo. After 6 mo, numbers of the crystal former were 1.6 (low inoculum) and 1.1 (high inoculum) $\log_{10}$ CFU/g lower than in control cheese. The starter adjunct did not affect the growth of the starter. These data establish the potential for using bacteriocin-producing adjuncts to inhibit the growth of crystal-forming bacteria during cheese ripening.

Significance to the dairy industry

The frequent association of racemase-positive LAB with Cheddar cheese and the continued occurrence of crystal formation suggests that additional strategies are required to eliminate this problem. Although good manufacturing practices and sanitation can reduce the probability of bacterial defects, our results demonstrate that biopreservatives can significantly reduce the numbers of calcium lactate crystal-forming bacteria in Cheddar cheese. As such, LAB and associated bacteriocins offer great potential for improving the quality and shelf-life of certain cheese varieties.

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

"Update on biocontrol and subtyping of foodborne pathogens". Scientific Lectureship, Institute of Food Technologists, Honolulu, Hawaii. January 25, 1995.

"Advances in applications for biopreservatives and pulsed-field fingerprinting in Food Microbiology." Invited speaker at University of Hawaii, Department of Microbiology, Honolulu, Hawaii. January 25, 1995.

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

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

"Microbiological safety of reduced fat Cheddar cheese". Invited speaker at the International Business Communications Conference on Fat and Cholesterol-reduced Foods. New Orleans, Louisiana. March 30, 1995.

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

"Knowing and controlling cheese pathogens." Invited speaker at the International Cheese Technology Exposition, LaCrosse, Wisconsin. April 7, 1994.

"Applications of biotechnology to detect, type, and control foodborne pathogens." Invited speaker at the Biotechnology Conference of the Green Bay Area Chamber of Commerce, Northwest Wisconsin Technical College, Green Bay, Wisconsin. April 21, 1994.

"Overview of pathogen typing and control." Annual Meeting of the Food Research Institute, the Wisconsin Center, Madison, Wisconsin. May 25, 1994.

"Use of new generation preservatives to control pathogenic and spoilage organisms associated with food." Invited speaker at the 3rd International Aseptic Processing Association (ASEPT) Conference on Food Safety. Laval, France, June 2, 1994.

"Applications of biopreservatives and genomic fingerprinting to food safety." Invited speaker at the National Animal Disease Center, Ames, Iowa. August 16, 1994.

"Molecular tracking and biocontrol of foodborne pathogens." University of Wisconsin, Department of Food Science, Madison, Wisconsin. Student seminar series (FS 900); September 27, 1994.

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

"Overview of pulsed-field typing and biocontrol of foodborne pathogens." Invited speaker at the University of Wisconsin-River Falls Food Microbiology Symposium, River Falls, Wisconsin. October 13, 1994.

"The influence of lactic cultures on human health and food safety." Presentation to the general staff and the new science team of Gerber Products Company, Fremont, Michigan. July 1, 1993.

"The use of biological controls in food safety." Invited speaker at Symposium: Detection and control of foodborne pathogens. Joint Meeting of the Society for Industrial Microbiology and the Canadian Society of Microbiologists, Toronto, Ontario, Canada. August 5, 1993.

"Efficacy of bacteriocins in Queso Blanco and Cheddar cheese." Presentation and participation at a research review meeting on bacteriocins for the National Dairy Promotion and Research Board, Hilton Hotel O'Hare, Chicago, Illinois. September 30, 1993.

"Processing factors in cheese and low-fat cheese that control growth and survival of *Listeria*." Invited speaker at the "Conquering Listeria" symposium sponsored by the Dairy Research Foundation, Westin Hotel O'Hare, Rosemont, Illinois. October 5, 1993.

"Advances in the application of biopreservatives to control undesirable bacteria in foods." Invited speaker at the Food Microbiology Research Conference (XIV), Chicago, Illinois. October 22, 1993.

"Progress towards control of *Listeria* in foods using lactic cultures and associated antimicrobials." Invited speaker at the University of Wisconsin-River Falls Food Microbiology Symposium, River Falls, Wisconsin. October 29, 1993.

FINAL REPORT

Improving the Flavor of Enzyme-Modified Cheeses by Control of Lipase Action in Supercritical (SC) CO_2

Personnel: R. W. Hartel, associate professor, J.M. Johnson, research assistant, Dept of Food Science

Dates: July 1989 – October 1994

Funding: Cheese Research Institute 1-89

Objectives

1. To assess the feasibility of using supercritical CO_2 to control lipolytic action on milkfat.

2. To determine the specificity of lipase action on milkfat in supercritical CO2 as influenced by the type of lipase used, the level of water as an entrainer and the reactor operating temperature and pressure.

3. To develop a fundamental understanding of how lipase action on milkfat in supercritical CO_2 may be controlled to improve the flavor balance and fatty acid profile of enzyme modified butterfat.

Summary

Several experiments have been performed to document lipase activity in supercritical carbon dioxide (SC CO_2). Initially, pure triacylglycerides (TAGs) were used to determine if hydrolysis of the TAGs by lipase could be achieved under various SC CO_2 conditions. The mole fraction of tripalmitin in the SC CO_2 phase was determined to be 4.57×10^{-5} at 40°C and 4500 psi (300 bar). At the same reaction conditions, the percentage of palmitic acid hydrolyzed by a crude extract of porcine pancreatic lipase after 1 and 3 hours reaction time was 5.8 and 11.9% respectively. Hydrolysis of tricaprin was also achieved under these conditions, although the liberated capric acid was not quantified. In this series of experiments, the SC CO_2 was saturated with deionized water subsequent to entrance into the reaction vessel by passing through a surge tank containing the water. This was done to prevent the SC CO_2 from stripping water from the enzyme. However, this method introduced too much water into the system and caused clumping of the enzyme.

The presence of water is crucial for controlling the enzyme hydrolysis reaction. Thus, the crude extract of porcine pancreatic lipase was placed in relative humidity chambers at 5°C for 24 hours to control the amount of water present in the reaction. The enzyme was equilibrated to water activities (Aw) of either 0.09 or 0.28. The reaction temperatures were 40 and 60°C, and reaction pressures were 14.7 (1 bar) and 4500 (300 bar) psi. Tricaprin was chosen as the substrate. The sample concentration extracted remained constant throughout the reaction at 10 mg /100 g CO_2 for all conditions tested when using low enzyme concentration (10% of substrate weight). There were changes in the capric acid concentration. After approximately 6 minutes reaction time, the concentration of capric acid ranged from 1 to 16 μmoles in the sample extract. However, after three hour reaction time (approximately 38 μmoles/g sample)the concentrations of the capric acid were similar for all the conditions studied. At very high enzyme concentrations (10 times the substrate weight), the concentration of extracted sample increased at a rate of 77.8 mg per 100 g CO_2 per hour. The capric acid concentration after three hours was higher under atmospheric pressure conditions as compared to the SC CO_2 conditions tested. The capric acid concentration ranged from 61 to 131 μmoles/g sample. Largest differences occurred between 40°C (0.09 A_w) and 60°C (0.09 A_w). In summary, the presence of water (as measured by water activity of the enzyme) and differences in temperature did not seem to effect the final capric acid concentration in the sample after three hours reaction time under SC CO_2 conditions.

In the next series of experiments, the substrate was changed to anhydrous milkfat (AMF). The lipase

remained the same as in the previous experiments and was placed at the bottom of the reaction vessel. The reaction temperatures were 40 and 60°C, enzyme water activity was either 0.18, 0.28 or 0.47, and the pressures were 138 (2000 psi) and 275 (4000 psi) bar. The amount of AMF added to the reactor resulted in at least a two phase system (liquid AMF and supercritical phase) at the pressures tested. Samples were extracted from both phases at various time intervals and analyzed for fatty acids by HPLC. Maximum fat concentration in SC CO_2 phase was obtained within 20 minutes. The maximum apparent rate of hydrolysis for any fatty acid depended on temperature for short chain (SC, ≤C8, 60°C), medium chain (MC, ≥C10 and ≤C14, 40°C) and long chain (LC, ≥C16 and ≤C18:1, 40°C) fatty acids. The maximum apparent rate of hydrolysis also depended on A_w for MC (0.28) and LC (0.18) fatty acids and pressure (in liquid phase only) for MC (275 bar) and LC (138 bar) fatty acids. Proteins have little to no solubility in SC CO_2. Therefore, the hydrolysis reaction takes place mainly in the liquid phase. However, the reaction products and substrate partition between the two phases and the concentration of these lipids change in each phase as the hydrolysis reaction progresses. Hydrolysis of AMF by lipase under SC CO_2 conditions is quite complex due to the simultaneous extraction in the supercritical phase and hydrolysis in the liquid phase.

Current efforts focus on using immobilized enzymes, which can be placed in either the SC or liquid phases in the SC reactor. Three enzymes (crude extracts from *Pseudomonas spp., Candida rugosa* and porcine pancreas) were immobilized onto polypropylene beads and tested for hydrolytic activity to anhydrous milk fat (AMF) at 35°C and atmospheric pressure. The lipase from *Pseudomonas spp* was found to be seven times more reactive than the other two enzyme preparations, and continued studies focused on this enzyme. Optimal conditions for this enzyme for hydrolysis of milk fat were found to be pH of 8.6 and temperature of 45°C. Increasing water content (from 1 to 10%) generally increased extent of hydrolysis as did using higher agitation rate (200 vs. 100 RPM).

Significance to the dairy industry

Use of supercritical (SC) fluid technology coupled with enzymic modification has the potential to produce specific milk fat fractions for use as food ingredients. In this research, we used SC CO_2 to produce dairy flavors through hydrolysis of butterfat triglycerides and selective extraction of the lipid products. Control over lipolyzed butter flavors for use in processed cheeses may be achieved in this way. A better understanding of the enzymic processes involved in modifying milk fat will allow development of value-added ingredients from milk fat.

Quality and Safety Summary

Chapter 4

Q & U S A A L F I E T T Y Y

Maintaining and enhancing consumer confidence in dairy food safety and quality is the cornerstone of CDR's safety and quality program. Projects investigating bacteriocins, or natural antimicrobials, HACCP (hazard analysis critical control points) and CIP assessment demonstrate our proactive approach. Highlights of these projects follow.

Nonstarter lactic acid bacteria (NSLAB) can produce a variety of defects in Cheddar cheese, such as undesirable flavors, gas formation or the white haze of calcium lactate crystals. Wong and M. Johnson have now demonstrated that these organisms can form biofilms on stainless steel and Buna N rubber. After forming biofilms, these organisms show increased resistance to heat and sanitizers. Normal cleaning and sanitizing, and even steam cleaning, reduced but did not always eliminate these tenacious organisms. More research is necessary to identify effective cleaning and sanitizing methods that eliminate problematic NSLAB.

Continuing their work with pathogen survival and growth in reduced fat and full fat Cheddar cheese, E. Johnson and Luchansky report that the microbial safety of both of these cheeses can be enhanced by adding natural antimicrobial compounds to cheese milk. The efficacy of adding lysozyme, Alta 2341, or Nisin was evaluated against *C. botulinum, L. monocytogenes,* and *Salmonella* spp. added to standardized, pasteurized cheese milk used to make full fat and reduced fat Cheddar cheeses. In general, the antimicrobials enhanced the safety of the cheeses but the effect varied among the pathogens tested.

Kaspar's project produced some good news for the dairy industry. The association of *E. coli* 0157:H7 and cattle prompted testing a variety of samples (air ducts, brine, condensate, drains, foot baths, raw milk, worker's hands, etc.) from fifteen dairy production/processing plants. Kaspar found no positive samples. The results suggest that the organism is present infrequently, survives poorly or is adequately controlled by current sanitation practices.

FINAL REPORT

Characterization of Biofilm Formation by Nonstarter Lactic Acid Bacteria (NSLAB) in Dairy Environments

Personnel: Amy C. Lee Wong, associate professor, Food Research Institute, Mark Johnson, senior scientist, CDR, and Eileen Somers, senior research specialist, Food Research Institute

Funding: Dairy Management Inc. WG294

Dates: July 1993 – June 1995

Objectives

1. To determine biofilm formation and survival characteristics of NSLAB from defective cheeses.

2. To determine heat resistance of NSLAB biofilm bacteria.

3. To determine biofilm formation in a cheese manufacturing environment.

Summary

Nonstarter lactic acid bacteria (NSLAB) are common causes of defects in cheeses, producing undesirable flavors, texture defects, gas formation and the white haze of calcium lactate crystals. The surfaces of food processing equipment are recognized as major sources of microbial contamination. Even with clean-in-place systems, microorganisms can remain on equipment surfaces through attachment and initial biofilm formation. Further development of biofilms on equipment surfaces may contaminate food products.

Biofilm characteristics and resistance

We investigated the ability of nine NSLAB isolates to form biofilms on two different materials commonly found in the dairy environment, stainless steel and Buna N rubber. Strains included heterofermentative lactobacilli and lactobacilli responsible for crystal development. Results obtained from two day old biofilms developed in skim milk medium showed that NSLAB could readily produce biofilms on both surfaces and the levels of biofilm bacteria varied with the isolate (Table 1). In general the number of biofilm bacteria for all isolates were higher on stainless steel than on Buna N rubber. As shown in Table 2, the availability of nutrients influenced the level of biofilm bacteria for any given strain. Strain RM 21 and strain #4, heterofermentive NSLAB are less affected by the nutrient level in the different media than JBL 2126 and LB26R, homofermentive isolates.

NSLAB are considered heat-sensitive organisms that normally do not survive pasteurization. However, it has been shown that biofilm bacteria can be more heat resistant than planktonic or non-attached cells. We tested two NSLAB (JBL 2126 and strain #4) for their ability to survive in skim milk tempered to 71.6 °C and held at that temperature for up to 30 seconds. Planktonic and biofilm bacteria (on stainless steel chips) were grown for two days in MRS broth, skim milk or skim milk diluted 1:5 in distilled water. Planktonic cells and stainless steel chips were removed from the culture and added to the tempered milk. There was an initial rapid decrease (2-3 log) in viable cells for both the planktonic and biofilm bacteria. After 5 to 10 seconds the rate of decrease tapered off and viable cells were recoverable in low numbers ($\sim$100/cm^2) at 30 seconds. Biofilm cells of both strains were always about 10 fold more resistant than the planktonic cells. In general all cells grown in MRS broth were the most heat resistant and cells grown in 1:5 skim milk were the most sensitive. Strain #4 was more resistant than JBL 2126 under all conditions.

Biofilms in a cheese manufacturing environment

Studies designed to identify process steps or locations where biofilms occur during the manu-

facture of stirred-curd Cheddar were conducted at the pilot plant in Babcock Hall with the technical assistance of John Jaeggi. To follow the process steps, presterilized chips of stainless steel were glued to the inside of a vat for cheese manufacturing, cleaning and sanitizing. The chips were scraped and biofilm bacteria were enumerated using selective and nonselective media. Cheese made from the study was assayed, at various time intervals during storage at 45° F, for total counts, numbers of NSLAB and for the presence of both the d- and l- isomer of lactic acid. The d-isomer is responsible for the formation of calcium lactate crystals. Lactic acid analysis was performed by Bill Tricomi at the CDR. Two NSLAB isolates were used. *Lactobacillus casei* (JBL 2126), a racemase positive homofermentive organism and a racemase negative gas forming heterofermentive NSLAB (strain #4). Both strains are erythromycin resistant (5 ug/ml) and can be differentiated from the starter culture and other NSLAB by plating on Lactobacillus MRS Agar (Difco) with erythromycin (MRSE). In addition Rogosa SL agar (SL) was used as a plating medium to detect and recover potential environmental NSLAB contaminants.

In the interim report we briefly described the results of a study which showed that JBL 2126 when inoculated into the pasteurized milk, could survive cheese manufacture, and the cleaning and sanitizing of the vat and persist into the next round of cheese. Table 3 summarizes the starter and JBL 2126 counts during the process for both the spiked (vat #1) and unspiked vat (vat #2) of cheese. The untreated vat was made in the same vat as the inoculated milk, but after it was washed and sanitized. The high initial inoculum level and high numbers of JBL2126 in the cheese curd of vat #1 resulted in cheese with d-lactic acid at 48 % of the total lactic acid content and JBL 2126 numbers of 10^7 CFU/g after only two weeks of ripening. The maximum amount of d-lactic acid produced at equilibrium would be 50%. The JBL 2126 numbers, total count and d-isomer levels in the cheese from the uninoculated vat are shown in Table 4. The numbers of JBL 2126 and total count increased until three months and then slowly declined. The % of d-lactic acid increased throughout storage.

A second approach was designed to mimic possible contamination of product by contact with biofilms on equipment surfaces. Either JBL 2126 or strain #4 was inoculated into skim milk medium and biofilms were allowed to develop on stainless steel chips (1 inch sq.) for two days at 30° C. Four chips with preformed biofilms were added to the cheese vat along with the pasteurized milk. Approximately 10^7 CFU were present in the biofilms giving a potential inoculum of around 100 CFU/g milk. Results from two representative experiments are shown in Table 3. JBL 2126 could not be detected in the milk at the time of the addition of the starter culture or in the curd at the time of hooping. This could indicate that very low numbers of bacteria were detached from the biofilm. The MRSE and SL counts in these experiments were the same, indicating no outside contamination, so the SL numbers were not included in the respective tables.

In contrast to JBL 2126, strain # 4, a heterofermentative lactobacilli could be detected in low numbers both at the time of starter addition and in the curd. During cheese ripening, JBL 2126 numbers increased and by 4 months had accumulated to $>10^6$ CFU/g (Table 5). The d-lactic acid content remained low initially but increased to 42% by 4 months. Strain #4 persisted in the cheese but did not increase during the ripening period (Table 6). However, the d-lactic acid content increased with time and was 37.5% at 2 months. This increase was likely the result of another NSLAB contaminant in cheese, evidenced by the increasing numbers of bacteria enumerated with Rogosa SL agar (SL). Isolated colonies picked at random from the SL and MRSE plates and tested in hetero/homofermentation broth (APT broth plus 1% glucose and 0.5% sodium citrate) were all negative for gas formation with the SL isolates and all positive for the MRSE isolates.

Steam cleaning the vats after cheese manufacturing reduced but did not always eliminate contamination. Swabbing areas in the pilot plant and enriching in MRS broth showed that NSLAB could be isolated on vat surfaces, counter tops, drains, hoops and the inside of the vacuum packager. Research designed toward better cleaning regimens may be helpful.

Significance to the dairy industry

Our research supports the hypothesis that biofilms can be a source of NSLAB contamination in the dairy environment. Normal cleaning regimens cannot eliminate biofilm bacteria, which can contaminate cheese and cause defects. Information generated in this study will be useful in designing strategies to manage and/ control contamination by NSLAB, preventing defects in cheese and improving the quality of cheese products. Further research targeting improved cleaning regimens and/or the need for steam cleaning would provide important information.

Presentations

E.B.Somers, A.C.L.Wong, and Mark Johnson. Biofilm formation by nonstarter bacteria and contamination in the dairy plant. Poster presentation at the University of Wisconsin Food Research Institute Annual Meeting, May 25-27, 1994, Madison, WI.

A.C.L.Wong. Survival and inactivation of biofilm bacteria in dairy and milking equipment surfaces. Dairy Symposium II - Sanitation and New Approaches to Better Dairy Products. 81st Ann. Meeting of the International Assoc. of Milk, Food and Environmental Sanitarians. July 31 - Aug.3 1994.

A.C.L.Wong. Biofilms - their implications for hygiene and quality in the dairy industry. International Dairy Federation Hygiene Week. Special session. April 25, 1995.

Publications

Wong, A.C.L. Biofilms in cheese processing environments. Proceedings of the Marschall Italian and Specialty Cheese Seminar. Sept. 28-29, 1994.

Somers,E.B., A.C.L.Wong, and Mark Johnson. Biofilm formation by nonstarter bacteria and contamination in the dairy plant. ADSA Abstract, J. Anim. Sci. 72:, Suppl 1, J. Dairy Sci.: 77, Suppl 1, 1994.

Table 1. Biofilm formation in Skim Milk Medium by NSLAB Isolates on Stainless Steel and Buna N Rubber

		Log CFU/cm^2	
Strain	Source	Stainless steel	Buna N rubber
201 TC1	aged Cheddar	5.0	4.6
201 TC3	" "	5.3	5.3
201 TC4	" "	5.6	5.4
Strain #4 (A2)	" "	5.4	5.3
RM 21	raw milk	5.8	5.5
RM 38	" "	4.6	4.1
RM 40	" "	4.2	3.7
LB 26	aged Cheddar	5.1	4.8
LB26R	" "	5.9	5.7

Table 2. Effect of Different Medium on Biofilm Formation by NSLAB Isolates on Stainless Steel

	Log CFU/cm^2		
isolate	MRS[a]	Skim milk[b]	1/5 Skim milk[c]
RM 21	6.1	6.0	5.5
LB26R	6.3	5.4	5.0
JBL 2126	6.2	5.6	3.8
Strain #4	5.2	5.2	5.1

[a] - Lactobacillus MRS medium (Difco)
[b] - Skim milk medium (Difco)
[c] - Skim milk medium diluted 1:5 in distilled water

Table 3. Summary of Bacterial counts of NSLAB and Starter Culture During Cheese Manufacturing Experiments

		Log CFU/ml or g		Log CFU/cm^2 on chips after			
Treatment		Starter addition	curd	whey	rinse	scrub	sanitizer
JBL 2126 bacteria added to milk							
Vat #1	MRS[a]	6.4	7.4	6.9	7.1	4.7	3.8
	MRSE[b]	4.0	5.1	4.0	4.7	2.9	2.3
Vat #2 (no JBL2126 added)	MRS	7.0	7.8	7.8	5.7	4.8	3.9
	MRSE	ND[c]	2.2	1.7	1.4	1.8	2.4
JBL 2126 biofilm chips added to vat							
	MRS	7.8	8.0	7.1	6.2	5.3	3.7
	MRSE	ND	ND	ND	ND	ND	2.3
Strain #4 biofilm chips added to vat							
	MRS	8.6	9.5	9.3	4.7	3.8	3.7
	MRSE	3.1	2.9	1.5	2.9	ND	ND

[a] - Total counts, starter plus NSLAB enumerated on Lactobacillus MRS agar
[b] - NSLAB bacteria enumerated on Lactobacillus MRS agar with 5 ug/ml erythromycin
[c] - Not detectable, less than 100 CFU

Table 4. Bacterial Count and d-Lactic Acid Content of Cheddar Cheese Cross Contaminated With JBL 2126 From Previous Vat.

	Log CFU/g		
Sample time[a]	MRSE[b]	MRS[c]	% d-lactic acid[d]
O wk	2.2	8.5	NT[e]
2 wk	3.0	8.1	2.9
1 mo.	3.5	8.7	15.7
2 mo.	5.7	8.7	21.9
3 mo.	7.9	9.9	35.3
4 mo.	<7.0	8.2	40.7
6 mo.	5.7	7.0	48.7

[a] - Cheese stored at 45° F
[b] - Number of JBL 2126 bacteria enumerated by plating on Lactobacillus MRS agar with 5 ug/ml erythromycin.
[c] - Total number of bacteria in cheese enumerated by plating in Lactobacillus MRS agar.
[d] - d-isomer content of lactic acid as percent of total lactic acid.
[e] - Not determined

Table 5. Bacterial Count and d-Lactic Acid Content of Cheddar Cheese Inoculated with JBL 2126 Biofilms

	Log CFU/g		
Sample time[a]	MRSE[b]	MRS[c]	% d-lactic acid[d]
O wk	ND[e]	7.8	NT[f]
2 wk	3.2	9.8	2.8
1 mo.	3.4	9.5	4.8
2 mo.	5.2	8.8	35.2
4 mo.	6.3	7.5	41.9

[a] - Cheese stored at 45° F
[b] - Number of JBL2126 bacteria enumerated by plating on Lactobacillus MRS agar with 5 ug/ml erythromycin.
[c] - Total number of bacteria in cheese enumerated by plating in Lactobacillus MRS agar.
[d] - d-isomer content of lactic acid as percent of total lactic acid.
[e] - Not detectable, less than 100/g.
[f] - Not determined.

Table 6. Bacterial Count and d-Lactic Acid Content of Cheddar Cheese inoculated with Strain #4 Biofilms.

	Log CFU/g			
Sample time[a]	MRSE[b]	MRS[c]	SL[d]	% d-lactic acid[e]
O wk	3.1	8.6	3.1	NT[f]
2 wk	4.0	10.1	5.8	4.1
1 mo.	3.6	9.2	6.3	5.1
2 mo.	4.0	8.7	7.2	37.5
4 mo.	3.0	9.6	7.4	NT

[a] - Cheese stored at 45°F
[b] - Number of JBL 2126 bacteria enumerated by plating on Lactobacillus MRS agar with 5 ug/ml erythromycin.
[c]- Total number of bacteria in cheese enumerated by plating in Lactobacillus MRS agar.
[d]- Total number of lactobacilli.
[e]- d-isomer content of lactic acid as percent of total lactic acid
[f]- Not determined

INTERIM REPORT

Biological Significance of Conjugated Dienoic Derivatives of Linoleic Acid

Personnel: Michael Pariza, professor, Food Research Institute, Wei Liu, research specialist, Jayne Storkson, senior research specialist, Karen Albright, senior research specialist, Kisun Lee, graduate student, Xiaoyun Yang, graduate student

Dates: June 1990 – November 1995

Funding: Wisconsin Milk Marketing Board 89-27

Objectives

1. Determine the function of CLA in protecting cell membranes from oxidation

2. Determine the effects of CLA in regulating/modulating various key membrane enzymes and enzyme systems

3. Determine practical way(s) to synthesize CLA for commercial application

Summary

We have obtained preliminary data that cytokine-mediated expression of cell adhesion proteins on endothelial cells is inhibited by CLA. This finding is important because it may explain how CLA inhibits atherosclerosis. We have also made progress in establishing the mechanism whereby CLA acts as an antioxidant. We have evidence that the conjugated double bond system of CLA chelates iron as we originally proposed. In addition, we have isolated and identified a harmless bacterium from rat colon that produces CLA from linoleic acid.

Four gram-positive bacterial strains, capable of converting linoleic acid to CLA, were isolated from the rat intestinal tract. They were subjected to physiological and biochemical tests and found to be lactobacilli, similar to *Lactobacillus reuteri*. These apparently newly recognized lactobacilli convert linoleic acid to CLA via a membrane-bound isomerase. The isomerase has been partially characterized, and is being purified for full characterization. These organisms or the purified isomerase(s) isolated from them represent a possible mechanism for synthesizing "natural" CLA.

We have obtained preliminary evidence that CLA inhibits the synthesis of cell-adhesion proteins that are expressed on the surface of endothelial cells following exposure to certain cytokines (IL-1, TNF) that are released from immune cells following immune stimulation. This finding is of great potential importance because the expression of these cell adhesion proteins on endothelial cells are thought to be directly involved in the induction of atherosclerosis. Hence, our finding indicates a biochemical mechanism whereby CLA may inhibit atherosclerosis.

We have obtained evidence that CLA regulates the level of arachidonic acid in membranes. This is a potentially important finding in that arachidonic acid metabolism is tied with prostaglandin synthesis and signal transduction pathways.

Finally, we have obtained direct evidence that the conjugated double bond system of CLA may chelate iron. This observation is important for explaining the antioxidant activity of CLA, and may also provide the basis for predicting how best to commercialize CLA as an antioxidant (covered by a WARF patent that was supported in part by WMMB funds).

Significance to the dairy industry

Despite the potential of commercial production, dairy products are the principal natural dietary source of CLA, which consumers may prefer. Hence, work aimed at possible health benefits of CLA is expected to enhance that already fine image of dairy products as important for sound health. The fact that CLA is a component of dairy fat is particularly intriguing in this regard. Additionally,

CLA is potentially of great importance as a natural antioxidant and mold inhibitor for use in food systems.

Publications/Presentations

Chin, S.F., Storkson, J.M., Liu, W., Albright, K.J. and Pariza, M.W. 1994. Conjugated linoleic acid (9.11- and 10, 12-octadecadienoic acid) is produced in conventional but not germ-free rats fed linoleic acid. J. Nutr. 124:694-701.

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Benjamin, H., Storkson, J.M., Liu, W. and Pariza, M.W. The effect of conjugated dienoic derivatives of linoleic acid (CLA) on mouse forestomach protein kinase C(PKL)-activity. FASEB J. 6, abstract #2666 (1992).

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INTERIM REPORT

Cleanability Assessment of Milking Equipment

Personnel: Douglas J. Reinemann, associate professor, Department of Agricultural Engineering, Amy C. Lee Wong, associate professor, Food Research Institute, Anton Muljadi, research assistant, John Patoch, research specialist

Dates: June 1993 – June 1995

Funding: Dairy Management Inc. RNM 94, Babson Brothers Company

Objectives

1. Characterize the interaction of mechanical, chemical and thermal processes on air injected Clean-In-Place (CIP) of milking systems.

2. Use information from objective 1 to develop design criteria and recommendations for installing and operating CIP systems which assure effective cleaning of milking systems.

Summary

The dynamics of air injected clean-in-place (CIP) systems are not well understood. Many different materials and several distinctly different flow regimes are encountered in milking CIP systems. Materials include: stainless steel, synthetic rubber hoses and gaskets, and various types of plastic used in hoses and milking system components. Cleaning flow dynamics and the resulting mechanical cleaning action vary considerably depending on the location in the system and the way the milking CIP system is installed and operated.

We have performed extensive studies of the flow dynamics of milking CIP systems on full scale systems at the University of Wisconsin Milking Research and Instruction Laboratory (UW-MRIL) and in the field. We have also developed and tested several methods of assessing mechanical cleaning action at the UW-MRIL. These studies were carried out in collaboration with Dr. Albrecht Grasshoff of the Federal Center for Dairy Research in Kiel, Germany.

We have developed methods to apply a standardized milk soil and bacterial residue to test chips. The chips are then used to assess the effectiveness of various cleaning regimes. Preliminary work has been performed to evaluate the use of the ATP bioluminescence method for hygiene testing on milk contact surfaces in the lab and in the field. Further work will examine methods for combined bacterial and milk soil deposit and assess removal and biofilm deposit and removal assessment.

Significance to the dairy industry

The dynamics of air injected CIP systems are not well understood. Most developments have occurred as a result of trial and error, although industry recognizes the need for research to develop a rational design criteria. Improved methods of cleaning and sanitizing milking equipment will lead to safer and better milk quality, maintaining consumer confidence in dairy products.

INTERIM REPORT

Microbiological Safety and Quality of Reduced Fat Cheddar Cheese

Personnel: Eric A. Johnson, associate professor, John B. Luchansky, assistant professor, Alvaro Quinones, research specialist, Al Degnan, research specialist, Greg Kulman, student , Food Microbiology and Toxicology

Dates: July 1993 – June 1995

Funding: Dairy Management Inc. JNLC94

Objectives

1. Determine viability of *Listeria monocytogenes* and *Salmonella* and toxin formation by *Clostridium botulinum* in reduced fat Cheddar cheese. Compare the behavior of these pathogens in reduced fat Cheddar to conventional full fat Cheddar.

2. Evaluate the efficacy of natural preservatives including bacteriocins (e.g., pediocins, sakacins, enterocins, nisin), monoglycerides, antimicrobial peptides from lactoferrin or conalbumin, and lysozyme for control of L*isteria monocytogenes*, *Salmonella* spp., *Clostridium botulinum*, and spoilage bacteria in reduced fat cheese.

Summary

Due to the higher moisture and unique physical properties of reduced fat cheeses, concerns have been raised regarding their microbiological safety. The overall goal of this project is to evaluate the microbiological safety and quality of reduced fat Cheddar cheese.

The behavior of *Clostridium botulinum*, *Listeria monocytogenes*, and *Salmonella* spp. was compared in reduced fat and full fat cheeses made with milk containing lysozyme (100 ppm), Alta 2341 or Nisin (1,000 arbitrary units [AU]/ml milk). Reduced fat cheese was prepared using low fat (1.3% butterfat) milk with a carbohydrate-based fat substitute (4% w/w Stellar, from A.E. Staley Mfg. Co., Decatur, IL). Milk was pasteurized, tempered to 31˚C, and inoculated with 3-6 strains of each pathogen. After 5 min., antimicrobials were separately added to the milk, and a standard Cheddar cheesemaking schedule was followed. Control vats were inoculated with pathogens, but antimicrobials were not added. After pressing, 100 g portions were vacuum packaged, stored at either 12 or 4˚C, and sampled for botulinal toxin and *Listeria* and *Salmonella* at 0, 1, 7, 14, 21, 30, 60, and 90 d.

In the absence of antimicrobials, counts of *Salmonella* (1-2 $\log_{10}$ cfu/g) and Listeria (2-3 $\log_{10}$ cfu/g) were lower in low fat compared to full fat cheese at both 12 and 4˚C. Using the mouse lethality assay, toxin was detected within 30 d in all cheese treatments at 12˚C, and in control and lysozyme-treated cheese stored at 4˚C. However, in samples treated with Alta and Nisin and stored at 4˚C, toxin was detected at 60 days. *Salmonella* counts were reduced (1-2 $\log_{10}$ cfu/g cheese) after 30 d in low fat and full fat samples treated with Alta (12 or 4˚C), while lysozyme and nisin treatments in comparison to controls did not affect counts. Listeria counts in low- and full-fat cheese treated with lysozyme were similar to control counts at both 12 and 4˚C. However, L*isteria* counts were reduced (1-2 $\log_{10}$ cfu/g cheese) after 30 d in low- and full-fat samples containing Alta (12˚C), while counts were similar to the control in samples stored at 4˚C. *Listeria* counts were reduced (up to 4 $\log_{10}$ cfu/g; 1d) in low fat and full fat samples containing nisin at 12 and 4˚C compared to populations in controls. These data show that the microbial safety of low fat and full fat cheese may be enhanced by the addition of natural antimicrobials to milk.

In conclusion, our data suggest that reduced fat Cheddar cheese may actually be safer from a microbiological perspective than full fat Cheddar cheese. At this time, it appears the effect is due to lower fat, but the carbohydrate replacer may be playing a significant role. We are currently repeating many of the experiments with the pathogens and antimicrobials using the industry practice of

washing the curd. These experiments are ongoing and will be completed in the fall of 1995.

Significance to the dairy industry

Reduced fat cheeses are potentially a large market for the Wisconsin cheese industry. They have considerable appeal to consumers and have desirable nutritional attributes. Some reduced fat cheeses display certain quality defects including off-flavors, a rubbery texture, and a tendency to develop gassiness. Consequently, much of the previous research has been directed to improve the physical properties and flavor. The safety of reduced fat cheeses has not been assessed. These cheeses might support pathogen survival since some contain slightly elevated moisture and pH compared to traditional Cheddar. The results of this project will be valuable in preventing foodborne outbreaks in reduced fat cheese and should also be helpful in extending the shelf-life of this promising class of dairy products.

INTERIM REPORT

Control of *Clostridium botulinum* and Related Sporeformers in Full Fat and Reduced Fat Cheddar Cheese

Personnel: Eric A. Johnson, associate professor, Ann E. Larson, research specialist, Food Microbiology and Toxicology

Dates: July 1994 – June1996

Funding: Dairy Management Inc. JHN95

Objectives

1. Identify clostridia responsible for spoilage of commercial Cheddar cheese obtained from manufacturers in Wisconsin. Determine growth and spoilage by these isolates in full fat and reduced fat Cheddar cheeses.

2. Determine growth and toxin production by *C. botulinum* and toxigenic *C. butyricum* in Cheddar, reduced fat Cheddar (25 and 50% fat reduction), and in commercial reduced fat cheese and cheese sauce.

3. Determine the limiting nutrients and physical conditions that govern growth of *C. botulinum* and *C. sporogenes*. Examine the location of spores within the cheeses by scanning electron microscopy (SEM) and by confocal microscopy.

4. Prepare peptide (cationic) and lipid fractions from the cheese and examine these for inhibitory activity against pathogens: gram-positive (*Clostridium* and *Listeria*) and gram-negative (*Salmonella*, *Campylobacter*, *E. coli*).

Summary

In order to obtain samples of gassy/spoiled cheese, more than 40 letters were kindly sent by the Wisconsin Cheesemakers Association to various commercial cheese factories. These cheeses were examined by standard microbiological methods for the presence of anaerobic sporeformers. Sporeformers were either absent or in very low concentrations in all of the cheeses. Enrichments for anaerobic sporeformers also failed to yield anaerobic sporeformers. We conclude that in Wisconsin and in the cheese varieties examined, spores are not a major cause of gassing. Most of the cheese samples were sent to CDR for examination for the presence of heterofermentative lactic acid bacteria. We hope that commercial cheese makers will continue to send our laboratory gassy/spoiled (e.g. butyric acid/sulfury) cheeses throughout the next year.

During the past year, two botulism outbreaks and one potential recall occurred in commercial cheese sauces due to temperature abuse foodservice operations. We have obtained lots of the commercial products and the causative *C. botulinum* isolates from the FDA. *C. botulinum* grows very well in these low-acid products. We are currently defining the conditions that promote or control growth of *C. botulinum*.

Significance to the dairy industry

Reduced fat cheese has the potential to be a large market for the Wisconsin cheese industry. However, to date much of the research has been directed to improve the physical properties and flavor. We don't know much about protection against *Clostridium botulinum* and related sporeformers in these products. Recent national outbreaks of botulism in cheese sauce and recalls of nonfat commercial cheese are potent warnings that these outbreaks can be detrimental to the Wisconsin cheese industry. The results of this project may help to prevent botulism outbreaks and maintain the safe reputation of Wisconsin cheese. Isolating novel peptide inhibitors from cheese and milk could also lead to pharmaceutical products of value for the dairy industry.

INTERIM REPORT

Application of Biopreservatives as Antilisterial Agents in Queso Fresco and Cheddar Cheese

Personnel: John B. Luchansky, associate professor, Food Microbiology and Toxicology, Mark E. Johnson, senior scientist, CDR, and Nana Y. Farkye, research scientist, California Polytechnic State University

Funding: Dairy Management Inc. LCH 95

Dates: July 1994 – June 1996

Objectives

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

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

3. Evaluate the effect, if any, of biopreservatives on sensory or biochemical qualities of Queso Fresco and Cheddar cheese during storage at refrigeration and abuse temperatures.

Summary

Queso fresco cheese was made according to a traditional process resulting in a final product composed of about 52% moisture, 38% fat, 1.8% NaCl, and pH 6.5. Sample batches of cheese were inoculated with a 4 strain mixture of *Listeria monocytogenes,* stored at refrigeration (4°C) or abuse (12°C) temperature, and sampled on days 0, 3, 7, 14, 21, 30, and 60. Treated batches included Nisaplin™ (1 g/L milk 1 g/g curd) added directly to inoculated milk (100 lbs. or 27 liters) or "salted" onto curd produced from inoculated milk.

In control batches, counts of the pathogen increased about 2.2 $\log_{10}$ cfu/g during 60 days storage at 4°C. At 12°C, counts of *L. monocytogenes* increased 3.5 $\log_{10}$ within 14 days, before declining to about 1.8 $\log_{10}$ cfu/g above initial levels. The decline in inocula populations at 12°C probably resulted from lactic acid production, as related to cheese pH, which declined relatively rapidly from day 14 (pH 5.8) to day 60 (pH 4.8). Cheese pH in batches stored at 4°C remained stable over the 60 days, with final values ranging from pH 5.7-5.9.

In the presence of Nisaplin "salted" onto curd, counts of *L. monocytogenes* declined below detection (10 cfu/g cheese) within 3 days at 4°C but recovered to original inocula levels within 60 days. At 12°C, populations increased 3.0 $\log_{10}$ cfu/g cheese, Cheese pH remained stable in samples stored at 4°C, while at 12°C, pH values declined less dramatically compared to control samples (pH 5.7 at 60 days).

In the presence of Nisaplin added to inoculated milk, counts of the pathogen initially declined 1-2 $\log_{10}$ below control levels (day 0), however populations recovered to at least 3 $\log_{10}$ cfu/g over initial inocula levels within 60 days. Cheese pH remained stable at 4°C (pH 6.5 initial; pH 5.9 final) while at 12°C a sharp pH decline at 14 days coincided with a population decline.

Significance to the dairy industry

Queso fresco is a Mexican-type cheese preferred by a rapidly growing Hispanic population in the U.S.A., especially in the South and West. The high pH (6.0-6.4), minimal starter culture activity, elevated storage temperature, and considerable manipulations during manufacture favor bacterial survival in Queso Fresco. Thus, additional barriers are needed to control the occurrence and proliferation of L. *monocytogenes* in Queso Fresco. Inclusion of biological preservatives (lactic acid bacteria and/or associated bacteriocins) along with good manufacturing practices and sanitation in a multifaceted approach to food safety/quality will

provide an additional measure of safety in Mexican-type cheese varieties.

Publications/Presentations

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

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

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

"Update on biocontrol and subtyping of foodborne pathogens." Scientific Lectureship, Institute of Food Technologists, Honolulu, Hawaii. January 25, 1995.

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

INTERIM REPORT

Verification of Dairy Product Safety System (HACCP=hazard analysis critical points safety system) Incorporated into Cheese Manufacturing

Personnel: J. Russell Bishop, director, CDR, Mark Johnson, senior scientist, CDR, Eric Johnson, assoc. professor, Food Microbiology and Toxicology, Steve Ingham, assist. professor, Dept. of Food Science, Marianne Smukowski, research specialist, CDR, Ann Larson, research specialist, FRI

Dates: July 1994 – June 1996

Funding: Wisconsin Milk Marketing Board UWA9403

Objectives

1) Develop a verification/sampling procedure for implementing HACCP

2) Deliver verification/sampling procedure for Wisconsin's dairy industry by:
   (a) general information shared during a seminar setting
   (b) on-site establishment of procedure

3) Assist other Wisconsin cheese manufacturers to implement HACCP

Summary

The International Dairy Foods Association (IDFA) has provided leadership to the dairy industry by emphasizing the importance of safe dairy products. IDFA's food safety system, based on HACCP, a management tool that stresses prevention by identifying and controlling potential hazards is being applied for HACCP programs in cheese manufacturing. Three cheese plants of various sizes and end-products were sampled quarterly for microbiological analyses. Each "sampling" was conducted two days in a given week and three times per day at the following sites: starter tank, balance tank (raw milk), pasteurized milk, fines, whey, curd (packaging and drain tables), chill water, brine, condensed whey, whey cream, press drippings, and one month cheese.

The following organisms are targeted for detection: *Listeria monocytogenes, Salmonella, Bacillus cereus,* Anaerobic sporeformers, Enterococci, *Staphylococcus Aureus,* Coliforms, Lactobacilli,Yeast/mold.

As of May 1995, 4100 samples were analyzed. We will continue to sample for another year. At this time, we are unable to test for pathogens in the finished product due to Wisconsin Ag 80.56 regulations. This rule requires dairy facilities to report results of microbiological tests conducted on pasteurized, or ready-to-eat dairy products, that confirm the presence of pathogenic organisms in the product. We are working with the WI Dept. of Agriculture to have this reporting of pathogenic organisms waived for research purposes.

Significance to dairy industry

HACCP systems, as part of a total safety system, represent a method for assuring production of safe dairy foods. There exists a great need to verify whether an implemented HACCP system is effective, especially from a microbiological standpoint. This study will establish a microbiological sampling regime that cheese manufacturers can use to verify the effectiveness of their HACCP programs.

FINAL REPORT

Sources and Fate of *Escherichia coli* O157:H7 in Cheese

Personnel: Charles Kaspar, assistant professor, Susan Ansay, research specialist, Food Research Institute

Dates: July 1994 – June 1995

Funding: Dairy Management Inc. KSP95

Objectives

Identify sources, if any, of *E. coli* O157:H7 in cheese-production facilities.

Summary

Escherichia coli O157:H7 has emerged as a significant foodborne pathogen and is estimated to account for 7,000 to 20,000 cases, 150 to 300 deaths, and $230 to $600 million in medical and productivity costs each year in the United States. *E. coli* O157:H7 is a concern of the dairy industry because of the organism's possible presence in raw milk, ability to produce toxin, low infectious dose, transfer by healthy animals and humans, and stability in high-acid foods (i.e. apple cider; dry, fermented sausage; yogurt). Additionally, an *E. coli* O157:H7 outbreak last May in Scotland affected over 100 individuals. This incident, involving pasteurized milk, emphasizes the need for data on the prevalence of this pathogen in dairy processing environments.

Fifteen dairy production/processing plants participated in the survey to determine possible sources of *E. Coli* 0157:H7 in production facilities. Participating plants produce a variety of products, including American style, Hispanic, Mozzarella, Muenster, and Swiss cheeses, butter and dried whey. They provided a variety of samples for testing. For example, samples of air ducts, brine, condensate, curd, drip pans, drains, equipment, floors, foot baths, packaging material, utensils, raw milk and whey, worker's apron/hands, etc., were tested for *E. coli* O157:H7.

Of the 1,104 samples tested, none tested positive for *E. coli* O157:H7. These results suggest that this pathogen is infrequently present, survives poorly, or sanitation practices are effective in eliminating it from cheese manufacturing facilities. However, non-O157:H7 E. *coli* were isolated from some samples suggesting that a contamination pathway is present.

Significance to the dairy industry

Our results are significant because they establish baseline data for the presence of this important foodborne pathogen in cheese production/processing environments. This project demonstrated that *E. coli* O157:H7 is infrequently present in dairy processing environments, most likely because it survives poorly or sanitation practices are effective in eliminating it.

Communication

CHAPTER 5

CDRC

CDR Communications

Personnel: Sarah Quinones, outreach program manager, Karen Paulus, associate editor, Linda Hewitt, program assistant 2, CDR

Funding: Wisconsin Milk Marketing Board

Summary

The communications group provides support for all CDR outreach functions, below is a list of all the primary activity areas for FY95.

Outreach events

Producing Safe Dairy Foods, August 9-10, 1994, Madison, Wisconsin

The program content was designed by Professor Elmer Marth. The instructors were Elmer Marth, Steve Ingham, Rusty Bishop, Eric Johnson, and Charles Kaspar. The course covered the key dairy foods pathogens and their control (staphylococcus, clostridia, mycotoxins, *E. coli*, campylobacteriosis, yersiniosis, listeriosis,brucelosis, tuberculosis, salmonellosis) and injured pathogens, animal drug residues, and HACCP. An in depth reference manual was prepared for the course.

The attendance was excellent with 40 industry and university personnel attending. The evaluation of the course was outstanding with much mention of the quality of the presentations and the manual.

World Dairy Expo, October 5-9, 1994 Madison, Wisconsin

CDR prepared a booth for World Dairy Expo. Three program areas, Cheese as a food ingredient, Milkfat fractionation, and Specialty cheeses were highlighted with handouts, cheese samples and tabletop display items. A large number of producers were reached with the CDR message, and it seemed a valuable effort that would be continued in subsequent years.

Cheese Functionality — Limitations and Potential, November 16, 1994

This industry discussion group was designed to provide industry with an update on CDR research projects focusing on cheese as an ingredient, especially those of Professor Norm Olson and Assoc.Professor, S. Gunasekaran. This event was attended by 15 industry representatives, and several UW staff. The event conveyed the accomplishments of the research and provided industry with the oppurtunity to discuss what questions they felt still needed to be addressed. This event also included one on one meeting sesstions with companies attending; these meetings led to three on-site meetings with compa-

	nies to discuss their cheese as an ingredient issues. A technical fact sheet which placed the research information in a less technical format was prepared. Events such as this one continue to build the critical CDR-industry working relationship which will lead to valued research and technology transfer.
Wisconsin Cheese Industry Conference, March 22-23, 1995, Green Bay, Wisconsin	This conference was co-sponsored with the Wisconsin Cheese Makers Association. CDR held its technical program concurrently with WCMA's program. CDR's program focused on lower fat cheese, cheese quality, cheese ingredients, milkfat fractionation, and cheese safety. This was the first joint conference with WCMA and was a resounding success, with over 800 attendees. Proceedings are being pubished for CDR's technical section.
Institute for Food Technologists (IFT), June 3-5, 1995, Anahiem, California	CDR prepared a booth for IFT. The highlighted programs were cheese as a food ingredient and milkfat fractionation. A profile of CDR's cheese plant research services was also profiled. CDR's annual reports and Pipeline were made available. Numerous informational materials were prepared for the event including an update to the dairy researcher directory, a promotional brochure for the cheese as an ingredient research program, a promotional brochure for the milkfat consortium, and miscellaneous informational sheets. A follow-up evaluation determined that numerous valuable contacts were made and everyone agreed that next year CDR should have a booth at IFT again.

Publications, Videos, Databases

Support for the Master Cheesemaker Program	A promotional brochure and administrative binder was prepared for Jim Path's Wisconsin Master Cheese Maker Program. A press release was also done for the program.
Brochures/press releases	Support was given in developing brochures, program documentation and press releases for the following events: *Making Mexican Cheeses, Wisconsin Processed Cheese Shortcourse, The Art of Making Dutch-Eyed Cheeses*.
Technical fact sheets	New Standards for Sizing Milklines This fact sheet resulted from a scientist exchange (Grasshoff, Germany) hosted by Assoc. Professor Doug Reinemann. Making Quality Reduced-Fat Cheddar (Mark Johnson-Carol Chen research) Milkfat Fractionation — Emerging Value of Milkfat (Kerry Kaylegian program)
Dairy Foods Safety Compendium 1994	Dr. Elmer Marth's review of the literature for dairy foods pathogens is nearly complete, with development of the compen-

dium book now in progress. In FY95 literature searches were done and editing of search results was compiled. The compendium will have sections covering each pathogen with an industry-focused summary preceding the section. The associated database will be designed in FY96 and accessible on the internet.

CDR Annual reports

CDR continues to publish and distribute two types of annual report. The technical annual report summarizes all current research projects and is distributed to funding agencies and scientists in academia, industry and at other dairy research centers around the world.

We also publish a shorter, general annual report which we use to describe CDR and highlight several research projects. We have taken this report to the Dairy Expo, IFT, and the joint meeting with Wisconsin Cheesemakers.

Dairy Pipeline

This has been a reorganizing year for the Dairy Pipeline. We have a new database with the most current list of addresses we can get. Adding "Address Correction Requested" to the return address allowed us to remove addresses with no forwarding address and correct many others. Currently, we have 1124 subscribers.

The Dairy Pipeline also looks different. We added our logo, changed the font, and we also changed the ink colors on the front and back page.

CDR databases

The databases were completed February, 1995 and staff members have been using them. The information in the databases has been printed and now staff are reviewing it for accuracy. For example, Tom Szalkucki is reviewing the Projects database data for errors and a student hourly was hired to take his results and make corrections to the databases. At the same time consideration is being given to how the databases can be improved for ease of use.

Currently changes in how the databases are accessed are being placed on hold as the database specialist (Tom Rowe) is in the process of setting up CDR's World Wide Web server. This server will place CDR on the Internet. This will make the information more accessible to the industry.

Use of the CDR electronic bulletin board (BBS) is up a little over the course of the year. The software was upgraded and the BBS was placed on a dedicated computer to prevent any problems that had occurred when it was also being used as a workstation.

CDR training video and software sales

CDR's training video activity is now self-supporting. One new video was released in FY95: Producing Safe Dairy Foods — this is Elmer Marth's 2-day workshop on video. There are now 14 videos for sale. Two software programs by Brian Gould were added to CDR's Resources brochure, CHEESE-ECO and CHYIELD.

Scientist exchanges

Jim Steele, Associate Professor, Dept. of Food Science
Attended International Dairy Lactic Acid Bacteria Conference, February 19-23, 1996
Palmerston North, New Zealand

Purpose for attending the conference:
To interact with investigators who are conduccting "state of the art" research on the physiology and genetics of lactic acid bacteria and their effect on cheese flavor development. Additionally, the researcher made presentations on his research.

How does this information benefit CDR's research and information transfer programs, and the dairy industry? It is essential that if CDR is to stay internationally competitive in dairy research that it sends its researchers to international meetings in the area of their expertise. This allows CDR investigators to stay at the cutting edge of research developments in their research area. Research in the area of cheese flavor development is a rapidly moving field, as is its allied fields of lactic acid bacteria physiology and genetics. By attending the International Dairy Lactic Acid Bacteria Conference in Palmerston North, New Zealand the scientist learned of the latest advances in research on cheese flavor development and lactic acid bacteria. This information has helped shape the future directions of his research efforts. Additionally, numerous contacts were made with other researchers working in this area; these contacts may in the future, develop into collaborative research efforts.

This information also strengthens the scientist's contributions to Cheese Makers Short Course and the other cheese outreach events that CDR sponsors.

Two trends driving the cheese industry today are the growing interest in low-fat cheeses and the use of cheese as a food ingredient. If the dairy industry is going to be able to exploit these trends, it is imperative that we are able to consistently produce cheeses with high-quality flavor. Unfortunately, traditional cheese flavor is not presently available in reduced-fat cheese varieties and significant variation in the flavor of full-fat varieties exists. Previous researchers have clearly demonstrated that the most important factor in the development of cheese flavor is the microorganisms present in the ripening curd. Therefore, to consistently produce cheese varieties with acceptable flavor characteristics, especially low-fat varieties, we must develop a better understanding of how these microorganisms generate cheese flavor compounds. This trip will benefit the dairy industry by enhancing my research efforts directed at

cheese flavor development. Only by expanding our understanding of how cheese flavor develops will researchers be able to consistently produce cheeses with the high-quality flavor attributes that consumers and food manufacturers demand.

To interact with investigators who are conducting "state of the art" research on the physiology and genetics of lactic acid bacteria and their effort on cheese flavor development. In addition, the researcher gave four presenations on his research program.

Sundaram Gunasekaran, Associate Professor, Dept. of Ag. Engineering
Attended training Rheology Symposium, TA Instruments, Inc.
May 22-24, 1995, 109 Lukens Dr., New Castle, DE 19720

This training applies to the following CDR research:
1. Evaluating microstructure of reduced-fat cheeses by computer image processing
2. Structure-function relationships during melting and cooling of lowfat cheeses

Training objectives:
To gain greater understanding of rheology of foods and polymeric materials with specific emphasis on structure-function relationships.

How will this benefit CDR's research and information transfer programs and the dairy industry?

CDR researchers are currently investigating the structure-function relationships of lowfat cheeses. The overall goal of the project is to improve the textural attributes of lowfat cheese so it is an acceptable alternative for consumers. This will enhance widespread use of low fat cheese as a food ingredient.

Knowledge and understanding gained at the symposium will be applied to design and conduct rheological experiments to fully characterize the functional properties of low fat cheeses. Using appropriate rheological techniques, the effect of composition, cheesemaking procedures, and temperature regimes imposed on cheeses while the food is prepared, etc., will be carefully evaluated. The observed physical (rheological) property changes will be related to structure development and/or breakdown.

Dr John Lucey, National Dairy Products Research Centre, Ireland, March 20-31, 1995

Participant in scientist mentor program and gave presentation at 1995 Cheese Research Conference, Green Bay, WI "Effect of Rotational Grazing on Cheese Yield and Especially Cheese Functionality as it Relates to Cheese as a Food Ingredient"

Comments from mentor meetings:
C. Chen
There was a discussion on projects concerning the influence of

the casein to fat ratio on stirred curd Cheddar cheese yield; effect of coagulum firmness on yield, moisture and quality of 50% reduced fat Cheddar cheese; evaluation of mixer molder temperature and speed on lower fat Mozzarella cheese; and effect of different rennets on cheesemaking, yield and quality. Comments included when varying the casein to fat ratio the actual contents of milk solids also varied contributing to yield differences, gels with very different coagula, e.g., UF gels, are usually cut with specialised equipment to minimise losses and alteration of the cutting conditions are possible for different coagula firmness; comparison of the cheese yield performance with difference rennets should be performed as was done in this CDR study, i.e., standardisation of rennet levels by firmness at cutting not just RCT; it may be possible to use confocal microscopy to examine the properties of curd surfaces.

W. Wendorff
His main area of interest was the seasonality effect on cheese manufacture. The material prepared for the proceeding of the conference, UW pipeline article and the responses to the curd clinic will be useful for discussions with producers. There may also be interest in seasonality of milk production for sheep's milk.

D. Bogenrief
Discussions on the project concerning tailoring cheese for specific physical properties. There has been a huge amount of work done on this project. To assist with the visualising of these results it may be possible to put the data into 3-dimensional graphs, like surface response plots. This would help in examining interactions between a number of variables. On the current trials with trypsin the work done by Donnelly et al. 1984 (details given to Dave) may be useful. If one wishes to independently vary cheese pH, the curd can be placed in an ammonical atmosphere or cabinet, similar to some commercial Camembert producers. Some of the observed changes in textural properties during ripening may be influenced by the water holding properties of cheese. Young cheese retains moisture poorly, i.e. it is easily expressed. However, as the cheese matures and proteolysis proceeds it becomes increasingly difficult to express any moisture.

N. Olson
Many of the comments similar to those of D. Bogenrief. In addition we had a very long discussion of my post doc work with P. Walstra on the properties of acid gels made from Na caseinate with GDL. The objective of this project was to understand why some acid gels exhibited wheying-off. The methods used and the main conclusions were discussed. Gelation temperature had a major effect on all rheological

properties. Confocal microscopy was used to study the microstructure of the gels using the approach of Bremer (1992).

M. Johnson
Discussions ranged from a whole series of topics including types of seasonal cheesemaking problems encountered in Ireland, possible solutions to these problems, use of late-lactation milk in Ireland for the manufacture of low fat cheese, defects that occur in cheese, use of bacteriocins in cheesemaking, effect of acidification and pH on cheese properties, role of CCP in cheese texture, etc. We also visited a local cheese plant that was having problems in making stirred curd Cheddar.

CDR Publications

APPENDIX 1

Wisconsin Center for Dairy Research Publications 1995

Bhowmik, T.; Steele, JL (UW Madison, Dept. of Food Science). Cloning, Characterization and Insertional Inactivation of the *Lactobacillus helveticus* D(-) lactate dehydrogenase gene. Applied Microbiology Biotech. 1994 Jan; 41: 432-439.

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