wisconsin

center for dairy research

annual report 1999

University of Wisconsin—Madison
1605 Linden Dr.
Madison, WI 53706-1565

608/262-5970
fax 608/262-1578
http://www.cdr.wisc.edu

cdr annual report

Published March 1, 2000, 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 1999. This document was prepared for organizations funding CDR and for fellow dairy researchers. Although it describes projects in progress and interpretations of data gathered to date, it is not a peer-reviewed publication.

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

Contents

Chapter 1, Milkfat

Investigation of baked milkfat flavor development in milkfat ingredients for the bakery and food industries 3

Improvement of functionality, flavor, and stability of butter and milkfat fractions 9

Use of immobilized lipases to prepare dairy products enriched in conjugated linoleic acid (CLA) 10

Using immobilized esterases/lipases to modify the composition of milkfat 15

Determination of caloric bioavailability and apparent lipid digestibility of liquid milkfat fractions 18

Milkfat applications research program 19

Use of butterfat fractions and emulsifiers in dairy-based reduced-fat spreads 21

Rheological and structural properties of dairy-based lipids 24

Effects of milkfat source and composition on crystallization kinetics 29

Chapter 2, Cheese

Intensified flavors in Cheddar cheese and cheese ingredients for enhanced applications in foods 37

Characterization of interactions between ingredients and cheese constituents for improved functionality of fat-free processed cheese 40

Improvement of Cheddar cheese quality through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese 46

Succinate production by *Lactobacillus casei*: pathways responsible and development of strategies to control its accumulation. 48

Glutathione and Cheddar cheese flavor development 50

Growth of nonstarter lactic acid bacteria in reduced fat Cheddar cheese 54

Optimizing the standardization of milk to manufacture 50% reduced fat Cheddar cheese 56

Cheese applications program 61

Extending the cheese net paradigm to include economic evaluation and optimization in cheese manufacture 64

Technology for improving the flavor and consumer acceptability of fat-free Cheddar cheese 65

Whey applications research program 66

Dairy marketing and economics program 68

A multi-country analysis of household food demand: Implications for U.S. food exports (phase I) 71

Development and application of a cheese shred/texture map delineated by cheese rheological, sensory and chemical analysis 76

CDR specialty cheese applications program 77

Chapter 2, Section 2 Cheese Safety

Microbiological safety of reduced fat and fat free pasteurized process cheese products 81

Safety/quality applications program 83

Chapter 3, Fluid Milk

Identification and characterization of components of the proteolytic enzyme system of Lactobacillus helveticus which affect bioactive peptide accumulation 87

Application of milk powders in milk chocolate, butter and butter spreads 88

Growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder 89

Reports unavailable at publication date:

Cheese

Minimizing the watering-off of unripened lower fat and no fat Mozzarella cheese
Carol M. Chen

Pizza cheese II: Shelf-life evaluation and tailor manufacturing of pizza cheese
Carol M. Chen

Fractionation of κ-casein glycomacropeptide from whey for nutraceutical uses: scale up of the ion exchange membrane technology
Mark. R. Etzel

Improved quality of shredded cheese—antimycotics, oxygen scavengers and modified atmosphere packaging
Joseph E. Marcy

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

Investigating reasons for hardening of reduced-fat Cheddar cheese during heating
S. Gunasekaran

Effect of Water Distribution on Physical Properties of Pizza cheese and LMPS Mozzarella cheese during early stages of Maturation and Freezing and Thawing
Sundaram Gunasekaran

Large Amplitude Nonlinear Viscoelastic Behavior of Mozzarella Cheese During Twin-Screw Extrusion
Sundaram Gunasekaran

"Whey Refinery" for producing proteins for beverages and nutraceuticals.
Mark Etzel

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

CDR staff

J. Russell Bishop, director

Administration

Tom Szalkucki, administrative coordinator
Curtis Blevins
Joe Bissonnette
Carmen Huston
Lisa Lokken
Jackie Utter
Sandra Sekel

Applications Staff

Carol Chen, cheese applications coordinator
Kim Burrington, whey applications coordinator
Brian Gould, marketing and econ. coordinator
John Jaeggi, cheese applications coordinator
Kerry Kaylegian, milkfat apps coordinator
Jim Path, specialty cheese coordinator
Juan Romero, analytical coordinator
Marianne Smukowski, safety & qual.coordinator

Research Staff

Gene Barmore
Amy Dikkeboom
Rani Govindasamy-Lucey
Bill Hoesly
Kristen Houck
Mark Johnson
Karen Smith
William Tricomi
Matt Zimbric

Communications Staff

Mary Thompson, coordinator
Joanne Gauthier
Tim Hogensen
Karen Paulus

Program Area Coordinators

Cheese– Robert Lindsay, Dept. of Food Science, University of WI-Madison
Milkfat–Rich Hartel, Dept. of Food Science, University of WI-Madison
Whey–Mark Etzel, Dept. of Food Science, University of WI-Madison
Quality and Safety–Eric Johnson, Food Research Institute, University of WI-Madison

CDR's Cheese Industry Team

Alto Dairy
Beatrice Cheese
Chris Hansen Labs
Dairy Management Inc(DMI)
Dean Foods Technical Ctr
Foremost Farms
Gist-brocades Dairy Ingredient Group
Grande Cheese
Hilmar Cheese
International Flavors and Fragrances (IFF)
Kraft Foods Technology Ctr
Land O' Lakes Inc
Plymouth Cheese/DFA
Rhodia Inc
Saputo Cheese
Schreiber Foods Inc
SKW Biosystems Inc
Wisconsin Milk Marketing Board (WMMB)

CDR Directory

Barmore, Gene	(608)262-5798	barmore@cdr.wisc.edu
Bishop, Rusty	(608)265-3696	jrbishop@cdr.wisc.edu
Blevins, Curtis	(608)265-6194	blevins@cdr.wisc.edu
Burrington, KJ	(608)265-9297	burrington@cdr.wisc.edu
Chen, Carol	(608)262-3268	cchen@cdr.wisc.edu
Dikkeboom, Amy	(608)265-2271	ald@cdr.wisc.edu
Gauthier, Joanne	(608)263-1874	larson@cdr.wisc.edu
Gould, Brian	(608)263-3212	gould@cdr.wisc.edu
Govindasamy-Lucey, Rani	(608)265-5447	rani@cdr.wisc.edu
Hoesly, Bill	(608)263-3215	hoesly@cdr.wisc.edu
Hogensen, Tim	(608)265-2133	hogensen@cdr.wisc.edu
Houck, Kristen	(608)265-6346	houck@cdr.wisc.edu
Huston, Carmen	(608)262-3416	huston@cdr.wisc.edu
Jaeggi, John	(608)262-2264	jaeggi@cdr.wisc.edu
Johnson, Mark	(608)262-0275	jumbo@cdr.wisc.edu
Kaylegian, Kerry	(608)265-3086	kaylegia@cdr.wisc.edu
Lokken, Lisa	(608)265-9113	lokken@cdr.wisc.edu
Path, Jim	(608)262-2253	jpath@cdr.wisc.edu
Paulus, Karen	(608)262-8015	paulus@cdr.wisc.edu
Romero, Juan	(608)265-9242	romero@cdr.wisc.edu
Sekel, Sandra	(608)262-5970	sekel@cdr.wisc.edu
Smith, Karen	(608)265-9605	smith@cdr.wisc.edu
Smukowski, Marianne	(608)265-6346	msmuk@cdr.wisc.edu
Szalkucki, Tom	(608)262-9020	tszal@cdr.wisc.edu
Thompson, Mary	(608)262-2217	thompson@cdr.wisc.edu
Tricomi, Bill	(608)262-1534	btricomi@cdr.wisc.edu
Utter, Jackie	(608)265-2117	utter@cdr.wisc.edu
Zimbric, Matt	(608)265-2271	zimbric@cdr.wisc.edu

Our Mission Statement

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

chapter 1

Milkfat

Investigation of baked milkfat flavor development in milkfat ingredients for the bakery and food industries 3

Improvement of functionality, flavor, and stability of butter and milkfat fractions 9

Use of immobilized lipases to prepare dairy products enriched in conjugated linoleic acid (CLA) 10

Using immobilized esterases/lipases to modify the composition of milkfat 15

Determination of caloric bioavailability and apparent lipid digestibility of liquid milkfat fractions 18

Milkfat applications research program 19

Use of butterfat fractions and emulsifiers in dairy-based reduced-fat spreads 21

Rheological and structural properties of dairy-based lipids 24

Effects of milkfat source and composition on crystallization kinetics 29

FINAL 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

Funding
Wisconsin Milk Marketing Board

Dates
February 1993–January 1995
(Extended to 6/30/96)

Objectives

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

2. To establish a model baking system for the assessment of baked milkfat 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 milkfat based ingredients which provide enhanced baked milkfat flavor (UW and NZDRI).

Summary

Establishment of Sensory Analysis Procedures
The NZDRI team established a sensory panel and appropriate guidelines for descriptive sensory analysis procedures for evaluating baked-milkfat flavors in baked products. Using an ADL-type descriptor profile generation technique, the NZDRI team identified aroma and flavor characteristics of butter shortbreads (called butter cookies in North America) which they felt their trained panelists could describe baked butter flavors using 9-point intensity scales. The NZ sensory descriptor terms and their technical definitions are listed in Table 1.

Concurrently, the UW-Madison team used experienced panelists to evaluate butter cookies (shortbreads), and found that panelists had difficulty in meaningfully separating the flavors into the flavor categories proposed by the NZDRI team. Thus, at the UW, a ballot was adapted which permitted a focus on the primary key flavor attributes of baked-butter flavor in butter cookies.

The UW primary scale for characterizing the distinct flavor of baked butter as selected at "baked butter flavor intensity" (7-point scale) and milkiness, a term that was ultimately returned to the baked butter flavor intensity. Because subtle flavors with less desirable connotations (animal, cowy, etc.) were encountered in evaluations of

Table 1. Aroma and flavor characteristics of butter shortbreads

Buttery	cooked butter, melted butter, overcooked butter, and butterscotch.
Oxidized	rancid butter, dripping, and plasticky; stale.
Vegetable oil	food cooked in oil, salad oil, margarine, beany, and oily.
Sweetness	sweetness provided by sucrose.
Saltiness	saltiness provided by sodium chloride.
Cereal/Wheatin	bran, stale bran, floury, uncooked flour, oat cereal, husky, porridge.
Browned flour	flavor of flour on bottom of scones, browned flour from greasing a baking dish.
Caramel/Butterscotch	caramelized sweetened condensed milk.
Milk Solids	condensed milk, milk powder, and creamy.
Cultured	cream cheese, sour cream, and cheesecake.

concentrated butter flavor ingredients, a general off-flavor intensity scale was incorporated routinely. For broad scale sampling from unguided experiments, it was found that use of the broader range of terms assigned at NZDRI gave more complete information. Thus, standardized procedures were developed and adopted.

Establishment of a model baking system for baked butter flavors

The establishment of a standardized, model baking system was pursued simultaneously with the development of descriptive sensory analysis protocols, and the formulas evaluated by NZDRI served as the basis for selection of the model shortbread baking systems at NZDRI and UW-Madison. This was a simple standard, shortbread (butter cookie) formulation consisting of the following:

Ingredients	% of Formula NZDRI	% of Formula UW-Madison
Butter	36.1	36
Sugar	12.9	14
Salt	1.0	–
Flour	49.9	50

The NZDRI formula (i.e., for very low salt butters) applied directly to US butter gave shortbreads that were too salty because of typical US salting rates of about 2%, and thus omission of added salt brought the saltiness into an appropriate intensity for UW-Madison formulations.

When shortenings or anhydrous milkfat were substituted as part of the experimental formulations, the basic formula was adjusted to accommodate the shifted constituents according to the following:

Ingredients	% of Formula NZDRI	% of Formula UW-Madison
Shortening/Milkfat	29.1	30.0
Skim Milk Powder	0.7	––
Water	5.8	5.0
Sugar	13.0	14.0
Salt	1.0	1.0
Flour	50.0	50.0

Because skim milk powder was found to contain important precursors (UW-Madison–alkyl phenol conjugates) for baked butter, the UW-Madison formulation omitted this ingredient to prevent interference from uncontrolled introduction of these important flavor substances.

Mixing protocols for the cookie dough also affected the characteristics of the cookies, and were standardized. The New Zealand protocol adopted an aerated dough (50-150 rpm) in mixer (to a fixed density) which produced a Danish style shortbread when dispensed with a piping bag. The UW-Madison protocol used a less vigorous mixing (low speed-Hobart) followed by flattening the dough which produced a more dense, disc-shaped butter cookie of the Scottish shortbread-type. Shortbread cookies were baked at 205°C for 16 min, and the length of time was varied to modulate the degree of Maillard browning occurring. Both styles of preparation yielded distinct baked-butter flavors when prepared with regular butter, and were very applicable for standardized experimentation.

Identification of key flavor compounds

Both the NZDRI and the UW carried out flavor chemistry studies on the volatile flavor compounds in heated butter and butter cookies which were isolated by headspace, vacuum steam distillation, carbon dioxide and solvent extractions. Compounds identified by gas chromatography and mass spectrometry included free fatty acids, methyl ketones, γ- and δ-lactones, aldehydes, esters, furanones, pyrazines, and pyrroles, the latter of which are Maillard browning products.

Two other groups around the world also have been researching heated butter flavors (G. Reiniccius, U. of Minnesota, Personal Communication; and P. Schieberle, U. Wuppertal, Personal Communication). Both these groups have used similar isolation methods, but also used aroma-dilution extract analysis to determine the most-potent aroma compounds in flavor isolates. However, unfortunately the method does not address the key aspect of flavor quality (i.e., identifiable flavor characteristics), and the compounds . They have found essentially the same groups of compounds identified at

NZDRI and the UW, and they have indicated several that should contribute to heated butter flavors according to their methods. These include d-decalactone (coconut-like), (E)-2-nonenal (green, fatty), (Z)-2-nonenal (fatty, tallowy), 4,5-epoxy-(E)-2-decenal (metallic), 2-acetyl-1-pyrroline (roasty), (ZZ)-2,4-decadienal (fatty, tallowy), skatole (fecal), (E)-6-dodecenolactone (fruity), 1-octen-3-one (mushroom), 2,5-dimethyl-4-OH-3(2H)-furanone, g-octalactone (peach-coconut), 1-hexen-3-one (ketone), and methional (cooked potato).

At the UW-Madison, in-depth flavor chemistry studies were pursured to investigate the chemical reactions involved in the development of baked butter flavors. Since elevated heat is involved in baking (205°C), the role of heat-accelerated oxidation of butterfat on baked butter flavor was investigated. When variously oxidized (none too distinctly) milkfat was used in butter cookies, the lower levels and less oxidized samples gave blended desirable, browned flavors, but higher levels and more extensively oxidized butterfats resulted in oxidized flavors. Notably, the true baked butter flavor in butter cookies was not intensified by additions of oxidized milkfat.

New literature reports from Germany had indicated that some new volatile compounds had been found in oxidized milkfats, and that these might be important in butter flavors. These reports had indicated that milkfat contained 3-methyl-2,4-nonadione (MND) and bovolide which have potent flavors, and they were derived via oxidation from minor furanoid fatty acids in milkfat. However, isolation of crude fractions containing the furanoid fatty acids from milkfat using an approach involving methanolysis and urea adduct fractionation followed by oxidation did not provide flavor concentrates that intensified butter or baked butter flavors.

Role of alkyl phenols in baked butter flavors

Research at the UW prior to the initiation of this project had revealed that alkyl phenols present in skim milk caused milky flavors, and that they broadly greatly enhanced dairy flavors. These dairy-type flavors were greatly intensified in milk-based systems when free forms were released from bound forms by conjugase enzyme activity. (Lopez and Lindsay, J. Agric. Food Chem. 41:446. 1993.) When concentrations of alkyl phenols were increased sufficiently, their flavor contributions were perceived as cowy, but nevertheless unmistakably characteristic of milk and dairy products, and were suspected as contributors in some manner to heated butter flavors.

Since the general survey approach to the flavor chemistry of baked butter flavors did not yield a viable direction for further intensive research, attention at the UW-Madison turned to investigating the alkyl phenol flavor system. Remarkably, adding alkyl phenols to butter cookies confirmed that they provided the missing note–the true baked butter flavor in butter cookies.

Chemical reactions in the baked butter flavors

The UW research then centered on studying the alkyl phenols that occur in butter, and defining the specific chemical reactions involved in the development of baked butter flavors. The free, active alkyl phenols are high boiling in nature, and are present in dairy products at very low parts per billion (ppb) concentrations. However, higher concentrations are present in bound or metabolically-conjugated forms which can be released by using the conjugases, sulfatase, phosphatase, and glucuronidase. Studies were then carried out using conjugase treatments of butter and churning cream, and these ingredient prototypes were evaluated in butter cookies

The conjugase enzymes that are commercially available are crude enzyme preparations with good specific activity (for example, that from *Helix pomatia)*, but they also contain other enzymes, including some lipase activity which releases free fatty acids. Thus, after specified reactions times, it was necessary to heat the samples to inactivate the enzymes. Because of this need, it was also discovered that applying the heat treatment in the preparation of intensely-flavored butter ingredients gave more full, sweet baked butter flavors than without the heat treatment.

The supporting flavors for the baked butter alkyl phenol flavor notes in the baked butter flavor concentrates were quite familiar, however, and included lactones, methyl ketones and volatile free fatty acids. It is well-established that heating milkfat yields several methyl ketones and lactones from the hydrolysis of hydroxy- and keto-fatty acids present in milkfat. Similarly, using lipolyzed

butter oil ingredients to provide butter-type flavors is a well-established commercial practice.

The collaboration with the NZDRI researchers provided several samples of New Zealand butter, buttermilk, and butter cookies for comparison with U.S. dairy products. These samples were analyzed with available quantitative (semi-quantitative) methods along with selected U.S. products for key baked butter flavor components.

The concentrations of methyl ketones as supporting-flavor compounds for alkyl phenols in baked butter flavor ingredients are missing from untreated U.S. butter, very low in N.Z. butter, but notably elevated in a heated, baked butter flavor ingredient. Similarly, the concentrations of key supporting lactone flavor components are substantially elevated in the baked butter ingredient. Contrary to what was hypothesized, crude conjugase (H. pomatia) did not provide an active lipase, but instead the final heat treatment notably elevated some of the longer-chain free fatty acids, but did not elevate the level of butyric acid. These results are useful in understanding the absence of rancid, butyric acid flavor notes in the intensely-flavored baked butter flavor ingredients. The analytical data illustrate the notable elevation of the alkyl phenols in the enzymically-derived baked butter flavor ingredient compared to the starting anhydrous milkfat employed in its manufacture.

Analysis methods for alkyl phenols

The initial methods that were available for analysis of alkyl phenols were extremely time-consuming, and subject to experimental error because of the nature of the flavor compounds and their conjugate precursors. Basically, the initial methods available were adsorptions, extractions, distillations, high-sensitivity capillary gas chromatography, and SIM mass spectrometry.

The literature indicated that some solvents caused alkyl phenol conjugates to spontaneously hydrolyze. Thus, solvent selection for extractions used in anlaysis was critical because functional levels of flavor-active free alkyl phenols in dairy products and ingredient were extremely low (<20 ppb) while the concentrations of non-flavorful bound alkyl phenols reached levels up to 2,000 to 3,000 ppb. As a result, any incidental hydrolysis would grossly distort the values obtained from subsequent instrumental analysis. A detailed study (Han and Lindsay, 1995. J. Food Science, 60:1100) provided the basic information needed to permit the selection of solvent systems for extracting free alkyl phenols from alkyl phenol flavor concentrates and dairy products. These were avoidance of polar solvents held against acidic aqueous phases, and instead using diethylether at nearly neutral pH along with excess water, saturation with sodium chloride, at ambient temperatures, and short solvent-exposure times.

Similarly, a detailed study was conducted for the development of a rapid high performance liquid chromatography analysis procedure, and the method is described in detail in a chapter in Q. Zeng's thesis (1996). Generally, free alkyl phenols are extracted from the sample, and then are analyzed by fluorescence detection using precolumn derivatization with dansyl chloride. When analysis of total alkyl phenols (free + conjugate-bound) is desired, the extracted conjugates are first hydrolyzed by a combination of enzyme and acid hydrolysis before derivatization. The detection limit of dansylated alkyl phenols was about 0.2 nanogram absolute amount. Calibration curves were linear for dansylated alkyl phenols from the detection limit to about 60 nanograms for phenols and cresols injected, and 80 nanograms for other alkyl phenols injected, and overall the correlation coefficient was 0.98 or higher. The reproducibility of the method was from 5.5 to 18%, and the recovery of alkyl phenols from skim milk ranged from 78-106%.

Our basic approach in this segment of the project was to use all-dairy starting materials except for enzymes and processing chemicals. Additionally, the intensely baked-butter flavor ingredients were structured to assume the form of a high-fat ingredient, such as anhydrous milkfat, recombined butter, or similar product. In these forms they would represent new generation butter ingredients.

Several approaches to the preparation of intensely alkyl phenol-flavor ingredients were used, including conjugase treatment of cream, milk, butter, nonfat milk solids, whey solids, and buttermilk solids. All of these ingredients provide a reservoir of alkyl phenols in the form of conjugates, but the dried products provide much more concentrated sources.

The preferred method for manufacture of intensely baked butter flavor ingredients employs enzyme treating a solution of dairy (approximately 30%) to effect the primary release of free alkyl phenols. This treatment is then followed by combining equal amounts of the enzymically-treated dairy solids solution and anhydrous milkfat, and then heating to effect extraction of freed alkyl phenols into the milkfat.

Alkyl phenols in dairy ingredients

Analysis of dairy ingredients for free and conjugated alkyl phenols revealed that considerable variation occurred between samples from both New Zealand and the U.S. Presumably, this reflects the feeding regime (New Zealand is pastured; U.S. is dry-lot) because alkyl phenols are generally accepted to derive from feed ingredients although the mechanisms are still incompletely understood (R. Lindsay, Unpublished Results; D. Rowan, NZDRI, Personal Communication).

Data for levels of free alkyl phenols in U.S. and N.Z. butters show that N.Z. butter has higher levels of flavorful free alkyl phenols, especially the summer N.Z. butter from pastured cows. This contrast in concentrations of alkyl phenols between U.S. and N.Z. butters is also observed for the reservoir of bound alkyl phenols in butter samples where the p/m-cresol levels are particularly higher than that found in dry-lot fed U.S. butter.

Further analyses of U.S. and N.Z. raw ingredients, including buttermilk powders and whey products used in the preparation of baked butter flavor concentrates, show similar trends of elevated concentrations in N.Z. pastured dairy products. This is especially note- worthy for the bound p/m-cresols in the N.Z. buttermilk powders. On the other hand, the 4(3)-ethylphenols are generally higher in the U.S buttermilk powders.

Influence of baked butter flavor

Intensely alkyl phenol-flavor baked butter concentrates have been prepared into a variety of butter-compatible foods, including butter, butter cookies, pound cakes, butter toffee, and milk chocolates, and their introduction intensified baked butter flavors. Free-choice descriptive sensory paneling was used to evaluate selected dairy or butter-compatible foods.

The difficult to describe “baked butter” flavor found in authentic butter cookies was routinely described as rich, milky, buttery. Generally, N.Z. butter yielded cookies with stronger baked butter flavors than those made with unmodified U.S. butter, and the concentrations of free alkyl phenols typically present in butter cookies in the cookies correlated with the more intense baked butter flavors.

Intensely-flavored baked butter flavor concentrate produced intensification of the milky flavor characteristic of milk chocolate. Similar intensification of the rich dairy-milky flavor attribute in butter toffee confection was seen and the overall acceptability was also significantly improved. The data has shown to date that the alkyl phenol concentrations must be carefully controlled at levels below where they produce slightly cowy or animal-like notes. The natural variablity of alkyl phenol concentrations in dairy ingredients used to prepare baked butter flavor concentrates makes this a very formidable task. To achieve constant flavor, the alkyl phenol profile must be blended to within some as yet undefined parameters. However, the basic contributions of alkyl phenols to baked butter flavors have now been established and shown to produce desirable baked butter flavors under controlled conditions. Further applications work should lead to overcoming the intensity and variability problems with alkyl phenols in flavor concentrates, and commercialization of concentrated baked butter milkfat-based ingredients should quickly follow.

Publications/Presentations

Han, L.-H., and Lindsay, R. C. 1995. Stability of Metabollically Conjugated Precursors of Meat and Milk Flavor Compounds in Various Solvents. Journal of Food Science 60:1100-1103.

Lindsay, R. C. 1992. The Chemistry of Flavours Associated with Milkfat. Presented to the New Zealand Dairy Research Institute Flavour Forum, Palmerston North, NZ, March 3-4, (Abstract).

Lindsay, R. C. 1993, Effects of Feeds on Milk Flavors. Presented at the Technical Workshop Parallels in Dairy Grazing in New Zealand and the Midwest, UW Babcock Institute for International Dairy Research and Development, AgResearch Corporation of New Zealand, and USDA Forage Research Laboratory, Arlington, WI, August 25-28, (Proceedings)

Lindsay, R. C. 1996. Bake-Through Butter Flavor. Presented at the Wisconsin Center for Dairy Research Open House Program, University of Wisconsin-Madison, March, Madison (Abstract).

Lindsay, R. C. 1996. Butter Flavor Technologies and Applications. Presented at the Milkfat Technology Forum '96, April 23, 1996, Madison; Also in: Proceedings of the Milkfat Forum '96, National Milkfat Program Consortium, Madison, pages 59-63, (Text).

Lindsay, R. C. 1996 Milkfat Flavor. Presented at the "Milkfat as a Food Ingredient Short Course, Center for Dairy Research, University of Wisconsin-Madison, October 22-23, Madison, (Slides & Course Information).

INTERIM REPORT

Improvement of functionality, flavor, and stability of butter and milkfat fractions

Personnel

R. C. Lindsay, professor, Ann Han, graduate student, and Sungjoon Jang, graduate student, Department of Food Science, University of Wisconsin-Madison

Funding

Dairy Management Inc.

Dates

July, 1997–December, 1999 (Extended to June, 2000)

Objectives

1. To develop an expanded understanding of the flavor properties of alkylphenols in butter and milkfat fractions to enhance the functionality of butter and milkfat fractions in food ingredient applications.

2. To develop information on the biochemical origin of alkylphenols in milk and butter through studies of alkylphenol precursors and their formation in milk.

3. To investigate the oxidative stability of milkfat fractions, and determine the effectiveness of antioxidant strategies for extending the stability and flavor quality of milkfat fractions and recombined butters.

Summary

Temperature-gradient milkfat fractionation produces a range of ingredient milkfats that exhibit enhanced physical properties that are applicable for specific applications, including cold-spreadable butter and more temperature-resistant pastry butters. Because of the inherent nature of temperature-gradient milkfat fractionation, soluble functional components of butter, including flavors, remain in the melt as crystalline fractions are harvested. Thus, higher melting fractions are characterized by low flavor intensities while lower melting fractions contain a disproportionate amount of the initial butter flavor.

In order to compensate and enhance the flavors in the higher melting fractions, the functionalities of a variety of butter-derived flavor components were investigated, including lactones, methylketones and short-chain fatty acids, for their ability to interact with alkylphenol flavors. From these studies, the role of alkylphenols in enhancing accurate sweet cream butter flavors was reinforced, and research has been centered on developing a further understanding of the flavor contributions of alkylphenols to butter flavors.

The flavor properties of naturally-occurring alkylphenols (9 isomers) have been determined in a variety of media, including water, butteroil, and salt and sugar solutions. At low concentrations (0.1 to 10 parts per billion), the phenolic flavors assocated with neat or pure compounds are not detectable, but instead a key butter flavor-enhancing effect is provided. These alkylphenols intensify butter flavors when present at appropriate concentrations through an apparently previously unrecognized mechanism.

This flavor effect is distinctly different from the umami flavor of monosodium glutamate, but the newly recognized flavor effect potentiates umami sensations. Combinations of alkylphenols and other milkfat flavor compounds provide enhanced flavors to milk chocolate, baked goods, and butter ingredients. Research is continuing on the flavor effects of the alkylphenols when introduced into the foods listed above.

Studies employing controlled feeding of cow and sheep ruminant models have been used to examine the importance of individual feed constituents upon the biochemical formation of alkylphenols in milk. Results have shown that the type of diet is influential in determining the specific alkylphenols that occur naturally in milk, and therefore the flavor functionality of butter depends on the feed. High forage or pasture diets contribute much lower levels of the key flavor functional alkylphenol isomers than certain concentrate diets. Therefore, an opportunity to enhance specifically enhance butter flavor intensity and functionality is provided through concentrate selection.

FINAL REPORT

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

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; Prima Sehanputri, research assistant, Colin Crowley, research assistant, Jose Arcos, postdoctoral fellow, Kurt Keough, undergraduate, Carlos Torres, postdoctoral fellow, Department of Chemical Engineering

Funding

Dairy Management Inc.

Dates

July 1996–December 1999

Objectives

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

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

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

Summary

Triacylglycerols containing conjugated linoleic acid residues have properties that make these materials appropriate for use in nutraceuticals as anti-oxidants, anti-atherogenic and anti-carcinogenic agents. These attributes make CLA very attractive for inclusion in the human diet, especially since the consumption level necessary to achieve efficacy is anticipated to be only *ca*. 3.5 grams per day for a 70 kilogram person. A particularly attractive route for incorporation of CLA in the human diet involves modification of conventional dairy spreads (or cheese) by either replacement of some of the fatty acid residues naturally present in the triglycerides which constitute milkfat or by addition of synthetic glycerides containing CLA residues to butter, butterine, and other dairy products which are rich in milkfat. Studies pursuing the avenues established by the proof-of-concept work reported in earlier progress reports confirmed the technical viability of several routes proposed for incorporation of CLA residues in triacylglycerols.

Our efforts in the past year have focused on two primary activities:
1. Studies of the kinetics of the reactions of CLA with glycerol and butterfat in the presence of immobilized lipases in order to determine the reaction conditions and types of reactors that will be most useful for commercial implementation of this technology. We have persuasively demon-

strated the technical feasibility of both routes for obtaining glycerides that can be incorporated in any dairy product that contains milkfat. This work will continue past the expiration of the CDR grant. Publications in archival journals and presentations at professional society meetings are being used to disseminate the results that we have obtained to date. It is important to note that substantially higher levels (by a factor of 10 to 100) of CLA residues in milkfat acylglycerols can be obtained by either of these immobilized enzyme routes than can be achieved by modification of the diets of dairy cows.

2. Studies of the production of CLA from linoleic acid using a bioconversion process involving *Lactobacillus ruteri*. To date we have demonstrated that we can accomplish this isomerization reaction in a manner which gives significant improvements in the yield of the desired biologically active cis-9, trans-11 isomer of CLA. Work in this area is continuing with a view towards obtaining further improvements in the yield of the bioactive isomer. This phase of the research will be followed by studies (with concomitant economic implications) involving immobilized cells. In addition, we have fabricated two chemostats that can be operated either independently or in a series flow configuration. These units have been employed in studies of the kinetics of cell growth and CLA production.

The work on producing acylglycerols containing CLA residues has been carried out primarily by Dr. Hugo S. Garcia (a visiting faculty member) and Jose Arcos (a postdoctoral fellow) and undergraduate students working with these individuals. Dr. Garcia's work focused on acidolysis reactions of conjugated linoleic acid with butteroil. Dr. Arcos concentrated on the direct synthesis of acylglycerols from CLA and glycerol.

The work by Dr. Garcia has led to several papers and presentations at professional society meetings that clearly demonstrate the technical feasibility of using immobilized lipases to catalyze acidolysis reactions of CLA and milkfat. These reactions, as well as other forms of transesterification reactions, serve to substitute a beneficial fatty acid residue for some of the fatty acid residues present in the native triacylglycerols that constitute milkfat, thereby producing a product with nontraditional health benefits.

An important result of this aspect of our research has been experimental validation of the working hypothesis that one can employ immobilized lipases to produce fats and oils which are substantially enriched in conjugated linoleic acid residues. For example, we have employed a *Candida antarctica* lipase (Novozym 435) in a substrates-only medium to increase the conjugated linoleic acid content of milkfat acylglycerols from the native value of 0.6 to 15 g/100 g fat. While we have not conducted experiments to assess the functional and sensory attributes of dairy spreads and other traditional products obtained from these modified milkfats, we believe that there would not be insurmountable problems in this regard, because the large majority of said products consists of traditional milkfat.

In an investigation of the direct synthesis of glycerides from glycerol and CLA, Dr. Arcos determined rates and product distributions for the consecutive esterification reactions of conjugated linoleic acid (CLA) with glycerol in the presence of an immobilized form of a *Mucor miehei* lipase (Lipozyme IM, kindly provided by Novo Nordisk). In a solvent-free environment (a substrates only medium), both rates and product distributions are affected by the ratio of reactants, temperature, and hydration level. Incorporation of up to 95 % of the original CLA into the product acylglycerols occurred at 50°C. Typical data indicating the observed product distributions are shown in Figure 1. (next page)

Inspection of Figure 1 reveals that the rates of esterification of CLA were comparable in magnitude, but increased slightly with increasing ratio of CLA to glycerol. The most evident disparities are those associated with formation of the triester. When the stoichiometric ratio of CLA to glycerol required for formation of the triacylglycerol is employed, both the original glycerol and the monoester are rapidly consumed. However, the subsequent reaction of the diester to form the triacylglycerol occurs much more slowly because it primarily involves esterification of a secondary hydroxyl group on the 1,3-diester. Because *Mucor miehei* is a 1,3-specific lipase, isomerization of the 1,3-diester to the 1,2- or 2,3- form must occur prior to esterification of the third hydroxyl group. This isomerization is responsible for the low rate of reaction observed after *ca*. 2/3 of the original CLA has reacted.

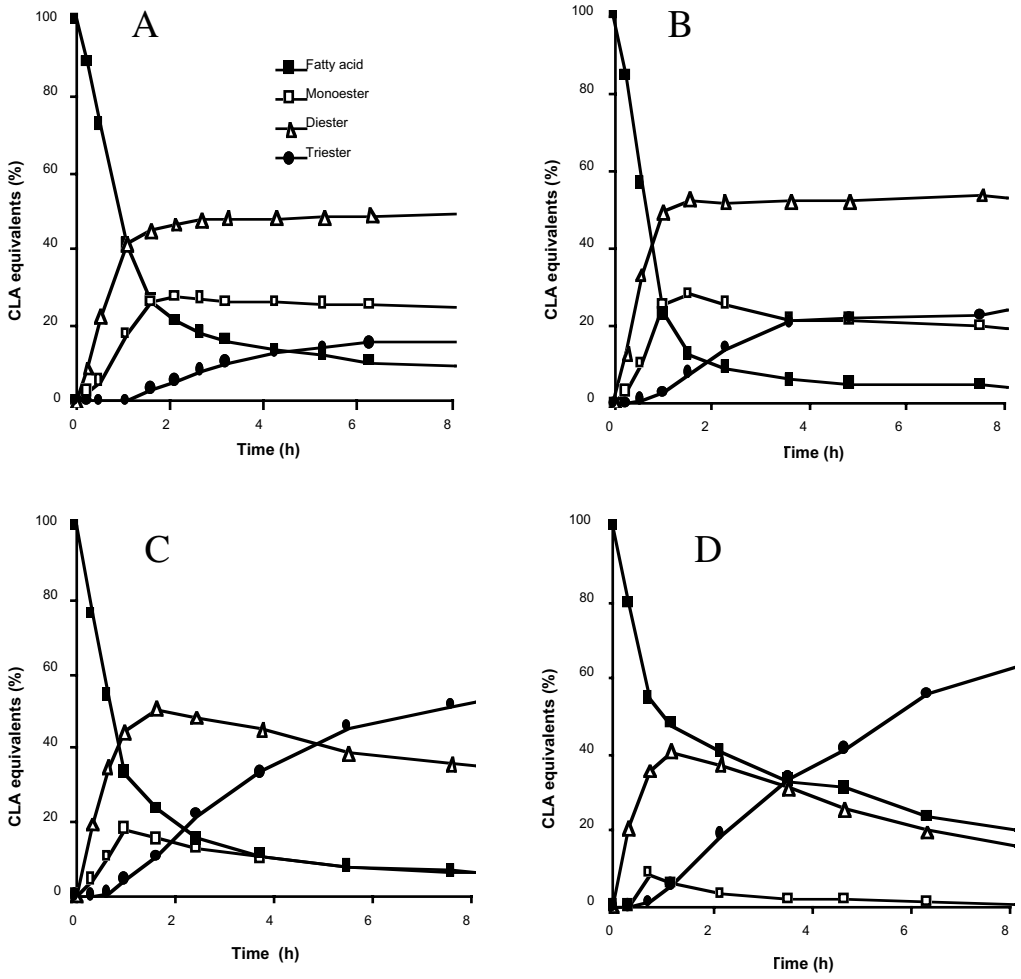


Figure 1. Percentage of CLA incorporated in various acylglycerols and unreacted CLA for different ratios of CLA to glycerol. A) 1/1, B) 1.5/1 C) 2/1, D) 3/1. Conditions: 2000 mg CLA, 300 mg Lipozyme IM, 50 °C, and 800 mg molecular sieves.

The most significant aspect of this portion of our work is that it clearly demonstrates the feasibility of obtaining a wide range of acylglycerol compositions that could be employed in a variety of situations to manufacture nutraceuticals. Via the direct synthesis route, we can obtain products in which all of the ester bonds in acylglycerols involve CLA residues. Via the acidolysis route, we can obtain very substantive degrees of substitution of CLA residues for the residues present in the original milkfat. The extent of substitution depends on the ratio of CLA to milkfat employed in the reaction, but extents as high as 60% should be readily achievable, although this level is not necessarily the level which gives the best process economics. That remains to be determined on the basis of future studies. Our technology permits one to obtain triacylglycerols characterized by a factor of 10 to 100 greater enrichment in CLA residues than can be achieved by modification of the diets of dairy cows. Moreover, by appropriate choice of operating conditions, one can obtain products covering a wide range of CLA contents.

In the light of the growing demand for nutraceuticals and the increasing health aware-

ness of consumers, dairy products containing milkfat enriched with CLA present an intriguing marketing option for the dairy industry. Such products could be able to partially counteract the negative image that milkfat has developed in recent years because of its relatively high proportion of saturated fatty acids, particularly those that have demonstrated hypercholesterolemic effects on humans. In practice, any dairy product that may be formulated using milkfat as an ingredient could be a potential product of the technology on which our research was based; in particular, butter, butterine, butteroil, and reconstituted dairy products prepared from skim milk and anhydrous (modified, CLA-rich) milkfat such as fluid milks, cream, cheese and frozen products represent very attractive marketing options. The modified milkfat products of interest thus have significant dietary implications with respect to not only nutrition, but also with respect to anti-atherogenic and anti-cancer activity. The use of immobilized enzyme technology for the production of tailor-made triacylglycerols offers the intriguing possibility of being able to produce specially designed foods for selected segments of the population, in particular, those individuals who are especially health conscious from a dietary standpoint or who are high risk candidates for cancer, atherosclerosis, hypertension, or other health problems. These products represent a very significant long-term marketing opportunity for the dairy industry.

Publications/presentations

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

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

"Enrichment of Butteroil in Conjugated Linoleic Acid Residues in a Continuous Flow Reactor Containing an Immobilized Lipase." By H. S. Garcia, K. J. Keough, J. A. Arcos, and C. G. Hill, Jr., paper presented at the 1999 annual meeting of the Institute of Food Technologists.

"Lipase-Catalyzed Interesterification (Acidolysis) of Corn Oil and Conjugated Linoleic Acid in an Organic Solvent," by C.E. Martinez, J.C. Vinay, R. Brieva, C.G. Hill, Jr., and H.S. Garcia, paper presented at the 1999 annual meeting of the Institute of Food Technologists.

"Immobilized Lipase-Mediated Acidolysis of Butteroil with Conjugated Linoleic Acid: Batch Reactor and Packed Bed Reactor Studies," by H.S. Garcia, K.J. Keough, J.A. Arcos, and C.G. Hill, Jr., poster presented at Biotrans '99, the Fourth International Symposium on Biocatalysis and Biotransformations.

"Rapid Solvent-Free Esterification of Conjugated Linoleic Acid and Glycerol in a Packed-Bed Reactor Containing an Immobilized Lipase," by J.A. Arcos and C.G. Hill, Jr., 12th International Congress on Catalysis.

"Continuous Interesterification of Butteroil and Conjugated Linoleic Acid in a Tubular Reactor Packed with an Immobilized Lipase," by H.S. Garcia, K.J. Keough, J.A. Arcos, and C.G. Hill, Jr., Biotechnology Techniques, 13, 369-373 (1999).

"Lipase-Catalyzed Interesterification (Acidolysis) of Corn Oil and Conjugated Linoleic Acid in Organic Solvents," by C.E. Martinez, J.C. Vinay, R. Brieva, C.G. Hill, Jr., and H.S. Garcia, Food Biotechnology, 13, 183-193.

"Biotechnology for the Production of Nutraceuticals Enriched in Conjugated Linoleic Acid: 1. Uniresponse Kinetics of the Hydrolysis of Corn Oil by a *Pseudomanas sp* Lipase Immobilized in a Hollow Fiber Reactor," by P. S. Sehanputri and C. G. Hill, Jr., Biotechnology and Bioengineering, 65, 568-579.

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

"Continuous Enzymatic Esterification of Glycerol with (Poly)unsaturated Fatty Acids in a Packed Bed Reactor," by J.A. Arcos, H. S. Garcia, and C.G. Hill, Jr., paper accepted for publication in Biotechnology and Bioengineering.

"Interesterification (Acidolysis) of Butterfat with Conjugated Linoleic Acid in a Batch Reactor," by H. S. Garcia, K. J. Keough, J. A. Arcos and C. G. Hill, Jr., accepted for publication in the Journal of Dairy Science.

Papers Submitted for Publication

"Immobilized Lipase-Mediated Acidolysis of Butteroil with Conjugated Linoleic Acid: Batch Reactor and Packed Bed Reactor Studies," by H.S. Garcia, K.J. Keough, J.A. Arcos, and C.G. Hill, Jr., submitted for publication in the Journal of Molecular Catalysis: B. Enzymatic.

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

."Increased Production of Conjugated Linoleic Acid by Modification of a Reaction Medium Containing Free *Lactobacillus ruteri* Cells, " by C.P. Crowley and C.G. Hill, Jr., submitted for publication in Enzyme and Microbial Technology.

FINAL REPORT

Using immobilized esterases/lipases to modify the composition of milkfat

Personnel

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

Funding

Dairy Management Inc.

Dates

July 1997 – December 1999

Objectives

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

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

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

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

Summary

This project was an extension of an earlier project funded in part through the Center for Dairy Research and in part through a grant from the National Science Foundation. Efforts in our laboratory focused on the use of pregastric esterases derived from the salivary glands of suckling animals (calf, kid goat and lamb) to effect the lipolysis of butteroil. We developed experimental protocols for partial purification of these enzymes, beginning with the crude preparation generously supplied by Systems Bio-Industries, Inc. Subsequent immobilization of these enzymes in a hollow fiber reactor provided a vehicle for obtaining lipolyzed butteroil products with significantly different sensory attributes than either typical commercial products or the effluent from a reactor containing an immobilized *A. niger* lipase. The three pregastric esterases gave products that differed in fatty acid composition from one another.

Two PhD candidates (Louis Lessard and Souheil Ghannouchi), a visiting professor (Hugo S. Garcia), and a visiting scholar (Julio Vinay) conducted the experimental work and developed kinetic models to characterize the performance of the reactor in terms of both the total amount of free fatty acids released (the uniresponse model) and the amounts of the individual free fatty acids present in the effluent stream (the multiresponse model). Funding for these activities was split *ca.* 50-50 between NSF and the University of Wisconsin Center for Dairy Research.

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

were able to approximate the C4-C10 fatty acid content of commercial lipolyzed butteroils while reducing the C12-C18 content by an order of magnitude or more. Varying the pH and temperature at which the reactor operates permits additional manipulation of the chemical composition (and hence the apparent flavor notes) of the product mixture. Our results clearly demonstrate that you can use an immobilized pregastric esterase reactor to tailor the product composition for specific applications by selecting operating conditions and source of the enzyme.

Louis Lessard focused on characterizing the kinetics of the lipolysis of anhydrous milkfat in a hollow fiber reactor containing an immobilized calf pregastric esterase, in particular, the effects of pH on reaction rates and product distribution. Butyric and capric acids (together with other short chain fatty acids) are largely responsible for desirable flavor components of the lipolyzed butteroil product. By contrast, palmitic and oleic acids, the two fatty acids present in highest concentrations in the product, are associated with undesirable flavor notes. At low conversions, the immobilized calf pregastric esterase favors release of butyric acid during hydrolysis. As the conversion increases, the relative rate of release of butyric acid decreases because fewer residues remain to be released.

Souheil Ghannouchi concentrated his efforts on an investigation of the lipolysis of anhydrous butterfat using immobilized forms of pregastric esterases derived from the salivary tissues of lambs and kid goats. Both hollow fiber and batch reactor experiments indicate that the optimum conditions (maximum conversion) correspond to a pH of *ca.* 6.0 (see Figure 1) and a temperature of 40 °C. A mathematical model based on Michaelis-Menten kinetics and a ping pong bi bi mechanism provides an appropriate fit of the data. Mr. Ghannouchi also employed a neural network model to analyze his data.

The results of our research have been described in several publications in the archival literature, presentations at professional society meetings, and the PhD thesis of Souheil Ghannouchi (see below). Additional publications and another PhD thesis will be generated in the future.

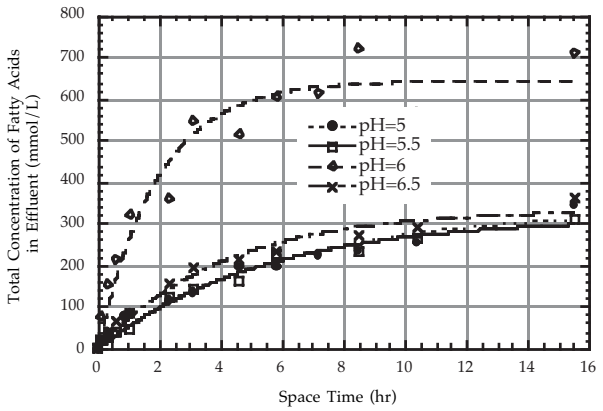


Figure 1. Effluent concentration of total free fatty acids released from butteroil by immobilized lamb lipase as a function of the space time and the pH of the buffer solution. Operating temperature = 40 °C. Solid lines indicate the best statistical fit of the data.

The thrust of this research project addressed that component of the 1996 National Milkfat Plan which was intended to create new uses for milkfat, modified milkfat and/or its components. Specifically, it focused on enzymatic modification of milkfat to produce lipolyzed butteroils and/or diacyl- and monoacyl-glycerides that can be employed as food grade emulsifiers. The various pregastric esterases produce lipolyzed butteroils which could find applications as flavoring agents within the food industry. Each type of enzyme produces a butteroil with somewhat different flavor notes and odors because of differences in the specificities of the different enzymes.

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

Representatives of a producer of flavoring agents met with us on several occasions to explore the commercial potential of this technology.

Presentations/publications

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

Manuscripts submitted
"Effect of pH on the Production of Lipolyzed Butteroil by a Calf Pregastric Esterase Immobilized in a Hollow Fiber Reactor: I. Uniresponse Kinetics" by L. P. Lessard and C.G. Hill, Jr., paper submitted for publication in Biotechnology and Bioengineering.

"Effect of pH on the Production of Lipolyzed Butteroil by a Calf Pregastric Esterase Immobilized in a Hollow Fiber Reactor: II. Multiresponse Kinetics" by L. P. Lessard and C.G. Hill, Jr., paper submitted for publication in Biotechnology and Bioengineering.

"Hydrolysis of Butteroil by Pregastric Esterases Immobilized in a Hollow Fiber Reactor," PhD thesis of Souheil Ghannouchi, University of Wisconsin - Madison (1999).

INTERIM REPORT

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

Personnel

Denise M. Ney, professor, Dept of Nutritional Sciences

Funding

Wisconsin Milk Marketing Board

Dates

July 1996 – December 2000

Objectives

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

Summary

The Center for Dairy Research provided 5 kg of a very low melting milkfat fraction (dropping point < 10° C) and intact anhydrous milkfat in August 1998. The liquid milkfat fraction contains a decreased proportion of 16:0 and 18:0 saturated fatty acids and an increased proportion of 18:1 monosaturated fatty acid compared to the intact milkfat. Both fractions contain approximately 10% of fatty acids with less than or equal to10 carbon atoms. During the last year we have obtained a profile of the triacylglycerol species present in the milkfat fractions using high temperature capillary gas chromatography and conducted an animal feeding study to determine apparent lipid digestibility.

The liquid milkfat fraction contains higher levels of triacylglycerols with unsaturated fatty acids, especially 18:1 and the intact milkfat contains higher levels of triacylglycerols with trisaturates including: tripalmitate, myristate-myristate-palmitate, myristate-palmitate-palmitate and stearate-stearate-myristate. The lower levels of trisaturated triacylglycerols in liquid milkfat compared to intact milkfat may improve the lipid digestibility of the liquid milkfat. An animal feeding study comparing the apparent lipid digestibility of diets containing corn oil, liquid milkfat, intact milkfat and medium chain triacylglycerols was conducted to test this concept. Liquid milkfat showed improved digestibility associated with the lower levels of trisaturated triacylglycerols such that the apparent lipid digestibility of liquid milkfat was not significantly different from corn oil (96%) and significantly improved compared to intact milkfat (90%). These data demonstrate that temperature fractionation of intact milkfat to reduce the proportion of trisaturated triacylglycerols significantly improves the lipid digestibility of milkfat.

APPLICATIONS PROGRAM REPORT

Milkfat applications research program

Personnel

Kerry E. Kaylegian, researcher, Gene Barmore, research specialist, Kathy Nelson, research specialist, Center for Dairy Research

Funding

Wisconsin Milk Marketing Board
Dairy Management, Inc.

Dates

January 1999–December 1999

Objectives

1. To provide technical support on butter and milkfat fractions to the dairy, bakery, confectionery, and food industries:
a. through direct inquiries, consultations, and on-site support
b. through the milkfat fractionation and specialty ingredient pilot plant program
c. through research trials to evaluate the functional properties of milkfat ingredients, and investigate potential new applications for specialty milkfat ingredients

Summary

Technical support questions come through our offices on a regular basis and run the gamut of topics related to butter, milkfat fractions, and other dairy ingredients. We provide answers and technical information to these inquiries from dairy and food manufacturers, university reseachers, trade organizations, the media, and consumers.

The milkfat fractionation and specialty ingredient pilot plant program continues to support the U.S. commercialization efforts of these products. The program provides samples of milkfat fractions that have a wide range of physical and chemical properties. We also use these fractions to make specialty ingredients, such as cold spreadable butters for consumer type markets and high melting pastry butters for the food processors. This past year we evaluated several production schemes to determine which sequence provides good manufacturing characteristics and valuable milkfat fractions. Some processing sequences result in difficulties in the separation of the solid and liquid fractions, and have low yields in the desirable fractions. Other schemes provide fractions with desirable characteristics but may be impractical for commercial use. We are currently evaluating the data from these experiments so that we can make recommendations to the industry for optimal production of new milkfat ingredients.

Cold spreadable butter and pastry butter prototypes were essentially finished this year. We went through multiple processing and evaluation trials of these products to obtain prototypes with the desired performance characteristics. Cold spreadable butter was evaluated for its spreadability directly from the refrigerator, flavor characteristics, and performance in a consumer home-type setting. Consumer-type testing involves cooking (e.g., frying eggs) and baking with the butter to simulate how a consumer might use this product at home in addition to using it as a bread spread. Some of the prototypes were spreadable but burned easily in a frying pan, others did not have good baking characteristics. This information will be used to highlight the benefits of spreadable butter, and also to provide warnings if necessary, such as “do not soften prior to use in baking,” because most recipes call for “softened” butter.

The pastry butter prototype performs very well in puff pastry applications. We achieved significant differences in plasticity and height of the pastries compared with conventionally churned butter. We think that the pastries made with our experimental butter showed better characteristics than pastries made with vegetable margarines that are designed for pastry use. The experimental butter was also evaluated using only 75% of the normal level for puff pastries, and the finished products were very similar to those made with 100% fat. This may allow a manufacture to use less of the specialty pastry butter and still have the characteristics they desire compared with the full fat product.

We began trials on an all-purpose bakery or cookie-type butter. Our first round of prototypes did not perform well, but gave us a good indica-

tion of what direction to take. These butters were based on solely on fractions that did not have other highly valued uses, largely the middle and low melting fractions that melt slightly lower than intact milkfat. It seems that we may not be able to rely only on these fractions, and our next round of prototypes will use a combination of these and other fractions to provide the correct characteristics for the target applications.

Other applications for milkfat fractions that we have begun to investigate, at industry's request, include soups, sauces, and cheese applications. We continue to respond to requests for samples of milkfat fractions, specialty butters, and product evaluations as the program allows.

Publications and Presentations

Kaylegian, K.E. Milkfat Fractions in Ice Cream. Invited presentation at the International Ice Cream Association Technical Council Meeting, Scottsdale, AZ. March, 1999.

Kaylegian, K.E. Properties of Milkfat Fractions. Laboratory demonstration at the UW Applied Dairy Chemistry Short Course, Madison. May 1999.

Kaylegian, K.E. Dairy Ingredients in Chocolate Products. Invited lecture for the American Association of Candy Technologists Chocolate Short Course, Chicago, IL. October, 1999.

Kaylegian, K.E. The Production of Specialty Milk Fat Ingredients. J. Dairy Sci. 82:1433-1439.

Kaylegian, K.E. Contemporary Issues in Milk Fat Technology. Lipid Technol. 11:132-136.

INTERIM REPORT

Use of butterfat fractions and emulsifiers in dairy-based reduced-fat spreads

Personnel

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

Funding

Dairy Management, Inc.

Dates

January 1996–December 2000

Objectives

1. Screen for formulations that yield stable, dairy-based, reduced-fat, water-in-oil emulsions.
2. Optimize formulations and processing protocols for preparing dairy-based, reduced-fat table spreads on a pilot scale.

Summary

The project continues to proceed along two lines of inquiry viewed as critical to success in reaching the objectives. We have almost completed an evaluation of interfacial dynamics at an oil/water interfaces, focusing on issues specific to using milkfat fractions and evaluating the effect of surface-active agents, primarily monoacylglycerols (MAG). The other area involves developing a bench-test method for screening table spread formulations in a manner that is predictive of processing efficacy on a pilot scale Gerstenberg & Agger texturizer.

Interfacial relationships between oil and water phases were evaluated for a liquid milkfat fraction using interfacial tension measurements, and for a solid milkfat fraction using contact angle measurements. Milkfat fractions were obtained from the UW-Madison CDR milkfat fractionation pilot plant, and are classified as 8L (liquid fraction isolated at 8°C) and 21S (solid fraction isolated at 21°C). Preliminary experiments indicated that a solvent extactable or sedimentable endogenous component of the 21S fraction was polar in nature and may be responsible for modulating the temperature-solidity profile (solid fat content, or SFC) of the 21S fraction. An extracted 21S fraction was prepared (E21S) and was found by thin-layer chromatography analysis to be diminished in polar components that constituted <0.1% of the native 21S fraction. Initial interfacial tension measurements between water and milkfat fractions ranged from 18-19 mN/m for the 8L fraction (at 25° and 50°C) to 19-21 mN/m (at 50°C) for a 8L/21S blend (82.5:17.5, w/w, suitable for a dairy spread formulation). These values are less than those (27-28 mN/m) recorded for a canola oil/water system for comparison. This indicates that surface active constitutents are present in the milkfat fractions, and these components may facilitate the stable incorporation of milkfat into table spreads compared to other native oils. In addition, surface tension values declined during the 15 minute analysis period to 13-15 mN/m for the milkfat fractions, implying dynamic and competitive interfacial absorption processes among surface active agents in the milkfat blends (no similar observation was made for the canola oil/water system).

The addition of MAG preparations at 0.25%, 0.75% and 1.5% levels of addition (w/w of oil phase) to the milkfat blends decreased surface tension values to 12-14 mN/m, 8-11 mN/m, and 4-6 mN/m, respectively. A time-dependent decrease in surface tension was again noted, but minimum values for surface tensions were achieved faster when blends contained the E21S (extracted 21S) compared to the native 21S milkfat fraction. This indicates that the removal of the trace component in the E21S fraction may lead to enhanced rates at which water can be dispersed in the milkfat blend during the dynamic process of table spread production. At equivalent levels of addition, MAG prepared from a milkfat fraction (12S fraction, crystallized at 12°C) by an enzyme process developed in this laboratory was as equally effective as two commercial MAG preparations.

Dynamics of interaction between solid milkfat water phases was assessed by contact angle (θ) measurements (the lower the θ measured, the greater the wetting of the lipid surface by an

applied drop of water). For the 8L/21S and 8L/E21S blends, q was 79-83° at 12°C, and no difference was evident between the two blends. For the 21S and E21S fractions, θ ranged from 95° to 107-108° as temperature of measurement was reduced from 35°C to 12°C, again with no difference observed between the fractions. Studies are continuing on the influence on θ of MAG added to these solid lipid systems. When these studies conclude, a set of predictions will be possible in terms of what compositional factors may be responsible for controlling the ease of dispersing water in the milkfat blends during table spread production. These predictions will then be tested using the bench-top (and possible pilot scale) studies that constitute the balance of the project.

The first of two phases of development of a bench-top scale system to evaluate efficacy of preparing table spreads has been completed. A scraped-surface heat exchanger with a thermostat was simulated with a batch processing scale of about 200 g. This series of studies focused on preparing 60% (reduced fat) spreads using the 8L/21S blend (82.5:17.5, w/w). MAG levels (0-3% of the milkfat phase) and the processing parameters of cooling rate, final product temperature (10-16°C) and extent of working were evaluated. Quality of the prepared products was indexed by measurement of emulsion stability (by a centrifugation technique), color/appearance (reflectance colorimetry), morphology (photomicroscopy), and textural analysis (total and peak force required to "spread" the product).

A central finding of this first phase of studies was that a compromise between the degree/rate of cooling and a need to "work" the spreads appears to be necessary to yield high/uniform quality, stable spreads. Although the conditions that afford this in the bench-top apparatus are empirical, an attempt will be made to relate these "near-optimum" conditions (chilling the product with dyanamic agitation from 50°C to 13°C in about a 5 minute time frame) to the scale of the pilot plant. Processing to a greater end-point temperatures (16°C vs. 13°C) led to a great variability in the finished products, and generally losses in product quality. Processing to lower product temperatures (10°C vs. 13°C) and greater process times (greater working) were associated with an apparent collapse of the product structure and losses in product quality/uniformity. A surprising finding was that within the range of processing parameters evaluated, MAG levels had no significant impact on the quality/uniformity of the 60% milkfat spreads. This may indicate that the level of polar/surface active lipids endogenous to the milkfat fractions are sufficient to stabilize the water/oil interface, obviating the need to add exogenous emulsifiers. We anticipate that added MAG may have a greater role in product/process performance as the level of fat in the spread is reduced further (to 40% and 20%).

At a constant heat removal rate during processing, statistically significant relationships were found between 1) processing time and lightness of appearance (positive correlation, based on reflectance "L" values), 2) processing time and both textural force parameters associated with spreadibility (negative correlations), and 3) between the two textural force parameters (positive correlation). When comparing processes at different rates of heat removal to a 13°C final product temperature, statistically significant relationships were found between 1) processing time and lightness of appearance (positive correlation), and 2) the two textural force parameters associated with spreadability (positive correlation). The relationships between the two textural parameters measured for the finished products appeared to be modeled by fairly simple relationships, yielding different models under different processing conditions. We speculate that *within a range* of process conditions that can yield high quality/uniform products, the *specific* processing conditions may be manipulated to provide *differential control* over the various textural attributes of spreadability. This may allow a given spread formulation to be processed in a manner that meets the specific needs (conferred by end-use) of a variety of finished table spread products.

Future work will focus on evaluating formulations of 40% and possibly 20% milkfat-based table spreads, and the effect of hydrocolloids and emulsifiers that are anticipated to be required for products most reduced in fat. Finally, the most (and least) successful formulations based on bench-scale testing will be processed on the pilot scale apparatus, to confirm the ability of the bench-top procedure for predicting processing efficacy of various table spread formulations into finished products of high and uniform quality.

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

FINAL REPORT

Rheological and structural properties of dairy-based lipids

Personnel

RW Hartel, professor, Baomin Liang, assoc. researcher, M. Lidia Herrera, Visiting Scientist, Dept of Food Science

Funding

Wisconsin Milk Marketing Board

Dates

July 1997–June 1999

Objectives

The primary objectives of this project were:
1. To determine the effects of processing conditions (time, temperature) on crystalline structure of mixed lipids of importance to dairy-based spreads.

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

Summary

Mixtures of high-melting (HMF) milkfat fractions (30, 40 and 50%) with low-melting (LMF) fractions or canola oil (CNL) have been crystallized under different processing conditions. The molten fat was cooled to crystallization temperatures (25 to 30°C) at different cooling rates (fast and slow). The fats were allowed to crystallize for several hours at different agitation speeds (50 to 300 RPM). The slurry in this vessel was then cooled to 10°C for 24 hours for further analysis. Images of the crystalline structure of the set product were recorded using Confocal Scanning Laser Microscopy (CSLM). The rheological properties of the product fats were determined using Dynamic Mechanical Analyzer (DMA). The effects of storage time on crystalline microstructure and mechanical properties were also studied.

Each of the processing conditions influenced both crystalline microstructure and mechanical properties for both model systems (HMF in CNL and HMF in LMF). The crystalline microstructure of the semi-solid samples contained dense, primary crystals (formed during agitation at crystallization temperature) surrounded by a matrix of secondary crystals (formed during cooling to 10°C). Rapid cooling of the sample with 30% high-melting milkfat fraction (HMF) resulted in many small primary crystals, as compared to slow cooling, and this led to slightly lower elastic modulus (from DMA). Increasing agitation speed (from 50 to 300 RPM) also resulted in decreasing primary particle size, which again led to lower elastic modulus. Lower crystallization temperature (from 30 to 25°C) also led to lower primary crystal size and lower elastic modulus. Note that even though the primary crystallization temperature was different, the final solid fat content (SFC) for these samples (all 30% HMF) was the same since they were all equilibrated to 10°C. However, increasing the ratio of HMF to low-melting fraction (LMF) caused an increase in SFC and resulted in an increase in elastic modulus. Longer storage times led to increased elastic modulus, as expected. Over time, the secondary crystalline structure appeared to get more dense, which could potentially have led to the higher elastic modulus. Some small differences in behavior were observed for the two systems (HMF in LMF and HMF in CNL). The secondary crystalline structure in the HMF-CNL system was less structured and more diffuse than the secondary crystalline structure of the HMF-LMF system. This suggests that the triacylglycerols (TAG) of the two fats were sufficiently different to promote diffuse crystallization. Differences in mechanical properties due to operating parameters were also smaller for the HMF-CNL system. In addition, the HMF-CNL samples did not undergo significant restructuring during storage so there was no increase in hardness during storage.

The effects of lipid composition and processing conditions on lipid crystalline microstructure have been studied for two model lipid systems (HMF in LMF and HMF in CNL). To prepare the samples for crystallization, mixtures of HMF and either LMF or canola oil were melted at 60°C for one hour to remove any crystal memory. The molten fat was cooled to crystallization temperature, where crystallization is allowed to proceed for several hours in the presence of agitation. The crystal slurry from the crystallizer

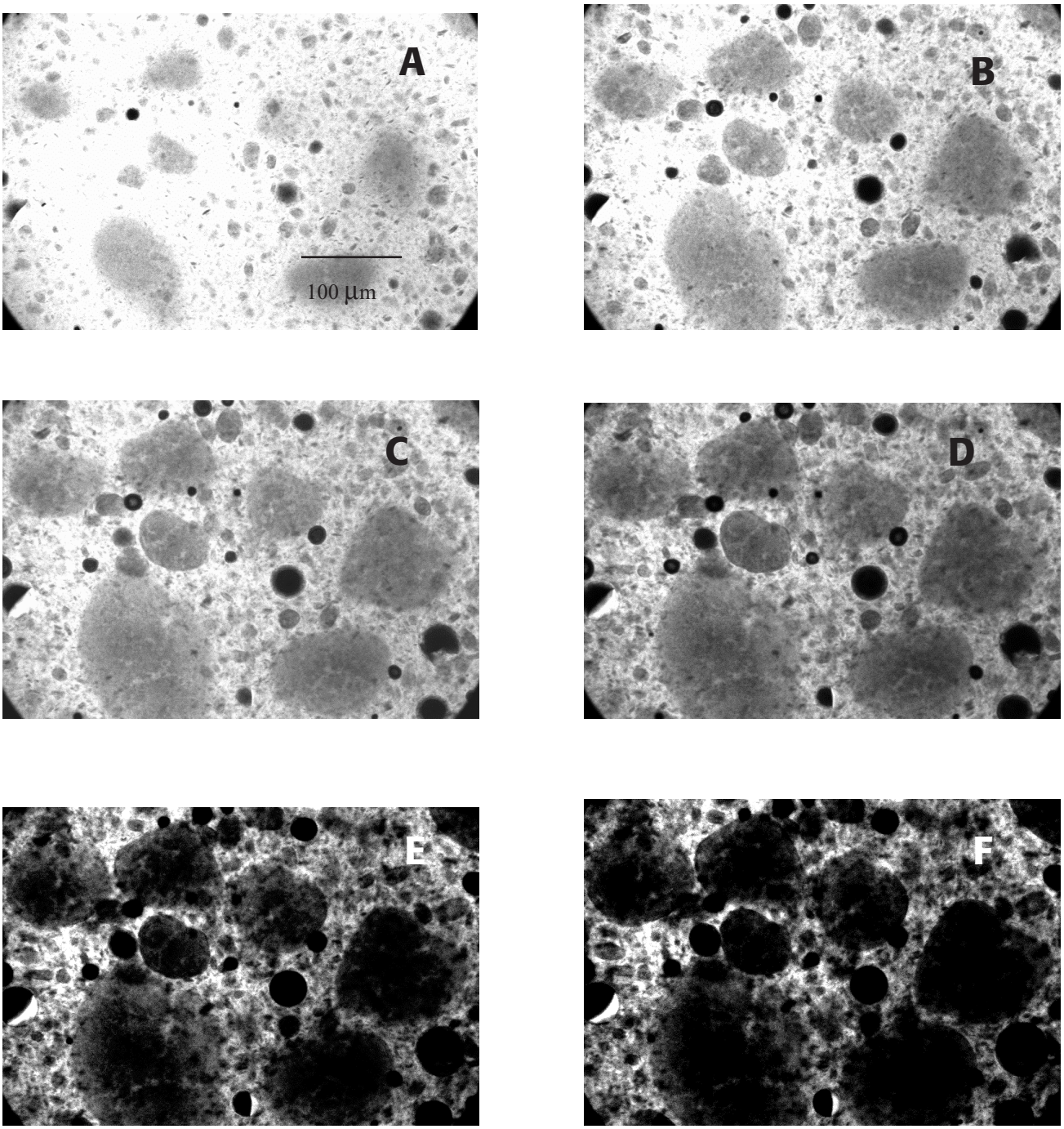


Figure 1. Confocal microscope images of a 50-50% blend of high-melting milk fat fraction (HMF) in low-melting milk fat fraction (LMF) crystallized first at 25°C (agitation rate, 50 RPM; cooling rate, 0.2°C/min) and stored for 24 h at 10°C. Images were taken at 3 µm increments from the surface: a) surface, b) 3 µm, c) 6 µm, d) 9 µm, e) 12 µm, f) 15 µm.

was then allowed to cool quiescently to 10°C and set into a semi-solid product. This process simulates commercial processing of lipids, where processing occurs in two steps, although the conditions are not exactly translatable to commercial conditions.

The variables studied in this experiment included:

- rate of cooling to crystallization temperature (fast, 5.3°C/min, and slow, 0.2°C/min),
- crystallization temperature (25 to 30°C),
- agitation rate (50 to 300 RPM),
- lipid formulation:
  - 30, 40 and 50% high-melting in low-melting milkfat fractions,
  - 30, 40 and 50% high-melting milkfat fraction in canola oil,
- storage time at 10°C (1 day to 3 weeks).

The following analyses were performed on either the slurry or the semi-solid sample.

- solid fat content of slurry (NMR),
- solid fat content of semi-solid product (NMR),
- optical microscopy of slurry crystals,
- confocal microscopy of crystals in semi-solid product,
  A small amount of dye was added to the molten product prior to crystallization to enhance the image from confocal micros copy. Initial experiments were performed to verify that this dye had no impact on crystallization kinetics of the fat systems.
- mechanical properties of semi-solid product (DMA).

The HMF-LMF system

Representative confocal microscope images of the crystalline microstructure in the semi-solid product are shown in Figures 1 and 2 for slow (0.2°C/min) and fast (5.5°C/min) cooling rates, respectively. These images clearly show two crystalline phases. The dense primary crystals were formed at the elevated crystallization temperature (25-30°C) under agitation. The primary crystals were surrounded by a more diffuse secondary crystalline structure, which formed as the slurry cooled under stagnant conditions to molding temperature of 10°C. The interaction between these crystalline structures, and the liquid matrix in which they are contained, gives rise to the characteristic mechanical properties of that semi-solid material. For comparison, the DMA frequency scan for the samples crystallized at different cooling rates is shown in Figure 3. These clearly show that crystalline structure influenced mechanical properties (elastic modulus) even though all of the samples had identical solid fat content at 10°C. Note that elastic modulus (e') represents the solid-like nature of the semi-solid material. Higher elastic modulus generally correlates with harder, more solid-like materials.

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

The HMF-CNL system

Similar results for crystalline microstructure were found when HMF was added to canola oil. The secondary crystalline microstructure seemed to have more distinct characteristics than the diffuse nature found in the HMF-LMF system. This suggests that the milkfat crystallized separately from the canola oil over the entire range of temperatures. One main difference between the HMF-LMF and HMF CNL systems appears to the effect of crystalline microstructure on mechanical properties of the solidified product. In the HMF-CNL system, no significant differences in elastic modulus were found for any of the operating conditions, despite the distinct differences in crystalline microstructure. The only parameter that lead to a difference in elastic modulus was the amount of HMF added to canola oil. In this case, the elastic modulus increased as HMF content increased according to the increase in solid fat content of the product. There were also no changes in elastic modulus in the HMF-CNL system during storage, whereas elastic modulus of the HMF-LMF product increased substantially over the first few weeks of storage.

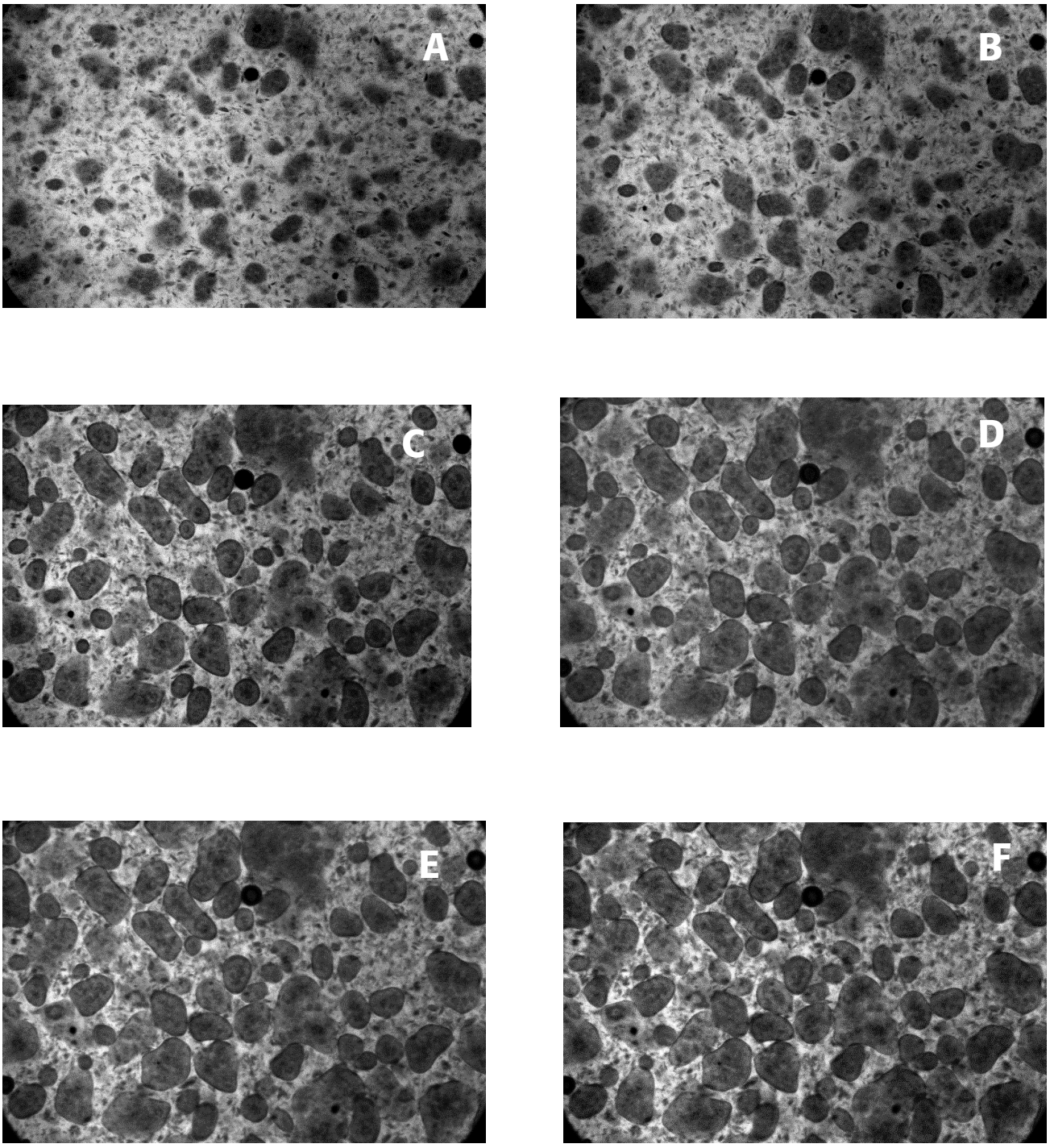


Figure 2. Confocal microscope images of a 50-50% blend of high-melting milk fat fraction (HMF) in low-melting milk fat fraction (LMF) crystallized first at 25°C (agitation rate, 50 RPM; cooling rate, 5.5°C/min) and stored for 24 h at 10°C. Images were taken at 3 μm increments from the surface: a) surface, b) 3 μm, c) 6 μm, d) 9 μm, e) 12 μm, f) 15 μm.

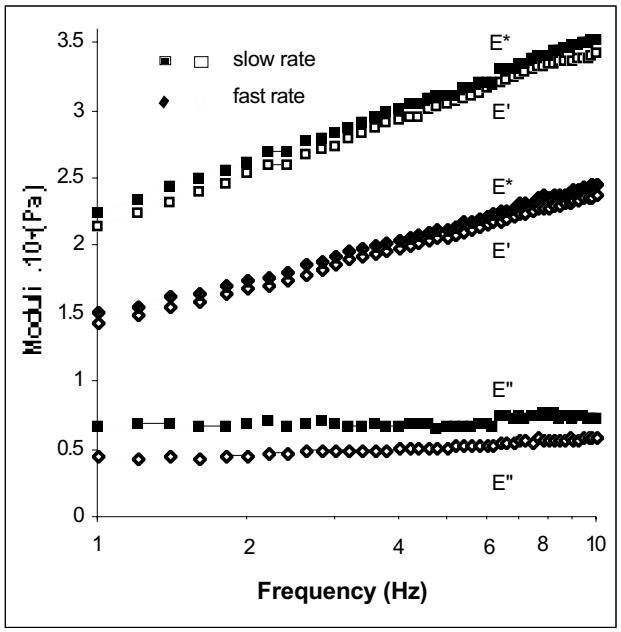


Figure 3. Effect of cooling rate on storage (E'), loss (E") and complex (E*) modulus values measured for a 50-50% mixture of high-melting (HMF) in low-melting (LMF) milk fat fraction initially crystallized at 25°C (50 RPM) and stored for 24 h at 10°C. Frequency scan from 1 to 10 Hz.

This work has led to substantial progress in our understanding of the effects of processing conditions on lipid crystallization kinetics, the lipid crystalline structure that is formed under these conditions and how these structures influence the mechanical properties of the product. However, this project has barely scratched the surface. Much more work is necessary to understand the relationships between lipid crystalline structure and the mechanical properties of lipid-based dairy foods. In addition, further work is necessary to correlate the mechanical properties with the organoleptic (sensory) properties, such as spreadability, of lipid-based dairy products.

Publications and Presentations

Herrera, M.L. and R.W. Hartel, Crystallization of a Model Milkfat System, J. AOCS (accepted).

Herrera, M.L. and R.W. Hartel, Effect of Processing Conditions on Physical Properties of a Milkfat Model System I. Rheology, J. AOCS (accepted).

Herrera, M.L. and R.W. Hartel, Effect of Processing Conditions on Physical Properties of a Milkfat Model System II. Microstructure, J. AOCS (accepted).

Herrera, M.L. and R.W. Hartel, Kinetics of Crystallization of a Model Milkfat System, paper presented at AOCS Conference, Orlando, FL (May, 1999).

Herrera, M.L. and R.W. Hartel, Crystalline Microstructure in a Model Milkfat System, paper presented at AOCS Conference, Orlando, FL (May, 1999).

Hartel, R.W. and B. Liang, Applications of Milkfat Fractions: Interactions With Other Fats, paper presented at International Society of Fat Research, Brighton, UK (Oct, 1999).

Hartel, R.W., Relationships Between Crystalline Microstructure and Mechanical Properties in Lipid Foods, paper presented at Eastern Analytical Science Symposium, Somerset, NJ (Nov., 1999).

FINAL REPORT

Effects of milkfat source and composition on crystallization kinetics

Personnel

RW Hartel, professor, Colleen Kubitz, research associate, Yuping Shi, assoc. researcher, Dept of Food Science

Funding

Dairy Management Inc.

Dates

June 1997– June 1999

Objectives

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

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

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

Summary

Milkfat samples were collected from various sources throughout the year. In particular, AMF produced from fresh cream obtained from the same source (in Minnesota) has been analyzed over the past year. In addition, AMF samples produced from whey cream were obtained from the same supplier. Further, several AMF samples were obtained from German, Irish and New Zealand sources for comparison.

All samples were analyzed for fatty acid profiles (GC analysis), acyl carbon profiles (GC analysis) and minor lipid content (TLC and HPLC analyses). Clear point, Mettler dropping point, solid fat content (SFC) curves and melting profiles (DSC) were also measured for each sample. Crystallization kinetics were measured by cooling the molten samples to 28°C in a temperature-controlled spectrophotometer in an agitated (250 RPM) cuvette. Change in turbidity was used to

Table 1. Milkfat samples used in this study

Sample Identity	Date Received	Source	Identification
Sweet Cream AMF*	January 1998	United States	1
Sweet Cream AMF*	March 1998	United States	2
Whey Cream AMF	March 1998	United States	3
Sweet Cream AMF*	April 1998	United States	4
Sweet Cream AMF*	May 1998	United States	5
Winter AMF	May 1998	Ireland	9
Winter AMF	June 1998	New Zealand	10
Winter AMF	June 1998	Germany	11
Sweet Cream AMF*	June 1998	United States	6
Whey Cream AMF	June 1998	United States	7
Sweet Cream AMF*	August 1998	United States	8
Winter AMF	December 1998	Germany	-
Summer AMF	December 1998	Germany	-
Whey Cream AMF	March 1999	United States	12
Sweet Cream AMF*	March 1999	United States	13
Butter	April 1999	Ireland	14

* Sequence for Sweet cream AMF through 15 months of collection

characterize crystallization rates for each sample. Since the turbidity technique does not give information about nucleation rate, a separate experiment was performed to determine the number of nuclei formed over a specified period of time under controlled conditions. In this procedure, the samples were cooled from 70 to 27.8°C. Before they crystallized, they were agitated for 30 s at 200 RPM to induce nucleation. Samples were then incubated at 30.5°C to allow nuclei to grow without new nuclei forming. After 3.5 h of incubation, a sample was prepared on a custom-built microscope slide and crystals were counted in a sample of known volume. An average nucleation rate was calculated from the number of nuclei counted per unit volume per time of inducing action (30 s).

Somewhat surprisingly, the AMF samples obtained from sweet cream from the same origin throughout the year showed only slight differences in chemical composition with no obvious trends that could be attributed to seasonal fluctuations. Even the whey cream samples showed only minor differences in chemical composition. The samples that stood out as having significantly different composition were the samples from Ireland. These had lower melting points, which were attributed to higher levels of unsaturated fatty acids. The Irish samples also had slightly (3-4%) lower levels of short-chain triacylglycerols (TAG) with acyl carbon number greater than 40 and higher free fatty acid content to go along with the lower melting points than the other samples.

Also somewhat surprisingly, no significant differences in crystallization rate were found among samples when using the turbidity technique, with the exception again of the Irish samples. For all samples except the Irish samples, the induction times and crystallization rates as measured by turbidity change were within standard deviations of the measurement. The Irish samples had significantly longer induction times before onset of crystallization (by turbidity). However, when the crystalline microstructure of all samples was investigated by using confocal microscopy, some differences became apparent. This led to utilization of a more accurate measure of nucleation rate for the milkfat samples and correlation of these nucleation rates against the minor differences in chemical composition (particularly TAG).

Based on nucleation rate data, the samples in Table 1 were ordered from 1 to 14. The relationships between nucleation rate and some indicators of chemical composition are shown in Figure 1. More specific differences in TAG composition are shown in Figure 2. Our conclusions are that there was not a single TAG component that correlated with nucleation rate. However, the sum of differences in composition led to significant differences in the solid to liquid (S/L) ratio, which correlated well with nucleation rate (Figure 2). For the purposes of this report, solid-like TAG were those with acyl carbon number between C46 to C54, excluding the C54 trans contribution. The liquid-like TAGs included those with acyl carbon number less than C40 plus the C54 cis contribution. As S/L increased, the nucleation rate increased dramatically. Samples with low S/L, such as the Irish samples, had the lowest nucleation rate, whereas samples with higher S/L, primarily the whey cream samples, had much higher nucleation rates.

It has been well documented that the composition of anhydrous milkfat (AMF) can vary based on source and processing conditions, and that these differences can have considerable effect on crystallization kinetics and fractionation efficiency. However, our seasonal analysis showed that there were no distinct trends in chemical composition among AMF samples made from sweet cream obtained from the same location over a 15 month period. Also, only slight differences were found in crystallization kinetics and these could not be correlated with seasonal fluctuations. Significant differences in crystallization kinetics were found for AMF samples obtained from whey creams and for some of the international samples. There are many compositional factors that influence crystallization kinetics, although no clear trends have been delineated in the past. These results show that the ratio of TAGs with solid-like characteristics to those with liquid-like characteristics provides a good indicator of crystallization kinetics of different milkfat samples.

Fig. 1 Major TAG components contained in AMFs from different sources

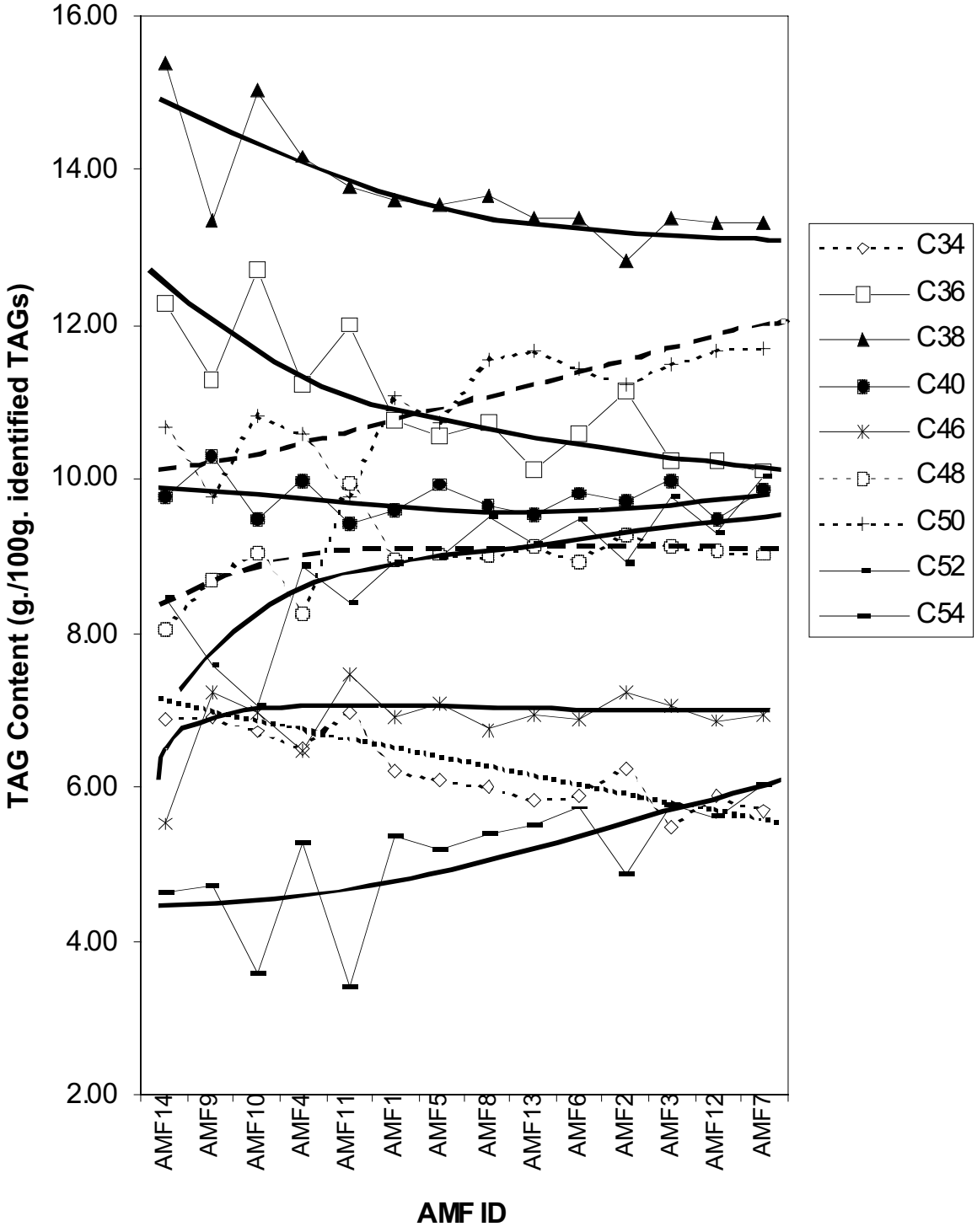


Fig. 2 Content of TAGs and nucleation rate for different AMFs

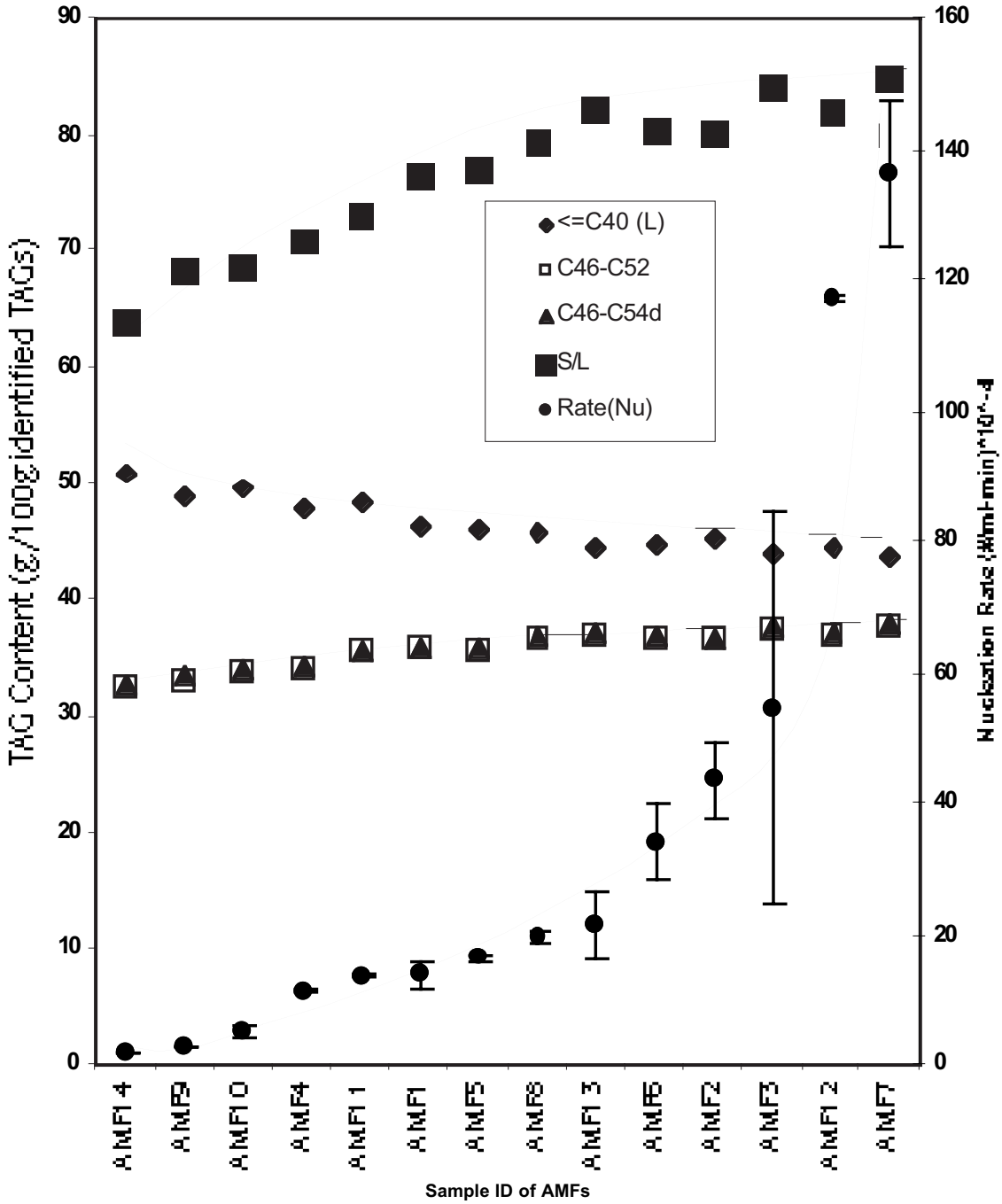
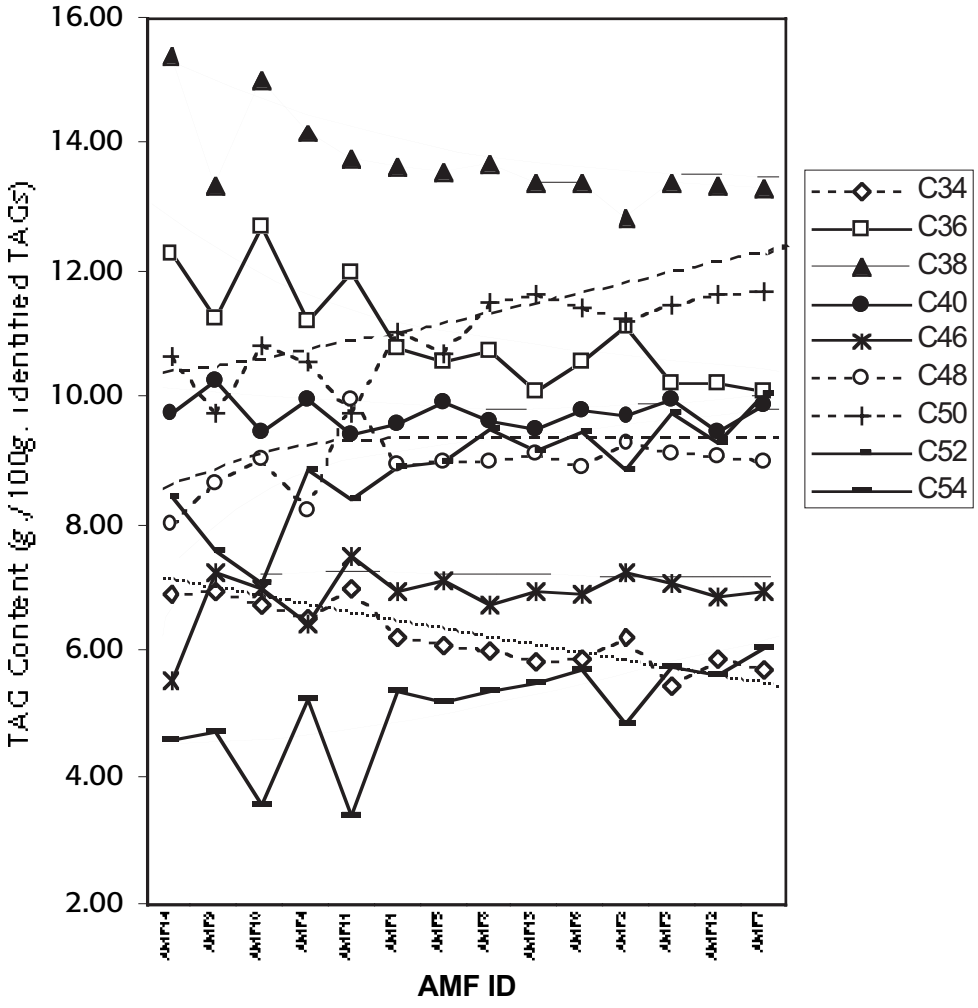


Fig. 3 Major TAG components contained in AMFs from different sources



chapter 2

Cheese

Intensified flavors in Cheddar cheese and cheese ingredients for enhanced applications in foods 37

Characterization of interactions between ingredients and cheese constituents for improved functionality of fat-free processed cheese 40

Improvement of Cheddar cheese quality through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese 46

Succinate production by *Lactobacillus casei:* pathways responsible and development of strategies to control its accumulation. 48

Glutathione and Cheddar cheese flavor development 50

Growth of nonstarter lactic acid bacteria in reduced fat Cheddar cheese 54

Optimizing the standardization of milk to manufacture 50% reduced fat Cheddar cheese 56

Cheese applications program 61

Extending the cheese net paradigm to include economic evaluation and optimization in cheese manufacture 64

Technology for improving the flavor and consumer acceptability of fat-free Cheddar cheese 65

Whey applications research program 66

Dairy marketing and economics program 68

A multi-country analysis of household food demand: Implications for U.S. food exports (phase I) 71

Development and application of a cheese shred/texture map delineated by cheese rheological, sensory and chemical analysis 76

CDR specialty cheese applications program 77

FINAL REPORT

Intensified flavors in Cheddar cheese and cheese ingredients for enhanced applications in foods

Personnel

Robert C. Lindsay, professor, Department of Food Science

Funding

Wisconsin Milk Marketing Board and Dairy Management, Inc.

Dates

July 1996–June 1998

Objectives

1. To determine the flavor systems and constituents of Cheddar cheese that are responsible for providing the desirable cook-through cheese flavors in foods, especially bakery products.

2. To develop technological means for intensifying desirable cook-through Cheddar cheese flavors in prepared foods, especially bakery products, through the selection and use of ingredients which interact during processing to provide products that amplify the impact of cheese flavors in these products.

3. To develop adjunct lactic acid bacteria culturing procedures for cheese ingredients which yield flavor compounds that intensify Cheddar cheese flavor in prepared foods, especially bakery products.

Summary

To assess the baked flavors of cheeses we modified a non-fermented dough batch-method to prepare cheese crackers. This model cracker contained levels of fat provided by butter and the fat from cheeses, and thus was characterized as a high fat cheese cracker. The high-fat model cracker system was selected because it amplified the cheese flavor notes that were formed either during baking or provided directly by the cheese ingredients for exposure to the baking conditions.

Descriptive sensory analysis ballots were developed and evaluated for effectively indexing bake-through cheese flavors. The most effective scales for documenting bake-through cheese flavors included: toasted flavor intensity (very weak to very strong), overall cheesiness flavor intensity (not cheesy to very cheesy), sourness/tartness (absent to very pronounced),varietal cheese flavor intensity (absent to pronounced; panelists provided cheese variety believed present), off-flavor intensity (absent to very pronounced). Sometimes overall acceptability (unacceptable to very acceptable) was also scaled.

Evaluating bake-through flavors

A wide range of cheese varieties, representing a range of ages, were added to cheese crackers. Then, expert flavor assessors and formal descriptive analysis sensory panels evaluated them to establish the types of cheese flavors that could be developed in baked cheese crackers. The cheese cracker varieties evaluated along with their distinguishing flavor characteristics as determined by expert flavor assessors included Cheddar-type, Swiss-type, hard Italian-type, and blue-type cheeses. These cheeses represented some mild, medium, and intensely flavored cheeses.

From a combination of the descriptive sensory analysis data and the expert assessor profiles of the baked cheese crackers, nine categories of bake-through cheese flavors were formulated. In addition to the categories, specific flavor notes were noted for the characterizing flavors in the cheese crackers, and supporting or modifying flavor notes were also identified. The main categories were baked true-Cheddar-type flavor, generic baked aged cheese flavor, baked dimethyldisulfide-type flavor, baked sweet Swiss-type flavor, baked blue-fatty acid-type flavor, baked fresh goat-type flavor, baked white mold surface-ripened type, and baked hard sheep's milk-type cheese flavors.

Cheese slurry systems

Modified cheese slurry systems were developed which were based on the initial work of Singh and Kristopherson (1969; Factors Affecting the Flavor Development in Cheddar Cheese Slurries, J. Dairy Sci. 55: 536) for use in systematic evaluations of both lactic bacterial adjunct strategies and

chemical flavor precursor additions. The overall slurry was composed of 60-62% Cheddar cheese component, and the remainder was added water and water-based ingredients. The Cheddar cheese component can be selected from freshly-made curd to very aged Cheddar cheese, and the cheese component can be prepared from mixtures of Cheddar cheeses with differing ages. Slurry components (Cheddar cheese, brine [to 38%], and other dissolved ingredients) are thoroughly mixed with a stomacher to homogeneity in sterile pouches, and then the pouches are incubated under a variety of oxygen-tension conditions (air exposure to anaerobic with Gas Pacs) for periods up to 21 days at 30°C.

Since contamination by advantitious innocuous microorganisms, as well as potential pathogens, could occur during manufacture, particular attention was given to the evaluation and selection of acceptable antimicrobial food additives for the cheese slurry systems. To accomplish this, the basic cheese slurry was prepared by adding an appropriate amount of sterile 5.2% sodium chloride to provide an elevated salt content to improve overall microbial stability. Then, through a series of trials, it was established that supplemention with 2% w/w MicrogardR (as a spoilage inhibitor; Wesman Foods, Inc), 1% w/w citric acid (to lower the pH; Mallinckrodt U.S.P.), and 0.2% w/w potassium sorbate (as a mold inhibitor; Pfizer) greatly enhanced the microbial stability of the cheese slurries.

Trials to determine the influence of pH conditions (4.4-7.1) on slurry aroma and flavor development were carried out employing pH adjustments with either phosphoric acid, citric acid, or *Lb. casei* growth and incubating for up to 72 hours at 30°C. A culture of *Lactobacillus casei*10, obtained from the culture collection of the Department of Food Science- University of Wisconsin where it was stored at -40 °C before cultivation in MRS broth (Difco) for 18 h at 32°C, was used to evaluate slurry culturing conditions. It was found that good flavor development occurred for combinations employing at least 20% of the total cheese ingredient as aged Cheddar cheese, when held at pH 5.0 and under carbon dioxide-nitrogen or aerobic atmospheres. However, accentuated cheese flavors were found to develop for samples prepared only from mild cheese and held under either aerobic or anaerobic conditions.

Studies were conducted on the susceptibilty of the Cheddar cheese slurry system to listeria hazards (objectives 2 and 3). Slurry samples were inoculated with *Listeria monocytogenes* Petite Scott A or NCTC 7973 at a level of 4 x 105 cells per gram, and then were incubated with 0.0, 0.5, and 1.0% citric acid at 30°C for 14 days with periodic examination for listeria counts. Listeria grew in the slurry system when the pH was adjusted to 7.1, but at lower pH values (pH 4.4 and 5.2), the listeria cells did not proliferate, and were depleted after about 150 hours. The inclusion of *Lactobacillus casei* in the cheese slurries provided some protection to the listeria, but cells were soon destroyed at pH 4.4 and 5.2. Based on the results of these studies, it was concluded that the cheese slurry system adopted provided a listeria-safe approach to production of concentrated cheese flavors.

Studies of adjunct cultures producing key bake-through cheese flavors identified in the baking trials were carried out, initially focusing on α-dicarbonyl production by lactic acid bacteria which had been earlier identified as an important route to overall cheese flavor development. The α-dicarbonyls, glyoxal, methylglyoxal, and diacetyl, react with amino acids to produce reaction flavor compounds that contribute to the general cheesiness category. These studies showed that selected lactic acid bacteria possessed the capability for producing elevated levels in cheese slurries used as ingredients in cheese crackers and other applications. These cheeses were made into cheese crackers, and higher α-dicarbonyl-containing samples which gave stronger toasted, baked cheese flavors.

Cheese flavors in crackers

Research was continued on the bake-through flavors of cheese crackers, and the data revealed that most commercial cheese crackers rely principally upon a generic methional, cheesiness character. These data also showed that the true Cheddar flavor was largely missing from the crackers, and they lacked much of the savory flavor provided by succinic acid. Using Cheddar cheese slurry systems prepared with intensified levels of succinic acid, aged Cheddar cheese, a-dicarbonyls, and volatile fatty acids, we produced Cheddar cheese crackers with substantially enhanced cheesiness. They were more intensely cheese-flavored than most commercial cheese crackers.

Investigations of the addition of selected ingredients or chemicals to mild Cheddar cheese-based cheese cracker doughs showed that methionine and a reducing sugar, such as glucose, provided distinct baked generic cheesiness to the resulting cheese crackers. Addition of succinic acid (75-300 ppm) greatly improved the savory cheesiness flavor, and it was stable to elevated temperatures encountered during baking. The discovery of succinic acid as a savory cheese flavor ingredient in Cheddar cheese flavors in this project has initiated substantial commercial activity by cheese culture companies, and it is the current focus of several University research projects involving adaptation during cheesemaking and/or genetic engineering to produce greater quantities in cultures.

Producing bake-through cheese flavor

Since volatile free fatty acids are key for cheesiness flavors (discovered in a parallel project), studies were carried out to demonstrate the production of volatile fatty acids by a variety of lactic acid bacteria in the cheese slurry system. Cell-associated lipases of adjunct lactobacillus and related bacteria produce substantial flavor potential in the cheese slurries. Levels of butyric acid provide an index for cheesiness, and concentrations over 150 ppm butyric acid in cheese ingredients provide desirable cheesiness. When selected slurries were incorporated into cheese crackers, distinctively elevated cheesiness flavors were obtained when compared to a mild Cheddar cheese control sample.

True Cheddar flavor

The chemistry of the true sulfury Cheddar compound was intensively studied using a variety of techniques for attempting to stablize the compound for analysis. Using an enclosed glove box provided an approach to maintain cheeses in an atmosphere free of oxygen, and it was conclusively shown using descriptive analysis that the true Cheddar flavor, hence compound, was greatly suppressed or destroyed upon exposure to oxygen. Only methanethiol could be demonstrated to be present regularly, but evidence was collected which indicated that the compound was an oxygen-heat sensitive compound that formed from diacetyl/acetoin, hydrogen sulfide, and methanethiol. For this reason, the adopted use of glutathione in the cheese slurry system was hypothesized to result from such a reaction.

Specific analysis of aged Cheddar cheeses described as “catty” by dairy products judges yielded the identification of 4-methyl-4-mercaptopentan-2-one in low concentrations. This compound is produced by the condensation of acetone to mesityl oxide, and then a subsequent reaction with hydrogen sulfide from amino acids or glutathione yields the mercaptoketone. Acetone is produced by some lactic acid bacteria, and light catalyzes the condensation to mesityl oxide. Based on its flavor properties, it was hypothesized that the mercaptopentanone may accompany and potentiate the as yet unidentified true Cheddar flavor compound.

Publications/presentations

Lindsay, R. C. 1997. Recent Advances in the Flavor Chemistry of Cheddar Cheese. In: The National Cheese Technology Forum, Proceedings ’97, December 9-10, Rosemont, IL, pp. 1-16.

FINAL REPORT

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

Personnel

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

Funding

Wisconsin Milk Marketing Board

Dates

July 1997– June 1999

Objectives

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

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

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

Summary

Manufacture of process cheeses
Pasteurized process cheeses were manufactured in a Blentech twin screw pilot cooker (Blentech Corp., Rohnert Park, CA) equipped with variable agitation and indirect steam heating capabilities. Batches of cheeses were produced by the formulation shown in Table 1. Disodium phosphate duohydrate was used as the emulsifying salt except where indicated. Throughout the study, fat and moisture compositions of fat-free process cheeses were measured, and mean values are reported in Table 2. Little variation in these values was observed throughout the study, which supported the view that the results reflected experimental treatments rather than compositional variations. Similarly, pH values of all fat-free process cheeses were monitored, generally showing little variation throughout the study.

Emulsifying salts

Effects of different emulsifying salts, at 3%, on textural attributes of fat-free process cheeses are reported in Table 3. Trisodium citrate and disodium phosphate produced significantly softer cheeses and melted more easily than those prepared with condensed phosphate Joha brand salts. For these trials, trisodium citrate produced the softest cheese, which also melted slightly more readily than the full-fat reference. In the case of Joha brand emulsifying salts, firm cheeses with limited melt and minimal spreadability resulted. Increasing the amount of emulsifying

Table 1. Fat-free process cheese base formulation.

Ingredient	%
Hard skim milk cheese	59.8
Water	26.8
Dried sweet whey	5.3
Nonfat dry milk	4.1
Emulsifier	3.0
Salt	1.0
Hydrocolloid	0
Total	100.0

Table 2. Typical composition[a] of experimental fat-free process cheeses.

Component	%
Fat	0.6 + 0.2
Moisture	58.5 + 1.0
pH	5.6 + 0.4

[a] Mean values + standard deviation of duplicate determinations for fat-free trials (n=88)

Table 3. Effect of emulsifying salts used at a 3 % level on the functional properties of fat-free process cheeses.

Emulsifying salt	Firmness[f] peak force (N)	Meltability[g] flow (mm)	Spreadability[h] total force (N's)
Trisodium citrate	21.3[d]	155.8[a]	521.6[a,b]
Disodium phosphate	25.4[c]	46.5[c]	493.5[b]
Joha S9	33.0[b]	9.5[e]	NSTC
Joha SE	32.9[b]	8.0[e]	NSTC
Joha C New	66.0[a]	19.5[d]	552.2[a]
Full-fat reference[i]	12.2[e]	145.5[b]	192.1[c]

[a,b,c,d,e] Means within a column with no common superscripts differ significantly ($p<0.05$).
NSTC = Not spreadable under test conditions.
[f] Means of triplicate determinations for two trials (n=6).
[g] Means of duplicate determinations for two trials (n=4).
[h] Means of duplicate determinations for two trials (n=4), smaller values equal greater spreadability.

Table 4. Effect of hydrocolloids used at a 2 % level on the functional properties of fat-free process cheeses.

Hydrocolloid	Firmness[f] peak force (N)	Meltability[g] flow (mm)	Spreadability[h] total force (N's)
Gelatin	45.0[b]	34.3[b]	524.0[b]
Carrageenan	45.1[b]	9.3[c]	485.3[c]
Locust bean gum	53.0[a]	12.0[c]	593.1[a]
Guar gum	38.1[c]	6.5[c]	228.9[d]
Full-fat reference[i]	12.2[d]	145.5[a]	192.1[e]

[a,b,c,d,e] Means within a column with no common superscripts differ significantly ($p<0.05$).
[f] Means of triplicate determinations for two trials (n=6).
[g] Means of duplicate determinations for two trials (n=4).
[h] Means of duplicate determinations for two trials (n=4), smaller values equal greater spreadability.
[i] Full-fat reference contained 3% disodium phosphate duohydrate as the emulsifying salt.

salt from 0.5 to 3% generally resulted in increased firmness, decreased melt and decreased spreadability in all cases (data not shown). Additionally, various mixtures of the emulsifiers did not provide advantages over single emulsifier usage for any of the textural attributes. The high degree of firmness for cheeses containing Joha brand emulsifying salts might be explained by an increase in the amount of protein-protein interaction facilitated by greater Ca++ sequestering abilities of these polyphosphate-containing ingredients. The similar consequences of increased firmness and decreased melt corresponding to increasing concentrations of emulsifying salts mentioned earlier also would be in agreement with this.

Hydrocolloids

Results of additions of commercial hydrocolloids to fat-free process cheeses formulated with 3% disodium phosphate are summarized in Table 4. Overall, an increase in the firmness of the cheese and a decrease in meltability occurred for all treatments compared to process cheese control samples without added hydrocolloids. Guar gum produced the softest texture of all hydrocolloids studied with gelatin exhibiting the greatest overall meltability. The heat reversible property of gelatin gels above 48.8∞C undoubtedly contributed to the melt characteristics of cheeses containing gelatin. However, the low meltability of cheeses containing carrageenan indicated that some polymers yielding heat meltable gels perform differently in fat-free process cheeses than in model aqueous systems.

In regards to spreadability, guar gum additions yielded significantly more spreadability compared to control cheeses prepared without guar gum. This may be due to the thixotropic nature of guar gum gels and may indicate that other hydrocolloids possessing this characteristic could enhance the spreadability of fat-free products. While addition of hydrocolloids did not provide fat-free process cheeses with textural properties simulating full-fat cheeses, qualitative observations indicate that products incorporating hydrocolloids had more uniform, smooth consistencies. Thus, certain hydrocolloids might be useful in process cheeses, especially when used at lower levels or in process cheeses with high water contents.

Cook time

The amount of time fat-free process cheeses were held in the cooker at 75°C was examined during incrementally increasing times (Table 5). In all cases, approximately four and one-half minutes was required to reach cook temperature. Cook time affected firmness and meltability. As cook time increased, significant decreases in the firmness of finished cheeses were observed. On the other hand, meltability tended to increase up to 10 minutes of cook time, after this further enhancement of melt was not observed. Spreadability was not significantly affected by cook time, although absolute values showed slightly greater spreadability resulted with longer cooking times.

Since the cheeses in this study were fat free, the consequences of increased breakdown of young cheese proteins as cooking progressed might be explained by a different mechanism. Perhaps less structure-building capability remained, resulting in less opportunity for protein-protein interaction. While this explanation appears consistent with the observations of this study, further investigation on molecular weight profiles of cooked cheese should be conducted to verify this hypothesis.

Cook temperature

The effect of cook temperature on the textural attributes of fat-free process cheeses is presented in Table 6. In all trials it took approximately four and one-half minutes to reach specified cook temperatures. Our results show that as the cook temperature was increased, firmness generally

Table 5. Effect of cook time on the functional properties of fat-free process cheeses.

Cook Time (min at 75°C)	Firmness[e] peak force (N)	Meltability[f] flow (mm)	Spreadability[g] total force (N*s)
0	31.7[a]	48.8[b]	518.0[a]
5	27.1[b]	57.5[b]	504.1[a]
10	26.3[b,c]	70.3[a]	500.2[a]
15	25.3[c,d]	78.0[a]	484.8[a]
20	24.1[d]	77.5[a]	485.5[a]

[a,b,c,d] Means within a column with no common superscripts differ significantly ($p<0.05$).
[e] Means of triplicate determinations for two trials (n=6).
[f] Means of duplicate determinations for two trials (n=4).
[g] Means of duplicate determinations for two trials (n=4), smaller values equal greater spreadability.

Table 6. Effect of cook temperature on the functional properties of fat-free process cheeses.

Cook Temperature (°C)	Firmness[e] peak force (N)	Meltability[f] flow (mm)	Spreadability[g] total force (N*s)
60	32.9[a]	18.5[d]	533.5[a]
70	28.2[b]	37.8[c]	493.7[b]
80	23.9[d]	89.3[b]	431.9[c]
90	26.2[c]	98.0[a]	342.7[d]

[a,b,c,d] Means within a column with no common superscripts differ significantly ($p<0.05$).
[e] Means of triplicate determinations for two trials (n=6).
[f] Means of duplicate determinations for two trials (n=4).
[g] Means of duplicate determinations for two trials (n=4), smaller values equal greater spreadability.

decreased over the temperature range studied (60°- 90°C), except in the case of the 90°C trial where cheese firmness increased slightly.
A marked increase in the ease of meltability, as well as spreadability, was observed as cook temperatures were raised from 60 to 90°C. These results clearly show that fat-free process cheeses produced at higher cooking temperatures exhibited enhanced melting and spreading characteristics. Observations in the present study indicate that, in the absence of fat, protein structural interactions and modifications, and not the fat emulsifying capability of proteins, govern the textural properties of fat-free process cheese.

pH

The effect of pH on cheese texture was evaluated by adding glacial acetic acid or powdered sodium bicarbonate to adjust the pH of the fat-free process cheese formulations. Physical properties of cheeses were examined over the range of pH 5.26 to 6.88, and results are reported in Table 7. As pH values increased, a corresponding increase in cheese firmness resulted, with significant increases above 6.0. In the case of meltability, cheeses produced with higher pH values melted more readily than those produced with lower pH values. Results of experimental process cheeses showed those produced at lower pH values were

Table 7. Effect of pH on the functional properties of fat-free process cheeses.

pH	Firmness[d] peak force (N)	Meltability[e] flow (mm)	Spreadability[f] total force (N*s)
5.26	26.0[c]	42.8[b]	381.8[c]
5.64	27.1[c]	47.5[b]	472.8[b]
6.09	32.8[b]	85.0[a]	568.2[a]
6.88	46.8[a]	82.3[a]	NSTC

[a,b,c] Means within a column with no common superscripts differ significantly ($p<0.05$).
NSTC = Not spreadable under test conditions.
[d] Means of triplicate determinations for two trials (n=6).
[e] Means of duplicate determinations for two trials (n=4).
[f] Means of duplicate determinations for two trials (n=4), smaller values equal greater spreadability.

more spreadable than those produced at higher pH values. Steady decreases in spreadability were noticed as pH values increased from 5.26 to 6.88. These observations may indicate the isoelectric point of cheese proteins plays a large role in resulting textures of process cheeses. At higher pH values, cheese proteins are further from their normal isoelectric point of about 5, and tend to exist in a more open conformation. This would facilitate protein-protein interactions that should result in increased firmness and decreased spreadability. However, the increased meltability of fat-free process cheeses at higher pH values would not be explained by this theory, indicating that other factors also must be involved.

Changes in pH, relative to the isoelectric point of cheese proteins, would affect the water-binding capacity of fat-free process cheeses. Presumably, cheese proteins function to bind greater amounts of free water at increased pH values. These changes in the ability to absorb water have been shown to play a major role in the textural performance of various foods and may have influences on the texture of fat-free process cheeses. Overall, the influence of pH on cheese texture occurs through a number of mechanisms and further investigation is needed to clarify its effects.

Results of this study indicate that fat-free process cheese physical functionality resulted from protein-protein interactions and protein modification mechanisms. Evaluation of emulsifying salts, hydrocolloids, cook time, cook temperature, and pH showed that all affected final texture to some degree. Emulsifying salt-type produced large variations in functionality, with trisodium citrate producing the most functional textural properties. Emulsifying salts containing polyphosphates increased firmness, while decreasing melt and spreadability, possibly by allowing for increased protein interactions after more extensive calcium chelation. Addition of hydrocolloids was not an effective means for enhancing the functional properties of the experimental fat-free process cheeses (49-60% moisture). However, hydrocolloids may be valuable for texture modification in higher moisture fat-free process cheese products.

Results from cook time and cook temperature trials revealed that increased heat and agitation yielded samples with enhanced functional properties. These treatments would disrupt and possibly break down existing protein (casein) structures producing softer textures with greater meltability and spreadability. Studies of the influence of pH on fat-free process cheese properties revealed that cheeses produced in the pH range of 5.0 to 6.0 had the softest and most spreadable textures. Elevated pH values above 5.6 (6.0-6.9) greatly enhanced the meltability of fat-free processed cheeses. A mechanism involving properties of proteins (caseins) at pH values around the isoelectric point was proposed to account for the effects of pH on firmness and spreadability of fat-free process cheeses.

Skin formation on process cheese

In recent years, industry personnel have reported a defect in pasteurized processed cheese referred to as skin-formation. Skin-formation is the presence of a shiny, rubber-like appearance on the surface of processed cheeses following manufacture. However, the problem has also been widely reported in low-fat and non-fat products during the heating or cooking process.

Observations on degree of skin formation on pasteurized processed cheeses, along with moisture and fat analysis, are given in Table 8. Quali-

Table 8. Apparent skin-formation on experimental processed cheeses.

Trial	% Moisture	% Fat	Skin Formation[1]
Fat-Free[2]	59.0	0.4	+++
Full-Fat[2]	49.1	20.8	++
Full-Fat[3]	49.2	20.9	+

[1] + = slight, ++ = moderate, +++ = heavy.
[2] Sealed in two-pound high-density polyethylene tubs.
[3] Sealed in two-pound loaf box with foil wrapping.

Table 9. Comparison of bulk versus surface moisture content[1] in experimental processed cheeses.

Trial	% Moisture (Bulk)	% Moisture (Surface)[2]
Fat-Free[3]	59.0	45.6
Full-Fat[3]	49.1	48.2
Full-Fat[4]	49.2	49.1

[1] Means of duplicate determinations (n=2).
[2] Determined by removing approximately 1 mm cheese from surface with a cheese cutter.
[3] Sealed in two-pound high-density polyethylene tubs.
[4] Sealed in two-pound loaf box with foil wrapping.

tative observations showed that of all samples monitored, fat-free processed cheeses exhibited the most skin formation. Of the full-fat cheeses examined, those stored in high-density polyethylene tubs showed a greater tendency to skin-over than the same product stored in foil-wrapped loaves. Therefore, initial observations on cheeses produced in this study indicate that moisture loss may play a role in the skin formation phenomenon.

Presumably, in the full-fat processed cheeses having the least amount of observable skin, foil wrapping efficiently slowed moisture migration from outer surfaces. Small degrees of skin-formation were still observed, which was probably caused by moisture losses through folded edges of the foil wrapping material. In the case of cheeses stored in high-density polyethylene tubs, moisture migration and loss from exposed surfaces was much more extensive through the open headspace and loose covers. Fat-free experimental samples stored in tubs exhibited more extensive skin formation than full-fat samples in tubs, apparently in part because of a lack of sufficient free milk-fat along surfaces to inhibit moisture vaporization.

Surface moisture losses were evidenced by removing noticeable areas of skin from cheese surfaces with a cheese cutter and analyzing and comparing their moisture contents to those of bulk cheese (Table 9). While clear moisture losses were observed in the case of fat-free samples, smaller losses were observed in full-fat samples. The small loss of moisture in full-fat cheeses could be attributed to increased proportion of bulk cheese removed by the cheese cutter in processed cheeses with thinner skin formation. Overall, losses of moisture from experimental processed cheeses may have facilitated skin-formation through physical alterations of protein structures in dehydrating cheese surfaces. Furthermore, losses in moisture may have had subsequent effects on the glass transition temperature of surface proteins, functionally causing the skin.

Observations made in this study have related moisture migration from cheese surfaces to the skinning-over phenomenon in processed cheeses. Modification of protein orientations and structures may be partially responsible for the formation of skin. Additionally, shifts in state relative to glass transition temperatures may be important to the development and overall control of skin formation. However, the results of this study are considered preliminary, and further study is needed to clarify the skin-formation mechanism in processed cheeses.

INTERIM REPORT

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

Personnel

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

Funding

Dairy Management Inc.

Dates

June 1997–June 2000

Objectives

1. Define the contribution of starter proteinase specificity on peptide pools and bitterness in Cheddar cheese.

2. Develop a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in Cheddar cheese.

3. Determine the bitter taste threshold for ß-CN(f193-209) and α_{s1}-CN(f1-9).

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

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

Summary

Variability in the degree of autolysis and intracellular peptidase activity among strains of Lactococcus lactis limited our initial effort to define the relationship between proteinase specificity and bitterness. To overcome this limitation, we constructed a series of isogenic strains which differ only in proteinase specificity and which lack the gene for the major lactococcal autolysin, AcmA. The proteinases which we evaluated included the *L. lactis* Wg2 group e proteinase, CEP, the L. lactis SK11 group a proteinase, and the group h proteinase from the bitter starter *L. lactis* S3. The proteinase specificity of each isogenic construct was confirmed by in vitro incubation of whole cells with α_{s1}-CN(f1-23) at pH 5.2 in 4% NaCl. Reduced fat Cheddar cheeses were then manufactured using these isogenic strains which differed only in their proteinase specificity. HPLC analysis has confirmed that peptide accumulation in the experimental cheeses is occurring as predicted by the CEP specificity of each starter. Trained sensory analysis of the experimental cheeses after 2, 4, and 6 mo of ripening has established a clear role for proteinase specificity in bitterness. As expected, strains carrying the group a, e, or h proteinase had low, intermediate, or high propensities for bitterness, respectively. These results confirm our previous findings that starter culture proteinase specificity is a key determinate of whether or not a cheese will develop bitterness.

In the past, researchers seeking to determine the contribution of specific peptides to bitterness in cheese have relied on sensory evaluation of peptides in aqueous solutions to measure bitterness. However, sensory studies have clearly established that taste thresholds for a compound increase when viscosity increases or when competing tastes are present. For this reason, the quantity of any peptide necessary to evoke a bitter response will always be much higher in cheese than in water, so water dispersion data cannot be reliably applied to cheese. Our group has demonstrated that dispersal of bitter compounds in a cheese model system is a representative and effective means to study bitterness in cheese.

To our knowledge, we are the first group to study the contribution of individual peptides to bitterness in model cheese system, and our work on bitter taste thresholds for ß-CN (f193-209) and α_{s1}-CN(f1-9) has provided valuable new insight into the role of specific peptides in bitterness. In the case of both peptides the bitter taste threshold was approximately 10-fold higher in the model cheese system than in water. When the bitter taste thresholds of these peptides in the model cheese system were compare to the levels of these peptides observed in a bitter cheese, it was concluded that the α_{s1}-CN(f1-9) was primarily responsible for bitterness in this cheese. While the ß-CN (f193-209) peptide likely had a complementary function, rather than a dominant role, in the perception of bitterness in this cheese.

The peptidase system of *Lactobacillus helveticus* CNRZ32, an adjunct that reduces bitterness in cheese, has been investigated in detail by Dr. Steele's laboratory. Genes for ten peptidases have been cloned and sequenced from this organism. Of these enzymes, the contribution of 2 general aminopeptidases (PepC and PepN), a proline-specific aminopeptidase (PepX), and two endopeptidases (PepO and PepE) to the hydrolysis of the known bitter peptides ß-CN (f193-209) and α_{s1}-CN(f1-9) have been evaluated. Growth studies and studies with cell-free extracts (CFEs) of CNRZ32 and isogenic strains lacking one of the five peptidases mentioned above revealed that all of the mutants hydrolyzed these peptides completely to free amino acids. These results indicated that overlapping specificities in CNRZ32 peptidases were masking the effect of individual peptidases. To overcome this problem, we evaluated the rate of hydrolysis and the transition peptides formed by cell-free extracts of CNRZ32 and the five isogenic peptidase-deficient derivatives described above. Differences in the hydrolysis of ß-CN (f193-209) were only observed between CNRZ32 and the mutant lacking PepN activity. These results indicated that PepC, PepX, PepO, and PepE have no detectable role in the hydrolysis of ß-CN (f193-209) and that PepN initiates the N-terminal hydrolysis of this peptide. The observation that 50% of the transition peptides identified from ß-CN (f193-209) had either a C-terminal Pro204 or Pro206 residue suggested that a post-proline endopeptidase was also involved in the hydrolysis of this peptide. Confirmation of a post-proline endopeptidase in CNRZ32 was obtained by the ability of CNRZ32 CFEs to hydrolyze C- and N-blocked ß-CN (f203-209). The identification of a post- proline endopeptidase in CNRZ32 is significant, as this enzyme's substrate specificity suggests it may contribute to the hydrolysis of numerous bitter peptides. Hydrolysis of the α_{s1}-CN(f1-9) by CFEs from CNRZ32 and its isogenic derivatives lacking one of the five peptidases previously described was evaluated. The primary peptide produced by all CFEs was α_{s1}-CN(f1-7), suggesting either that an endopeptidase distinct from PepO and PepE or a carboxypeptidase was responsible for the formation of this peptide. Currently, the possible involvement of the post-proline endopeptidase in the formation of this peptide is under investigation.

Publications/Presentations

Christensen, J.E., E.G. Dudley, and J.R. Pederson, J.L. Steele. 1999. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoek 76:217-246.

Steele, J.L. (1999). Peptidases and amino acid catabolism. Institute Food Technol. Abstr., 1999, 53-4.

"Peptidases and amino acid catabolism". Symposium on "Dairy Flavors and Biotechnology" at the 1999 IFT Annual Meeting. July 1999.

"Peptidases and amino acid catabolism." At the Sixth Symposium on Lactic Acid Bacteria. September 1999 in The Netherlands.

INTERIM REPORT

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

Personnel

James L. Steele, Professor, Dept. of Food Science,
Ed Dudley, Research Assistant, Bacteriology

Funding

Dairy Management Inc.

Dates

July 1997– September 2000

Objectives

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

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

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

Summary

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

Previously we reported the screening of two strains of *Lb. plantarum*, twelve strains of *Lb. casei*, and eight strains of *Lactobillus rhamnosus* (formerly *Lb. casei* subsp. *rhamnosus*) for succinate production. Cultures were grown to carbohydrate exhaustion in a complex medium under anaerobic conditions, and were resuspended in phosphate buffer saline pH 7.0 containing one of the following: 10mM citrate, 10mM L-lactate, 10mM citrate plus 10mM L-lactate, or 10mM Asp. After 3 days incubation at 37°C, succinate production was detected under all four conditions for *Lb. plantarum* ATCC 14917, and under all conditions except for Asp for *Lb. plantarum* ATCC 14431.

No succinate production was detected with any *Lb. casei* or *Lb. rhamnosus* strains studied. Whole cells of ATCC 14917 and ATCC 14431 converted approximately 44% and 15% of the citrate and 33% and 5% of the lactate to succinate, respectively. Additionally for both strains, the amount of succinate produced in the presence of both citrate and L-lactate was higher than the sum of the amounts produced by citrate and L-lactate alone. Therefore, this screen suggested *Lb. plantarum* possesses at least three distinct biochemical pathways for succinate production, and these strains are able to cometabolize citrate and L-lactate.

Additionally, the above screen was repeated with three strains of *Lb. plantarum* (ATCC 14917, ATCC 14431 and RL3), two strains of *Lb. casei* (ATCC 393 and ATCC 334), and one strain of *Lb. rhamnosus* (ATCC 7469). This second screen was performed similarly to the first screen, except all growth media and solutions included 0.5 g/l L-Cys and 10 mg/ml resazurin, were sparged with 0_2-free N_2 prior to autoclaving, and were stored in sealed bottles or tubes under a pressurized N_2 atmosphere. Resazurin is a redox-indicating dye that is colorless when E_h<-110 mV. Thus, this screen was done under redox conditions more typical of those found in Cheddar cheeses. Under these conditions, ATCC 14917, ATCC 14431 and RL3 produced 0.26mM, 1.94mM and 0.34mM succinate in 24h from 10mM citrate at pH 7.0 and 37°C, respectively. These strains also produced 0.75mM, 2.77mM and 1.0 mM succinate from the combination of 10mM citrate and 10mM L-lactate, respectively. No succinate production was detected from 10mM L-lactate or 10mM Asp. Additionally, no succinate production was detected from 10mM isocitrate, a precursor to succinate in other bacteria. No succinate production was detected from the *Lb. casei* or *Lb. rhamnosus* strains.

The above data suggests *Lb. plantarum* produces significant levels of succinate from citrate, while *Lb. casei* and *Lb. rhamnosus* likely divert citrate to

other products. While this suggests *Lb. plantarum* may be added to cheese during the manufacturing process to increase the level of succinate, recent research suggests *Lb. plantarum* does not effectively outcompete other nonstarter lactic acid bacteria found in Cheddar, including *Lb. casei*. Therefore, we have chosen the approach of engineering *Lb. casei* to produce succinate. As oxaloacetate is a precursor to succinate in other organisms, we began this strategy by targeting genes whose inactivation might increase intracellular pools of oxaloacetate. Two such genes are lactate dehydrogenase and oxaloacetate decarboxylase (*oadA*). We have recently isolated *oadA* from *Lb. casei* ATCC 393 using a degenerate PCR approach. The gene encodes a putative protein of 467 amino acids with sequence identity to oxaloacetate decarboxylases from other bacteria. Unlike previously described genes from *Salmonella* and *Klebsiella*, the ATCC 393 *oadA* is not associated with two other genes (*oadB* and *oadG*) which are involved in associating *oadA* with the cell membrane and the pumping of Na^+ ions across the membrane to generate a chemical potential. Upstream of *oadA* are the genes encoding the three subunits of citrate lyase and a protein with identity to the *Escherichia coli* CitW which currently has no defined function. These four genes appear to be cotranscribed with *oadA*. More sequence information is being obtained upstream of *oadA* to isolate the remaining gene (citrate lyase-ligase) expected to be associated with the citrate lyase gene cluster. Downstream of *oadA* two additional open reading frames (ORFs) were identified. One of these ORFs has identity to a family of transcriptional regulatory proteins. The second ORF has identity to CitG, a protein of unknown function found associated with the citrate lyase clusters of *Salmonella* and *Klebsiella*. Whether the putative transcriptional regulatory protein is functional on the citrate lyase cluster remains to be determined.

Currently, ^{13}C-NMR spectroscopy is being used to deduce the citrate catabolic pathways of *Lb. casei* and *Lb. plantarum*. Enzymatic assays will be used to support the ^{13}C-NMR data. Also, genetic techniques are being developed to inactivate *oadA* in *Lb. casei* and to determine the effect of this inactivation on citrate metabolism and succinate production. The results from this study will address industry needs outlined under Objective 1, Goal 1.1, Tactic 2 of the National Dairy Research Plan.

Publications/Presentations

Dudley, E.G., M.W. Atiles, and J.L. Steele. (1999). Characterization and physiological role of the branched-chain and aspartate aminotransferase genes of *Lactococcus lactis*. FEMS Sixth Symposium on Lactic Acid Bacteria Abstracts, 1999, H27.

Dudley, E.G. and J.L. Steele. (1999). Production of succinate by *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnous*. FEMS Sixth Symposium on Lactic Acid Bacteria Abstracts, 1999, G54.

Dudley, E.G. and J.L. Steele. (1999). Production of succinate by *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus rhamnous*. 99th General Meeting of the American Society for Microbiology, 1999, O-72.

FINAL REPORT

Glutathione and Cheddar cheese flavor development

Personnel

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

Funding

Dairy Management Inc.

Dates

July 1997–June 1999

Objectives

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

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

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

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

Summary

A variety of lactic acid bacteria were screened for the ability to obtain glutamic acid from glutathione (-glutamyl-cysteinyl-glycine) and for -glutamyl transpeptidase (-GTP) activity (Table 1). The results of this screen indicated that the ability to obtain glutamic acid from glutathione is strain dependent among lactic acid bacteria; -GTP activity is also strain dependent; and -GTP activity is necessary but insufficient for lactic acid bacteria to obtain glutamate from GSH. It is likely that the ability to transport GSH is also required for lactic acid bacteria to obtain glutamate from GSH. Additionally, the observation that -GTP activity was only detected in permeabilized cells and cell-free extracts indicates that -GTP is an intracellular enzyme.

Due to its -GTP activity and ability to obtain glutamic acid from GSH (suggesting the presence of a mechanism to transport GSH), *Lactococcus lactis* 1228 was selected for further investigation. The temperature sensitive integration vector pGH9::ISS*1* was introduced into 1228 by electroporation. Integration of the vector following propagation at the non-permissive temperature was demonstrated to occur randomly into *Lc. lactis* 1228 chromosomal DNA by Southern hybridizations utilizing a probe derived from ISS*1*. These 1228 derivatives were then screened for their ability to obtain glutamic acid from GSH. Although more than 5,000 individual integrants were screened, no derivatives lacking the ability to obtain glutamic acid from GSH were obtained. This result was surprising since we anticipated that this screen would identify integrants either -GTP activity or the ability to transport GSH. Currently, we are unable to explain this result. Subsequently, attempts were made to identify the gene encoding -GTP activity by complementation of a strain of *Escherichia coli* lacking this enzyme; this approach also failed to identify the -GTP gene. The inability to identify these genes made it impossible to construct isogenic strains differing only in their ability to metabolize GSH.

The ability of three strains of *Lc. lactis* to produce hydrogen sulfide from cysteine (Table 2) and GSH (Table 3) was investigated. These strains were chosen based on their ability to transport and hydrolze GSH. *Lc. lactis* 1228 is able to both transport and hydrolyze GSH (Table 1). *Lc. lactis* LM0230 possesses -GTP activity but is unable to utilize GSH as the sole source of glutamate (Table 1), which suggests it is unable to transport GSH. *Lc. lactis* Z8 is known to be able to transport GSH (previous published research) and lacks -GTP activity (Table 1). The results presented in Table 2 indicate that while all three strains were able to produce hydrogen sulfide from cysteine, differences exist both in the relative rates and quantity of hydrogen sulfide produced. The results presented in Table 3 indicate that all three strains were able to produce similar quantities of hydrogen sulfide from GSH; however, *Lc. lactis* 1228

Table 1. Ability of lactic acid bacteria to use reduced glutathione (GSH) as the sole source of glutamate and g-glutamyl transpeptidase (g-GTP) activity.

	Absorbance 600 values [1]				g-GTP Activity [2]	
Strain	Growth on Glutamate [3]		Growth on GSH [4]		Permeablized cells [5]	
Lactococcus lactis [6]		SD		SD		SD
1363	1.43	± 0.054	0.02	± 0.004	0.73	± 0.070
1228	1.46	± 0.032	0.97	± 0.022	1.08	± 0.118
1362	1.25	± 0.114	0.81	± 0.028	0.87	± 0.085
1361	1.21	± 0.051	0.73	± 0.045	1.36	± 0.165
ATCC 11454	0.99	± 0.222	0.61	± 0.003	1.14	± 0.109
C2O	1.07	± 0.051	0.13	± 0.112	0.77	± 0.048
C2	1.13	± 0.006	0.13	± 0.006	0.69	± 0.029
LM0230	1.40	± 0.015	BQL [7]		0.70	± 0.017
MG1614	1.51	± 0.035	0.08	± 0.002	0.44	± 0.058
S3	1.49	± 0.032	BQL		0.07	± 0.016
11007	1.19	± 0.107	0.53	± 0.011	1.14	± 0.078
1816	1.33	± 0.036	BQL		ND [8]	
DL16	1.05	± 0.0	0.08	± 0.015	0.32	± 0.052
Z8	0.98	± 0.063	BQL		ND	
Streptococcus thermophilus[9]						
ATCC 19258	0.93	± 0.040	BQL		0.02	± 0.008
ATCC 19987	0.98	± 0.019	0.35	± 0.053	0.10	± 0.006
Lactobacillus helveticus[9]						
10386	2.77	± 0.075	BQL		ND	
CNRZ32	2.78	± 0.037	BQL		0.40	± 0.011

1 Absorbance readings at 600nm were taken after a 42hr incubation period
2 Absorbance readings at 405nm correlating to g-GTP activity
3 Cultures grown in defined media (Christensen & Steele, unpublished) containing 2.7mM Glutamic acid
4 Cultures grown in defined media containing 2.7mM GSH
5 Cells permeablized with Triton X followed by a 25min incubation with g-GTP synthetic substrate
6 propagated at 30°C
7 Absorbance 600 values of < 0.01
8 Absorbance 405 values of < 0.01
9 propagated at 37°C

did so at a significantly faster rate. These results indicate that neither GSH transport nor hydrolysis is required for the production of hydrogen sulfide from GSH; however, strains capable of both transport and hydrolysis of GSH are likely to produce hydrogen sulfide at a significantly faster rate.

In summary, GSH is likely the primary source of cysteine in the cheese matrix and cysteine has been shown to play a critical role in the production of volatile sulphur compounds involved in the development of Cheddar cheese flavor. The ability of starter cultures to transport GSH, which is water soluble and hence most milk-derived GSH will be lost in the whey, is likely to increase the level of GSH in the cheese matrix. Additionally, lactococcal strains capable of both transport and hydrolysis of GSH are likely to produce volatile sulphur compounds at an enhanced rate resulting in more rapid Cheddar cheese flavor development.

Publications/presentations

Mikesell, D. and J.L. Steele. 1998. Enhanced Cheddar cheese flavor via starter culture uptake and hydrolysis of glutathione. J. Dairy Sci., Vol. 81, Suppl. 1, 1998, 16.

Christensen, J.E., E.G. Dudley, and J.R. Pederson, J.L. Steele. 1999. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoek 76:217-246.

Steele, J.L. 1999. Peptidases and amino acid catabolism. Institute Food Technol. Abstr., 1999, 53-4.

Mikesell, D. and J.L. Steele. 2000. Production of H_2S from glutathione and cysteine by lactic acid bacteria. Manuscript in preparation

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

"Enhanced Cheddar cheese flavor via starter culture uptake and hydrolysis of glutathione." By Debbie Mikesell at the 1998 ADSA Annual Meeting. June 1998.

"Peptdases and amino acid catabolism". By Dr. Jim Steele at the 1999 IFT Annual Meeting as part of the symposium on "Dairy Flavors and Biotechnology". July 1999.

"Peptidases and amino acid catabolism". By Dr. Jim Steele at the Sixth Symposium on Lactic Acid Bacteria. September 99 in The Netherlands.

Table 2. Hydrogen sulfide production from lactococcal strains incubated with cysteine.

	picomoles of H_2S/g (dry weight)							
	4hrs		8hrs		12hrs		24hrs	
Lactococcus lactis		SE		SE		SE		SE
Z8	BQL 1[b]		BQL [c]		36,000 [c]	2600	89,000 [c]	9500
1228	180,000 [a]	9000	280,000 [a]	9500	250,000 [a]	23000	500,000 [a]	35500
LM0230	BQL [b]		120,000 [b]	24000	200,000 [b]	33500	290,000 [b]	36000

	picomoles of H_2S/g (dry weight)			
	48hrs		72hrs	
Lactococcus lactis		SE		SE
Z8	97,000 [c]	20500	95,000 [c]	29000
1228	500,000 [a]	50000	600,000 [a]	50000
LM0230	470,000 [b]	55000	490,000 [b]	47500

1 Below quantifiable limits < 33,000 picomoles of H_2S/g (dry weight)

a,b,c Means within the same column followed by no common superscript letter differ ($P < 0.05$)

Table 3. Hydrogen sulfide production from lactococcal strains incubated with reduced glutathione (GSH).

	picomoles of H_2S/g (dry weight)									
	4hrs		8hrs		12hrs		24hrs		48hrs	
Lactococcus lactis				SE		SE		SE		SE
Z8	BQL [a]		BQL b		BQL [b]		36,000 [b]	4950	110,000 [b]	10000
1228	BQL [a]		40,000 [a]	1800	44,000 [a]	3700	140,000 [a]	8500	150,000 [a]	3050
LM0230	BQL [a]		BQL b		BQL b		38,000 [b]	7000	120,000 [b]	5000

1 Below quantifiable limits < 33,000 picomoles of H_2S/g (dry weight)

a,b,c Means within the same column followed by no common superscript letter differ ($P < 0.05$)

INTERIM REPORT

Growth of nonstarter lactic acid bacteria in reduced fat Cheddar cheese

Personnel

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

Funding

Dairy Management Inc.

Dates

July 1997– December 2000

Objectives

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

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

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

Summary

Microbial studies of ripening cheese reveal that numbers of starter bacteria decline during maturation while those of, while those of nonstarter bacteria (NSLAB; in particular lactobacilli) increase to levels of 10^7-10^8 CFU per gram of cheese. It is well established that starter, adjunct, and NSLAB can have a profound effect on the development of flavor in Cheddar cheese. The cause and effect relationship between these bacteria, however, has not been studied, nor is much known about mechanisms that enable these bacteria to maintain viability or proliferate in cheese. While the type and numbers of adjunct and starter bacteria can be controlled, the types of NSLAB still remain a matter of chance. It is the hypothesis of this project that certain adjunct bacteria can be used to control the NSLAB population to ensure proper flavor development. To test this hypothesis, we are investigating the effect of adjunct bacteria on the numbers and types of NSLAB in ripening cheese and the influence of cheese environment on NSLAB and adjunct populations.

The ability to address population dynamics between starter, non-starter, and adjunct bacteria during cheese ripening requires methodology that can detect and follow changes in that population, over time, at the strain level. Dr. Broadbent's group has found that random amplified polymorphic DNA (RAPD) fingerprinting by the polymerase chain reaction (PCR) can be used to differentiate between individual strains of *Lactococcus lactis*, *Lactobacillus casei*, and *Lactobacillus helveticus*. We have also been able to isolate bacterial DNA from commercial cheese and use this DNA as a template for the amplification of 16S rRNA genes to search for sequences from NSLAB population that cannot be cultured in the laboratory. These methodologies are now being used to analyze isolates collected from duplicate vats of 50% reduced-fat Cheddar and Colby cheeses that were manufactured with or without a *Lactobacillus paracasei* (lila) adjunct by Dr. Mark Johnson's group at the Center for Dairy Research. To date, we have prepared template DNA for PCR from isolates collected from each cheese by plating on Rogosa and Elliker's agar (8 vats total) at time 0 (press), 2 wks, and after 1, 2, 3, 4 and 6 mo of ripening. RAPD analysis of isolates from t = 2, 4 and 6 mo show that essentially all Elliker's isolates are the starter bacterium, *L. lactis* SCO213. In cheeses where lila was added, the adjunct clearly dominates NSLAB populations at 2 mo, but represents only 50% of isolates at 4 mo and was not detected after 6 mo. Heterogeneity clearly exists in the NSLAB population of our ripening cheeses, and DNA sequence analysis of 16S rDNA from some of these strains indicates that they are predominantly *Lb. paracasei,* but other species of lactobacilli are also present.

Lactobacillus casei strains capable of transforming D (-) -lactic acid to L (+)- lactic acid (racemase positive strains) were tested to establish specific enzyme activity; L and D-lactate dehydrogenases. There is evidence that both L and D-lactate dehydrogenase enzymes are responsible for the conversion of L-lactate to D- lactate. This needs to be confirmed. A separate racemase enzyme was not found. Both lactate dehydrogenase enzymes of *Lactobacillus casei* have been sequenced and work is now progressing to confirm that the sequences are identical to these enzymes found in other lactobacilli. Confirmation will help establish the roles of these enzymes in the growth of lactobacilli in cheese.

Lactobacillus casei is a predominant NSLAB found in Cheddar cheese, and is often added as starter adjunct in cheese manufacture. The substrates that enable *Lb. casei* to grow in ripening Cheddar cheese have yet to be determined. However, citrate (Cit) is thought to be a likely substrate for growth. We are investigating the possibity that citrate metabolism is required for growth of *Lb. casei* in ripening Cheddar cheese. A differential media has been developed that can screen Cit +/ Cit – phenotypes. Reaction between ferric ions and potassium ferricyanide due to utilization of citrate in the media gives a light to dark blue color to the Cit+ strains, and Cit- remain white. Ten strains of *Lb. casei* were identified by 16S rRNA sequencing, and were screened using the above media.

All of the 10 strains were determined as Cit+ when incubated at 37˚C for 48 hours under anaerobic conditions. *Lb. casei* ATCC 334 was determined to be transformable and having a strong Cit+ phenotype on the differential media.

Identification of a known citrate permease citP gene was attempted in *Lb. casei* via PCR. Degenerate primers resulted in amplification of a fragment of expected size from *Lb. casei* LB26R, ATCC 334 and Lila. Some of the subunits of citrate lyase were identified and sequenced from *Lb. Casei* ATCC 393 (Ed Dudley, Jim Steele's Lab). Work is now in progress to sequence the putative citP fragments obtained via PCR and also the other subunits of the citrate lyase. Subsequently, citP and citrate lyase will be inactivated via gene disruption resulting in a Cit - mutant of *Lb. casei* ATCC 334. Competition studies will be conducted to determine the ability of the Cit - mutant to grow in ripening Cheddar relative to its wild-type strain.

We will be establishing knowledge matrices relating flavor and the role of adjunct and non-starter microorganisms. We will do this by investigating the impact of adjunct bacteria on the growth of non-starter bacteria and flavor in reduced fat cheese.

FINAL REPORT

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

Personnel

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

Funding

Dairy Management Inc.

Dates

July 1996 – December 1998

Objectives

1. To determine the influence of a constant coagulant to casein ratio in the manufacture of 50 % reduced fat Cheddar cheese in which the milk was standardized by the addition of reconstituted nonfat dry milk.

Summary

This summary reports results from the last trial of this project. In previous trials, we observed a decrease in 12% soluble nitrogen when NDM was used to standardize whole milk for the manufacture of 50% reduced fat Cheddar cheese. In those trials, the amount of coagulant used was based on weight of milk. Therefore, less coagulant was used on a coagulant to casein basis as the amount of casein in the standardized milk increased. In this trial, the amount of coagulant was based on the amount of casein. Each vat of cheese was made with the same coagulant to casein ratio.

The final cheese making experiments were conducted in May 1999. Whole milk was standardized by the addition of reconstituted NDM (approximately 20% total solids). Total solids of the standardized milks were adjusted by adding water. Control milks were standardized by cream removal. The cheese making schedules were the same with one exception. The control milk (standardized by cream removal) was cut after a 50-minute set while the milks standardized by the addition of reconstituted NDM were cut in 40 minutes (to decrease moisture). Milk and cheese compositions are listed in Tables 1, and 2. Fat, nitrogen and solids recovery increased with increasing milk solids via NDM addition. However, fat recovery in the cheese was highest and nitrogen recovery in the cheese was lowest in milk standardized by cream removal (Table 6).

As expected, there is an increase in lactose in the milk with added reconstituted NDM, a concomitant increase in lactic acid in the cheese and a decreased pH (Table 3). Descriptive taste panels did detect an increase in acid flavor intensity with the higher acid cheeses (Table 7). Cheeses made from milk standardized by the addition of reconstituted NDM (above 10% solids) tended to become more grainy (mouthfeel) with age, softer (6 mo) but less smooth than cheese made from milk standardized by cream removal. There was no difference in Cheddar flavor intensity or preference between the cheeses at any tasting. However, these cheeses made with NDM were less preferred in both flavor and texture categories. Comments on the score sheets indicated the cheeses were less preferred due to brittle, grainy, crumbly body and unclean (stale casein) flavors.

The increase in milk solids retained in the cheese increased slightly when NDM was added. Most of this increase can be attributed to a higher nitrogen (protein) recovery.

As expected there was an increase in soluble nitrogen as the cheese aged but there were no differences in the 12 % TCA soluble nitrogen (indication of the extent of proteolysis) between the cheeses of similar age (Table 5).

The use of reconstituted NDM to standardize whole milk for the manufacture of 50% reduced fat Cheddar cheese has economic benefits but this must be weighed against potential decreased

Table 1. Pasteurized milk composition of whole milk standardized with reconstituted NDM for the manufacture of 50% reduced-fat Cheddar cheese.

Treatment (% solids)	Solids	Fat	Total Protein	Casein[1]	Lactose	C:F Ratio
			(%)			
control	10.26	1.49	3.14	2.46	4.56	1.65
NDM (10)	9.83	1.47	3.03	2.40	4.32	1.63
NDM (12)	11.49	1.79	3.61	2.85	4.98	1.59
NDM (14)	12.76	1.94	4.01	3.21	5.65	1.65

Table 2. Composition of 50% reduced fat Cheddar cheese made with whole milk standardized with reconstituted NDM. Compositional analysis completed on 2 week old cheese.

Treatment (% solids)	Moisture	Fat	Protein[1]	Salt	Lactic acid	Lactose	Galactose
				(%)			
control	47.73	16.31	29.12	1.43	1.92	.01	.01
NDM (10)	48.30	15.77	28.25	1.63	1.72	.07	.04
NDM (12)	48.15	16.20	28.55	1.38	1.84	.43	.02
NDM (14)	48.88	16.00	28.57	1.24	1.90	.65	.03

Table 3. Cheese pH of 50% reduced fat Cheddar cheese made with whole milk standardized with reconstituted NDM.

Treatment (% solids)	1day	2 wk	6 wk	13 wk	26 wk
control	5.11	5.05	5.05	5.13	5.25
NDM (10)	5.20	5.12	5.14	5.15	5.19
NDM (12)	5.17	5.07	4.98	4.96	5.08
NDM (14)	5.15	5.01	4.97	4.91	4.88

Table 4 Mean r-value, actual yield, and Van Slyke numbers for 50% reduced fat Cheddar cheese with whole milk standardized with skim milk or condensed skim milk.

		Van Slyke Recovery Values		
	Actual Yield	RF	RC	RS
control	8.20	89.75	.96	1.158
NDM (10)	8.06	86.90	.96	1.167
NDM (12)	9.77	88.41	.96	1.173
NDM (14)	10.88	89.62	.96	1.153

Table 5 12% TCA Soluble N as a percent of total nitrogen in cheese

	2 weeks	6 weeks	13 weeks	26 weeks
control	3.55	7.11	12.72	15.20
NDM (10)	3.61	7.56	13.28	16.15
NDM (12)	3.40	7.48	13.27	16.14
NDM (14)	3.09	6.67	12.05	14.36

preference by the consumer. Use of NDM (and increased solids) resulted in cheeses that did not age well (texture and flavor preferences were lower). Lower total solids could overcome some of the texture problems but not eliminate them. The results indicate that the decreased proteolysis observed in previous trials was due to lower coagulant levels.

Table 6. Mean percentages of mass, fat, and N recoveries of 50% reduced fat Cheddar cheese with whole milk standardized with skim milk or reconstituted NDM milk.

Treatment (% Solids)	Cheese	Whey	Pressed whey	Total
	Mass recovery (%)			
control	8.20	88.87	2.35	99.42
NDM (10)	8.06	88.97	2.30	99.33
NDM (12)	9.77	86.73	2.55	99.05
NDM (14)	10.88	84.89	2.35	98.12
	Fat recovery (%)			
control	89.75	9.90	.39	100.04
NDM (10)	86.90	12.43	.72	100.05
NDM (12)	88.41	10.78	.63	99.82
NDM (14)	89.62	9.83	.72	100.17
	N Recovery (%)			
control	75.37	23.37	.64	99.38
NDM (10)	75.36	22.05	.59	98.00
NDM (12)	77.18	22.51	.67	100.36
NDM (14)	77.60	21.95	.61	100.16
	Solids Recovery (%)			
control	40.58	57.14	.84	98.71
NDM (10)	41.10	56.77	1.33	99.20
NDM (12)	42.92	56.73	1.53	101.18
NDM (14)	42.47	56.34	2.03	100.84

Table 7. Descriptive taste panel results for 50% reduced fat Cheddar cheese with whole milk standardized with skim milk or condensed skim milk at 1.5, 3 and 6 mo of aging. Eighteen panels were conducted, each consisting of 6 to 10 experienced judges. Data are the means of the 6 taste panels conducted at each age.

Treatment (% solids)	Cheddar flavor intensity[1]	Acid flavor intensity[2]	Bitter flavor intensity[3]	Off-flavor intensity[3]	Flavor preference[4]	Body[5] Breakdown[6]	Body	Graininess[3]	Texture preference[4]
	Cheese at 1.5 mo of age								
control	2.9	3.6	1.3	2.1	4.5	5.0	3.9	1.7^{ab}	1.7
NDM (10)	2.7	3.5	1.2	2.1	4.7	4.7	4.2	1.4^{b}	5.0
NDM (12)	2.8	3.6	1.3	2.1	4.6	5.1	4.0	1.5^{ab}	4.8
NDM (14)	2.7	3.8	1.3	2.3	4.3	5.2	3.9	1.9^{a}	4.6
	Cheese at 3 mo of age								
control	3.7	3.8	1.5	2.7	4.3	4.8^{a}	4.4	1.6	4.8
NDM (10)	3.5	3.8	1.5	2.7	4.4	4.1^{b}	4.7	1.4	4.9
NDM (12)	3.3	3.7	1.4	2.6	4.2	5.1^{a}	4.3	1.7	4.7
NDM (14)	3.5	4.0	1.4	2.5	4.2	5.4^{a}	4.2	2.0	4.4
	Cheese at 6mo of age								
control	4.5	4.0	1.6	3.6	3.7	3.6^{bc}	5.1^{a}	1.4	4.6^{a}
NDM (10)	4.6	4.0	1.8	4.0	3.3	3.2^{c}	5.2^{a}	1.2	4.1^{ab}
NDM (12)	4.4	4.2	1.6	3.4	3.7	4.1^{ab}	4.4^{b}	1.7	4.4^{ab}
NDM (14)	4.4	4.7	1.5	3.5	3.3	4.6^{a}	4.0^{b}	2.6	3.8^{b}

APPLICATIONS PROGRAM REPORT

Cheese applications program

Personnel

Carol Chen, coordinator of cheese applications program, John Jaeggi, coordinator of CDR/ industry projects, Mark Johnson, senior scientist,Amy Dikkeboom, research specialist, Rani Govindasamy-Lucey, researcher, Bill Hoesly, research cheese maker, Kristen Houck, research specialist, Juan Romero, associate researcher, William Tricomi, assistant researcher, Matt Zimbric, research specialist, Wisconsin Center for Dairy Research

Funding

Wisconsin Milk Marketing Board

Dates

January 1999–December 1999

Objectives

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

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

3. Direct contact with industry to meet informational needs.

Summary

In addition to Wisconsin cheese industry activities, the 1999 Cheese Applications program annual report includes national cheese industry interactions. Approximately 75% of the work conducted by the Cheese Applications Program is for Wisconsin-based companies. Table 1 summarizes the Cheese Applications Program clients. In 1999, we worked with 60 Wisconsin and 42 national cheese industry clients. For Wisconsin, 75% of those clients are cheese manufacturers, which is similar to 1998 figures. On a national level, 35% of our interactions involve cheese manufacturers. The large number of interactions demonstrates the commitment between the Wisconsin Center for Dairy Research and the cheese industry.

A summary of technical transfer activities (cheesemaking, laboratory work, visits, consultations) can be found in Table 2. This past year we worked directly with Wisconsin cheese manufacturers to develop manufacturing protocols for cheeses, which target specific flavor profiles, texture and/or functional characteristics. For example, we outlined manufacturing protocols, demonstrated cheesemaking in the CDR pilot plant, then assisted in the commercial scale-up of several specialty Italian, English and other varieties of cheese. We worked directly with cheese manufacturers and end users to tailor manufacture Cheddar and Mozzarella cheeses for appetizer and pizza applications. For these projects it was critical to clearly understand the desired melt characteristics to ensure cheese functionality.

This past year we noticed an increase in laboratory work. The Cheese applications group conducts analytical, microbiological, applications and sensory testing on various cheese samples. Wisconsin 1998 and national 1999 figures were similar; 75% of laboratory work being completed in conjunction with CDR pilot plant cheesemaking. However, for Wisconsin companies in 1999, more than half of the cheeses analyzed were commercially manufactured. This shows that the cheese industry is placing a greater emphasis on understanding how the cheese composition/age affects the physical properties and thus the functionality of the cheese in the end application.

In 1999 we noted an increase in the number of companies visiting the CDR, and in CDR personnel traveling within the state of Wisconsin. We hosted several industry groups at the CDR to discuss application programs and current cheese research topics. CDR personnel traveled to cheese plants to provide one-on-one technical transfers of cheesemaking protocols, milk standardization and other cheese technology issues.

We noted several trends in cheese industry technology transfer requests. Controlling cheesemaking parameters, milk standardization, cheese yield, cheese defects and developing protocols for specialty cheeses continue to be of

Table 1. Cheese Applications Program Clients

Client	Wisconsin	National
Cheese Manufacturer	47	15
End User	9	8
Ingredient Supplier	5	7
Communications	4	1
Equipment Manufacturer	4	0
Organization	3	2
Consultant	3	1
Contract Lab	2	1
Milk Producer	1	1
Total	60	42

industry concern. Increasingly, questions are directed toward the use of UF technology in cheesemaking, methods of controlling and measuring cheese melt, and enhancing cheese functionality through the addition of whey proteins.

Publications and Presentations

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

"Wisconsin Center for Dairy Research Cheese Applications Program." by John Jaeggi at the Northwest Dairy Technology Society. January 1999.

"A newly developed melt test procedure." by Matt Zimbric, Carol Chen, S. Gunasekaran, Juan Romero at the 1999 Annual ADSA Meeting, Memphis, TN. June 1999.

"Lower fat Swiss cheese: Development of accurate Swiss-type flavors through adjunct / culturing." by Amy Dikkeboom, Carol Chen, Kristen Houck, Mark Johnson, Robert Lindsay at the 1999 Annual ADSA Meeting, Memphis, TN. June 1999.

"Comparative study of milk standardization methods and initial milk solids levels in the manufacture of 50% reduced-fat Cheddar cheese." by Carol Chen, Amy Dikkeboom, Mark Johnson at the 1999 Annual ADSA Meeting, Memphis, TN. June 1999.

"Wisconsin Center for Dairy Research Cheese Applications Program" by John Jaeggi at the Southwestern Wisconsin Cheesemakers Association Annual Meeting. October 1999.

Table 2. Cheese Applications Program Technical Support

Activity	Wisconsin	National
Cheese making in the CDR pilot plant	Worked with 9 companies: 5 manufacturers, 3 ingredient suppliers, 1 equipment manufacturer. Sixteen cheese making dates: 30% manufacturers, 70% ingredient suppliers & equipment manufacturers. Manufactured a wide variety of cheeses: Beaufort, Caerphilly, Cheddar, Cheshire, Fontina, LMPS Mozzarella	Worked with 3 companies: 2 ingredient suppliers, 1 consultant. Six cheese making dates Evaluated new ingredient functionality and demonstrated Mexican-style cheese manufacturing protocols.
Analytical, applications or sensory work	Worked with 22 companies: 14 manufacturers, 3 ingredient suppliers, 2 equipment manufacturers, 2 end users, 1 consultant. Thirty-eight sets of analyses: 40% of cheese manufactured in CDR pilot plant, 60% of cheeses commercially manufactured. Types of analyses: Composition, chemical, sensory, microbiological, physical properties, cheese functionality in end application.	Worked with 5 companies: 2 end users, 2 ingredient suppliers, 1 consultant. Eight sets of analyses: 75% of the cheese manufactured in the CDR pilot plant, 25% of the cheeses commercially manufactured. Types of analyses: Composition, chemical, sensory, physical properties, cheese functionality in end application.
CDR or Onsite visits	Met with 34 companies: 25 cheese manufacturers, 4 equipment manufacturers, 2 ingredient suppliers, 2 end users and 1 milk producer. Forty-four visits: 50% visits to the CDR, 50% onsite visits. CDR visits included discussion on current research, cheese applications, general cheese technology and methods of evaluating physical properties of cheese. Onsite visit included assisting in the scale-up of specific cheese varieties, discussions of milk standardization and cheese yield and general cheese technology questions.	Met with 4 companies: 3 ingredient suppliers, 1 end user. Four visits: 100% visits to the CDR. CDR visits included discussion on current research, cheese application program, general cheese technology
Consultations	Worked with 57 companies (34 manufacturers, 7 end user, 4 ingredient suppliers, 4 communications, 3 organizations, 3 consultants, 2 contract laboratories, 1 equipment manufacturer) Ninety-one different cheese topics discussed. Discussed general cheese technology issues, milk standardization, cheese yield, controlling the meltability of cheese, cheese defects, cheese process control, UF and RO technology, labeling/nutrient claims issues.	Worked with 31 companies (15 manufacturers, 6 end user, 4 ingredient suppliers, 2 communications, 2 organizations, 1 contract laboratories, 1 milk producer. Forty-three different cheese topics discussed. Discussed general cheese technology issues, milk standardization, cheese yield, controlling the meltability of cheese, cheese defects, cheese process control, UF and RO technology.

FINAL REPORT

Extending the cheese net paradigm to include economic evaluation and optimization in cheese manufacture

Personnel

John P. Norback, professor, Dept. of Food Science

Funding

Wisconsin Milk Marketing Board

Dates

January 1998–January 1999

Objectives

1. Devise an economic context for technical information about cheese.Exploit this context by developing and testing the models for usefulness and accuracy.

2. Create optimization models to assist in decision-making regarding the formulation of cheese and other dairy products.

3. Build a spread sheet implementation of the optimization models.

Summary

The cheese net flow of materials paradigm provides a context for determining materials used in dairy processing and the order of processing. This information is a base for organizing ingredient input costs and selling prices for cheese processing outputs, including cheese and various forms of processed whey. This approach, coupled with technical information about converting dairy inputs to cheese, has produced an optimization model for dairy product manufacture.

Interviews with dairy experts provided initial values for optimization coefficients, necessary to make a model. The challenge has been to interpret and extract the information necessary for optimization.Constraints that represent a mass balance are a key feature to any optimization of this sort.

We have built prototype optimization models and placed them in a spreadsheet to represent the Cheddar cheese making process. After creating test objective functions and applying optimization methods, in this case linear programming, the models look very promising. In addition, mass balance and component balance constraints have been built into these optimization models. The mass balance approach means that all outputs from processing are considered simultaneously during optimization. No output is considered a "by-product." In particular, whey is considered one of the valuable outputs from the cheese making process.

A model for Cheddar cheese has been created. Component balance constraints have been developed to allow the decision-maker to control measurable qualities of Cheddar cheese. For example, constraints for moisture, fat, various solids and fat in the dry matter are all part of the optimization model. The outputs from these models are realistic and show promise to help dairy processors make more efficient use of their resources. This model demonstrates the importance and economic impact of cheese milk standardization. It also provides a way to value whey output products.

In addition, we created a model for ice cream. This model includes constraints that allow the user to manipulate quality measures such as fat content, protein content and freezing point depression. The cheese and ice cream models have been coupled with other constraints to provide a 'product mix' optimization model. This model allows the incorporation of many such models into one overarching context. This will allow the decision maker to analyze plant wide utilization of incoming resources to achieve optimal production amount targets.

Publications/presentations

Norback, J. P., "Making Cheese Choices" presented at Cheese Technology Meeting, April, 1999, LaCrosse, Wisconsin

Barcenas, C. and J. P. Norback, "Spreadsheet strategies for diary optimization." Presented at the IFT annual meeting, July, 1999.

Spread Sheet Strategies for Optimal Management of a Dairy Plant,Copyright © by Candelaria Barcenas, 1999.Available at the Memorial Library, University of Wisconsin, Madison.

INTERIM REPORT

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

Personnel

R. C. Lindsay, professor, Meral Kilic, research assistant, Department of Food Science, University of Wisconsin-Madison

Funding

Dairy Management Inc.

Dates

January, 1997–December, 1999
Extended to June, 2000

Objectives

1. To identify the consumer acceptance-limiting brothy/umami off-flavor substance in the water soluble fraction of aged fat-free Cheddar cheese, determine its mechanism for formation, and develop means for its control in fat-free Cheddar cheese.

2. To develop basic technology for optimizing the desirable flavor properties, especially the fatty acid-based cheesiness flavor, in fat-free Cheddar cheese that is developed by the unique lipase-positive lactobacilli, *Lactobacillus casei* Lila strain.

Summary

Fat-free Cheddar cheese initially exhibits a bland flavor and rubbery texture, and upon aging it develops protein-like or brothy/umami type flavors that are often accompanied by various other off-flavors that are often associated with wild lactobacillus fermentations. Studies showed that quite high levels of glutamic acid generally accumulate in aged full-fat Cheddar, and similar levels also accumulate in fat-free Cheddar where the glutamate flavor dominates, partly because of the lack of balancing flavors. Thus, the absence or inadequate amount of influential flavor compounds in fat-free Cheddar cheese appears to be the key to consumer-acceptance limiting brothy flavor in fat free cheese.

Whey applications program

Personnel

Kimberlee J. Burrington, coordinator, Karen Smith, researcher, Center for Dairy Research

Funding

Wisconsin Milk Marketing, Board, Dairy Management, Inc.

Dates

January 1999–December 1999

Objectives

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

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

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

Summary

This year completed the second year of the Whey Applications program. In1999, the Whey Applications program was in contact with 17 Wisconsin-based companies and 41 national companies, consisting of whey processors, ingredient suppliers, and end-users. Activities were increased both in applications and processing support.

Whey applications were developed and presented at the following events, seminars, and companies: World Wide Food Expo, World Dairy Expo, Producer Value Showcases, the USDEC Latin American/CDR mission, Snaxpo, Pillsbury, WI Cheesemakers conference, CDR Mexican Cheese Seminar, and IFT. Applications development focused on energy bars, marshmallow, caramels, dulce de leche, yogurt, and smoothies. General whey processing, functionality, and applications information was presented 9 times over the course of the year.

Membrane processing support was initiated for the UF Cheese project commissioned by the Cheese Industry team this year. A project involving processing support for further processing of whey and quality improvements of whey for a member of the CDR Cheese Industry team was also in progress and will continue this year. Other processing support has involved further development of the whey processing pilot plant, with the purchase of a microfiltration/ultrafiltration unit, repair of an existing high temperature short time unit, and the ordering of ion exchange equipment and a spray dryer. Funding approval is underway for a pilot scale evaporator also. A cream cheese separator was also purchased to do cream cheese development with the CDR Cheese group. Many of the needs of the whey processors and end-users have been informational needs. Typical requests are for standard methods for chemical and functional analysis, specifications, whey ingredient sources, literature searches, formulations for specific applications, and processing troubleshooting questions.

Presentations

Whey Applications in Cultured Dairy Products, Chr. Hansen Cultured Dairy Products Symposium, Kimberlee J. Burrington. May 11-12, 1999, Milwaukee, WI.

Composition of Whey Ingredients, Latin American Candy Mission, Whey Applications in Confections, Karen Smith. March 15, 1999, Madison, WI.

Functionality of Whey Ingredients, Latin American Candy Mission-Whey Applications in Confections, Kimberlee Burrington, March 15, 1999, Madison, WI.

Whey Processing Effects on Functionality, DMI Seminar, Pillsbury Company, Karen Smith, July 15, 1999, Minneapolis, MN.

Functionality of Whey Ingredients, DMI Seminar, Pillsbury Company, Kimberlee Burrington, July 15, 1999, Minneapolis, MN.

Processing of Whey into Value-added Ingredients, USDEC Asian Mission on Whey Processing and Applications, Karen Smith, August 8, 1999

Whey Ingredient Functionality and Applications, USDEC Asian Mission on Whey Processing and Applications, Kimberlee Burrington, August 8, 1999.

Membrane Processes, CDR Seminar, Karen Smith, November 24, 1999, Madison, WI.

Ultrafiltration for Cheesemaking, Grande Cheese Annual Meeting, December 8, 1999, Fond du Lac, WI.

APPLICATIONS PROGRAM REPORT

Dairy marketing and economics program

Personnel

Brian W. Gould, senior scientist, Wisconsin Center for Dairy Research, Reyes Aterido, graduate student, Ulan Asanov, graduate student, Dept. of Agricultural and Applied Economics

Funding

Wisconsin Milk Marketing Board, University of Wisconsin-Madison, Hatch Funds

Dates

December 1998–December 1999

Summary

In 1992 researchers at the Wisconsin Center for Dairy Research developed a computer program (CHYIELD©) to analyze the use of alternative milk standardization procedures. CHYIELD© included a number of features that allowed for a sensitivity analysis of variables associated with the cheese making process. However, CHYIELD© had several weaknesses. In July, 1998 we started to upgrade and improve CHYIELD©. The resulting program, *EACY*© is near completion. All of the features contained in the original CHYIELD© program are incorporated within *EACY*© . Figure 1, contains an overview of the characteristics of this new and improved software program.

We currently have a test version of EACY©. Several cheese plants have been given copies of this software and we plan to incoporate suggestions from these plants when we convert the beta into a final version. The final step of this project consists of the development of an extensive on-line help system. A computer programmer is currently assisting with this phase of software development. We anticipate that we will have the program available for purchase by the end of the first quarter of 2000.

Development of Market-Related Information Systems for Dairy Industry Participants
There is no doubt that the marketing environment faced by both dairy manufacturers and farm operators has changed dramatically over the last decade. Figure 2 shows the MW/BFP since 1965. Prior to 1988, this figure shows the close correspondence between the BFP and U.S. milk price supports. With a more market oriented U.S. dairy policy, over the last decade the variability (risk) faced by dairy farm operators and processors is obvious. In response to this increased market risk, dairy industry participants are using some new tools to help control this risk –using forward milk pricing arrangements to lock in raw milk input costs (output price) and using dairy-based futures and options. Through the support of the Wisconsin Milk Marketing Board we established the University of Wisconsin Dairy Markets web site, (http://www.aae.wisc.edu/future) in 1998. The objective of this web site was to house within a single location information resources, data and specialized software that can be used by the Wisconsin dairy industry to more effectively produce market both raw milk and manufactured dairy products.

During 1999, this web site has been greatly expanded to include a more extensive collection of current and historical data associated with dairy markets. We have also included a more detailed graphical analysis of the U.S. dairy market which is updated daily. These analyses provide a quick and easy means of understanding current trends that influence milk and manufactured dairy product pricing.

Besides being a data archive for dairy producers and processors looking for information, this web site contains a number of software applications that assist with controlling dairy price risk and marketing of milk and dairy-based products. These applications can be divided into two general areas; spreadsheet models to help understand pricing under Federal Order Reform and models designed to help the dairy industry use dairy-based futures and options to control their price risk. For example, an interactive tutorial system can be downloaded from the above site to allow both producers and manufacturers understand how dairy-based futures and options can be used to control both input and output price risk. There are also a number of spreadsheet-based models covering the use of alternative price risk management strategies that can be downloaded from our site.

Figure 1. About Eacy©

EACY© is a user-friendly computer program designedto:

♦ Identify the components of your raw milk & track them in primary & secondary products

♦ Predict your cheese yield for alternative milk composition & standardization procedures

♦ Evaluate the economic consequences of changes in milk quality, cheese characteristics or market conditions

♦ Allow you to control your cheese yields by adopting alternative standardization strategies

EACY© is designed to be flexible. Users of this software can enter many types of data including:

♦ Raw milk components: total protein, casein, fat & lactose

♦ Cheese characteristics: fat, casein & other solids retention factors, final moisture content, price

♦ Standardization agent profiles: total protein, casein, fat, other solids, price

♦ Characteristics of your whey-based products: price, product composition, availability as a primary or secondary product, compatibility with other by-products

EACY © can help you manage your cheese yield data within a single database system.

♦ Enter data only once

♦ Easily modify previously entered data

♦ Match current & future production profiles

EACY © can generate a variety of analyses of your cheese yield. Examples include:

♦ Sensitivity analysis: Changes in milk composition and the impact on standardization methods

♦ Economic impact of product changes: cheeses produced, types of by-products

♦ Yield impacts of alternative standardization targets: fat content, FDB, casein-to-fat ratio

EACY © can accommodate future changes:

♦ Design new cheeses and whey by-products

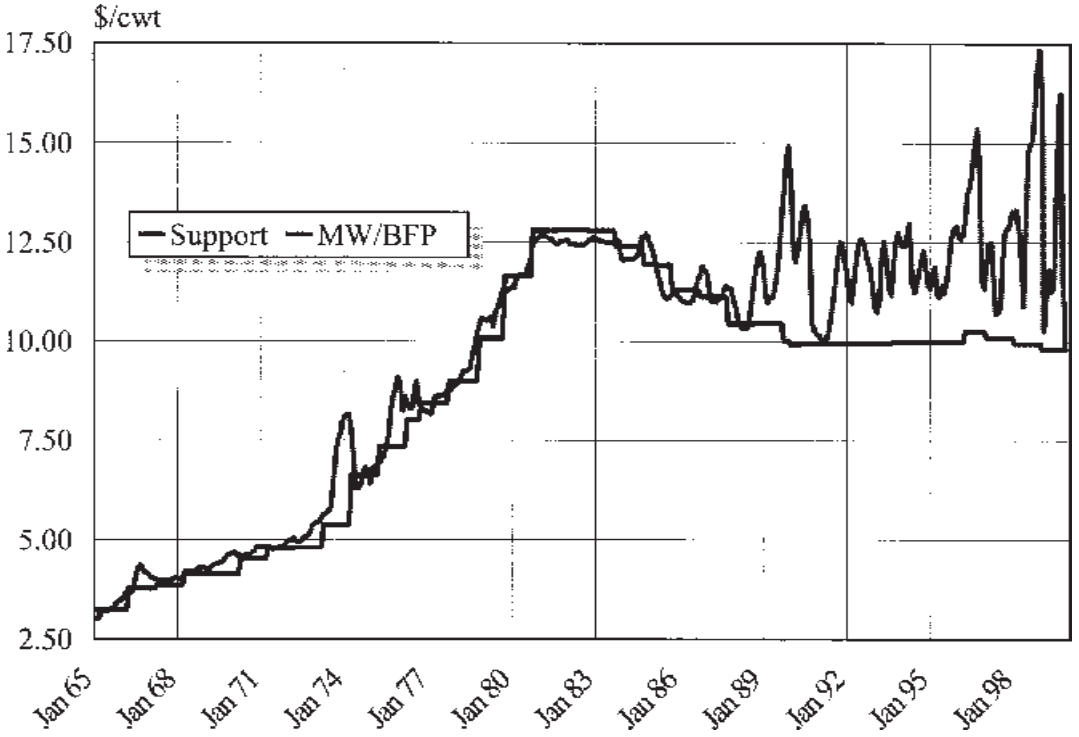
♦ Specify alternative standardization procedures

♦ Alternative dairy-based ingredients as suggested under proposed CODEX rules

Adoption of HACCP by the cheese industry

The third area of research undertaken in the Dairy Marketing and Economics Program is an analysis of the principles of Hazard Analysis of Critical Control Points (HACCP) and how they can be an integral part of the dairy product production process. The first step towards adopting these principles is the development of a HACCP plan reflecting individual production environment. Over the last year we developed a spreadsheet based system that will help small/medium dairy product manufacturers examine their product processes for critical control points (CCP's) and then develop a HACCP plan to monitor these CCP's. This software system is built on a number of menu driven options that allow the user to enter both production related data and points where production hazards might occur. We are close to having a prototype model completed and will be testing the spreadsheet model in the near future.

Figure 2. Relationship between support and MW/BFP



INTERIM REPORT

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

Personnel

Brian W. Gould, senior scientist, Wisconsin Center for Dairy Research, D. Dong, researcher, formerly with the Wisconsin Center for Dairy Research,W.S. Chern, professor, Ohio State University, B.K. Goodwin, professor, North Carolina State University, R. Mittlehammer, professor, Washington State University,T.I. Wahl, professor, Washington State University

Funding

U.S. Department of Agriculture, National Research Initiative Competitive Grants Program, Babcock Institute for International Dairy Development, University of Wisconsin Madison

Dates

October 1998–September 2001

Objectives

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

2. Identify an appropriate methodology to analyze disaggregated food (dairy product) demand in developing and developed countries that overcomes the limitations imposed by earlier two-stage estimation procedures.

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

4. Apply this methodology to a single county to verify its ability to accurately describe the structure of international food demand.

Summary

Understanding how food consumption responds to changes in relative prices, income, household composition and other exogenous factors is important for U.S. farm operators, processors, policy analysts and policy makers looking to expand the markets for U.S. farm products. This can be said not only with respect to domestic but also foreign markets. Given the recent economic problems in the former Soviet Union, Latin America and Asia, an understanding of the structure of food demand in current and potentially important export markets is more important than ever.

A good example of the importance of understanding the nature of international food demand can be found in the U.S. dairy sector where growth in domestic demand is relatively flat.The U.S. share of world dairy trade is small and competing dairy producing countries are attempting to increase their dairy export efforts. Previous research has indicated that the U.S. dairy industry will increase its export activity under trade liberalization via the passage of the North American Free Trade Agreement and the Uruguay Round Agreements of the General Agreements on Tariffs and Trade and the elimination of domestic dairy price supports after 1999. With reduced trade barriers, we need to understand the structure of dairy product demand in potentially new export markets. It will be important to quantify the sensitivity of consumption levels to changes in household income, the impact of changes on market price, the role of age/sex composition of households on dairy product consumption and the implications of future changes in these variables. This research project attempts to answer such questions not only for dairy products, but also for other foods that compete for dairy products' share of the consumer's food budget.

Previous analyses focused on identifying the determinants of the type of food (dairy products) and how much was purchased have used historical time-series (annual, quarterly, or in some cases, monthly) data and prices, incomes, and per-capita consumption. The inferences yielded by such analyses are important and useful. However, these inferences may be influenced by significant structural adjustments characterizing individual economies. An alternative approach to the estimation of demand system parameters uses cross-sectional data collected from individual households. This approach to demand analysis has several advantages. Cross-sectional analyses

make possible richer, more detailed inferences drawn from more detailed disaggregated household level data. In particular, the use of cross-sectional surveys enables the researcher to evaluate demographic and socioeconomic factors that are relevant to food demand issues. Perhaps the most important factor is that cross-sectional consumption data often provide the degrees of freedom necessary to permit an analysis to be conducted using very recent data. This advantage is important when considering food demand in countries that have undergone significant changes, such as the Eastern European countries, Latin America and China.

This project uses a number of international household expenditure/purchase survey data sets to evaluate, compare, and contrast dairy product and other food demand conditions in several countries important to US agricultural exports. The following general approach to the analysis and estimation of food demand parameters will provide a coordinated research effort across team members. First, a demand system comprised of aggregate commodity classes (e.g., beef, pork, vegetables, fruits, grains, dairy products) will be estimated. Second, less aggregated models permitting a greater degree of detail will be evaluated for the commodities of particular interest in this study. Demographic effects and the censoring imposed by observed zero purchases will be considered in the estimation process. Accounting for the censored nature of disaggregated food demand is essential for obtaining unbiased estimates of the structure of such demand.

For this project we emphasize the use of a demand system approach to characterize the food choices made by consumers. We use the system approach as it allows one to quantify the trade-offs associated with the consumption of one type of food versus another. Using household-level data for demand analysis requires the applied researcher to account for the censoring of commodity purchases. When analyzing food demand one commodity at a time, there are a number of fairly standard econometric techniques that can be used to address this censoring. The statistical issues become much more complex when expanding the analysis from a single equation approach to a demand system framework. This project will extend basic research to an analysis of food (dairy product) demand in a number of developed and developing countries.

Since starting this project we have developed a unique econometric model that accounts for both the censoring of commodity demands within a systems framework as well as addressing the issue of how to evaluate commodity prices for non-consuming households. An increasing number of cross-sectional surveys of food purchase behavior, especially those associated with developing countries, include both quantity and expenditure data. Division of observed expenditures by quantity (here referred to as unit-value) is often used as an estimate of a commodity's price. This method of calculating price reflects not only differences in market prices faced by each household but also differences in endogenously determined commodity quality. For example, observed differences in price paid for cheese across households may be reflecting not only local market conditions but also final product form. Households purchasing cheese in block form would be expected to pay a lower price than households purchasing cheese that is pre-sliced or shredded. The portion of product price determined by market forces is obviously beyond the control of the consumer.

During Phase I of this project we developed a methodology that will allow researchers to consistently estimate purchase price for non-consuming households along with the other food demand parameters. Using our demand system approach, researchers can investigate the determinants of the multi-stage purchase process faced by the consumer: whether-or-not to purchase a particular commodity versus the decision as to the amount to purchase given that an individual does indeed purchase the commodity. A detailed review of our methodology can be found in Gould and Dong (1999) and in Dong and Gould (2000). Some preliminary investigations of the structure of food demand in Mexico and the Former Soviet Union undertake as part of the project can be found in Gould and Kim (1998a,b).

Using our econometric structure, we have undertaken some preliminary model testing using Canadian food expenditure data. In this test case we present the results obtained from a five-commodity system composed of aggregate beef, pork, poultry, cheese and fluid milk. The example shown here is meant only to be illustrative. The expenditure data used in this preliminary testing was obtained from the nationwide 1992 *Canadian Family Food Expenditure Survey*. This survey con-

tains two-week diaries of food expenditures and quantity purchased where each expenditure item is coded according to a four-digit food code. In addition to purchase information, other data included in the survey are household member age distribution, pre-tax household income, male and female head country of birth, residential province, degree of urbanization and month during which the survey was undertaken. There are 10,848 households in the base data set. For this analysis we use a random sample of approximately 3,000 households. Table 1 provides an overview of data used in the analysis.

From the implementation of our econometric model to Canadian food demand, we estimate uncompensated price and expenditure elasticities (Table 2). These elasticities quantify the percent change in quantity purchased of each food as a result of a percent change in a variety of explanatory variables such as own price, other goods prices, and household income. All of the own-price elasticities are negative, statistically significant and of reasonable values compared to previous studies. We are currently developing the methods to separate the total impact of price

Table 1. Characteristics of Canadian Data Used With Preliminary Model Specification

Purchase Characteristics						
Commodity	Percent Purchasing	Bi-Weekly Expenditure Shares(%)		Bi-Weekly Expenditures ($) (CND$)		Price
		Uncond.	Conditional	Uncond.	Conditional	
Milk (litre)	92.5	33.2	35.9	10.29	11.12	1.10
Cheese(kg)	72.1	19.7	27.4	7.42	10.29	8.97
Beef (kg)	68.0	24.5	36.0	12.09	17.78	6.45
Pork (kg)	39.2	8.6	22.0	4.20	10.73	6.19
Poultry (kg)	51.1	13.9	27.3	6.78	13.25	5.21

Characteristics Used in Translating Functions		Household Characteristics Used In Unit-Value Equations		
Variable	Mean	Variable	Mean	Description
QRTR 1	0.251	HH Inc ($)	42,350 (28,555)	Annual Pre-Tax Income
QRTR 2	0.252	HH Size	2.68 (1.39)	Household residents eating out of household food supply
QRTR 3	0.246	Metro	0.618	Household located in urban area with population 100,000+
Single HH	0.210	Quebec	0.197	Dummy variables identifying province of residence
Two NOKD	0.252	Ontario	0.239	
		Manitoba	0.062	
		Sask	0.081	
		Alberta	0.081	
		BC	0.099	

Note: The conditional expenditure shares do not sum to one as these values are conditional on there being positive shares. The unconditional shares and expenditures are calculated as the mean across households. QRTR variables identify quarter data was collected. Single HH identifies single person households. Two NOKD identifies two-person households with no children.

(expenditure) changes on unconditional demand into the component impacts on conditional demand and market entry. This is similar to the decomposition shown by McDonald and Moffitt in their analysis of Tobit results. This decomposition will allow us to identify the relative importance of the market entry versus conditional demand price (expenditure) responses.

Comparing the results obtained under our approach, we present price and expenditure elasticities obtained from the estimation of two alternative model specifications: traditional Linear Approximate Almost Ideal Demand System (LA/AIDS) without accounting for censored quantity distributions and Heien and Wessells (1990) two-step censored demand system. Under the first alternative model we estimate the LA/AIDS model using the traditional SUR specification (referred to as the "uncensored" model). Similar to other researchers, we estimate missing prices using independent unit-value regression equations for consuming households. Under the Heien and Wessells (1990) specification, a SUR estimation procedure is used where all (including observations with zero-valued shares) are used. A series of explanatory variables calculated from univariate probit models of the decision-to-purchase stage are used as explanatory variables to account for censoring. Again, missing unit-values are calculated from a set of exogenous price regressions.

We obtained significant and negative own-price elasticities under the two alternative models. The most surprising result was the similarity of the own-price elasticities in spite of significantly different parameter values generated under our censored demand system. The similarity of the results between the censored versus uncensored systems is in contrast to the results obtained by Heien and Wessells (1990) who found, for a number of commodities, reduced own-price elasticities for their censored versus uncensored commodities with the difference being proportional to the degree of censoring. These are also surprising in light of the fact that we are using endogenous unit-values to estimate demand function parameters under our censored system while the other two procedures use the more traditional exogenously estimated unit-values. The cross-price elasticities were also very similar across model specification with the exception of the influence of milk price on cheese and beef purchases. Under all three specifications we found a complementary relationship but the size of the price response was much larger under our specification. Considering the similarity of the results obtained under the three specifications versus the differences found by Heien and Wessells (1990) in their analysis, points to the need for examining the robustness of our results to more disaggregated commodity definitions.

Future research

One problem that needs to be addressed is reducing the computation time required to estimate the econometric model. Our current algorithm is developed using the GAUSS software system. Given the short time since our initial funding (5 months) we developed the code necessary to obtain parameter estimates with little consideration for computational speed. We plan a number of approaches to improving computation speed during the last half of our current funding. First, we will modify our current computer code. Under the current configuration there are major sections of the GAUSS code that could be improved by the eliminating numerical loops and replacing them with algorithms based on faster matrix procedures. Second, we are currently using an algorithm developed by Breslaw to evaluate higher order integrals. We will experiment with alternative approximating algorithms to determine if there could be significant reductions in computational time. Finally, we will investigate whether computational time could be reduced if an alternative software system such as MATLAB or Mathematica were used to optimize the models likelihood function.

We currently have obtained food (dairy product) expenditure/purchase data for Canada, Mexico, Brazil, China and Eastern Europe. We are attempting to obtain 1996 data for Argentina. With this data, we will use the econometric lessons learned under Phase I of this project to better identify the important determinants of the structure of food (dairy product) consumption in thesecountries.

Presentations/publications

B.W. Gould and D. Dong, 1999. *Estimation of Censored Demand Systems with Endogenous Unit Values*, Working Paper, Department of Agricultural and Applied Economics, University of Wisconsin-Madison, October

D. Dong and B.W. Gould, 2000. *Quality Versus Quantity in Mexican Household Poultry and Pork Purchases, forthcoming*, Agribusiness.

Brian W. Gould and J. Kim, 1998. *The Structure of Meat, Poultry and Dairy Product Demand in the Former Soviet Union*, Babcock Institute Discussion Paper, Babcock Institute for International Dairy Development, University of Wisconsin-Madison, October.

Brian W. Gould and J. Kim, 1998. *Characteristics of Canadian and Mexican Dairy Product Purchases: A Comparison Using Household Expenditure Data*, Babcock Institute Discussion Paper, Babcock Institute for International Dairy Development, University of Wisconsin-Madison, July.

Mcdonald, J.F., and R.A. Moffitt, The Uses of Tobit Analysis, Review of Economics and Statistics, Vol.62:318-321.

Heien, D., and C. R. Wessells. Demand Systems Estimation with Microdata: A Censored Regression Approach. J. Bus. and Econ. Statis. 8(1990):365-71.

Breslaw, J.A., Evaluation of Multivariate Normal Probability Integrals Using a Low Variance Simulator, Review of Economics and Statistics, Vol. LXXVI:673-683.

Table 2. Comparison of estimated uncompensated price and expenditure elasticities under our preliminary censored system with other published model specifications

Commodity	Price Elasticities					Expenditure Elasticities
	Fluid Milk	Cheese	Beef	Pork	Poultry	
			Censored System			
Fluid Milk	-0.737*	-0.027*	-0.039*	0.008	0.023*	1.023*
Cheese	-1.456*	-0.464*	-0.038	0.105	-0.095	0.637*
Beef	-1.341*	-0.040	-0.690*	-0.104*	0.078	1.032*
Pork	0.325	0.021	-0.200*	-0.849*	-0.161*	1.100*
Poultry	0.307	-0.075*	0.021	-0.077*	-1.081*	1.221*
			Heien and Wessells			
Fluid Milk	-0.832*	-0.021	-0.163	0.117	-0.156	1.058*
Cheese	-0.097*	-0.581*	-0.094*	0.092	-0.252*	0.462*
Beef	-0.142*	0.039	-0.679*	-0.173*	-0.174*	0.975*
Pork	0.019	0.067	-0.072*	-0.896*	-0.078	0.882*
Poultry	-0.006	0.033	0.033	-0.021	-1.079*	1.740*
			Uncensored System			
Fluid Milk	-0.808*	-0.109	-0.201*	0.075	0.005	1.058*
Cheese	-0.136*	-0.571*	-0.106*	0.012	-0.105	0.542*
Beef	-0.153*	0.047	-0.686*	-0.312*	-0.060	1.093*
Pork	0.022	0.055	-0.103*	-0.804*	-0.069	1.125*
Poultry	0.017	0.037	0.002	-0.096	-1.039*	1.268*

Note: * identifies elasticities significant at the 0.01 level of significance. Elasticity variances are calculated using Monte Carlo methods. The elasticities were evaluated at the mean values of the exogenous variables. The price elasticities reflect the impacts on unconditional quantity demanded.

INTERIM REPORT

Development and application of a cheese shred/texture map delineated by cheese rheological, sensory and chemical analysis

Personnel

Carol M. Chen, researcher, Juan E. Romero,associate researcher, Mark Johnson, senior scientist, Brian Gould, senior scientist, Wisconsin Center for Dairy Research, Sundaram Gunasekaran, professor, Biological Systems Engineering

Funding

Wisconsin Milk Marketing Board and Dairy Management Inc.

Dates

July 1999–December 2001

Objectives

1. To develop a shred / texture map of cheeses based on rheological, sensory and chemical measurements.

2. To define manufacturing protocols of Cheddar and Mozzarella, tailored for shredding.

Summary

We started to work on this project in November,1999 by procuring the cheese shredding equipment. Electrical installation was then completed in the dairy plant. In order to do our tests, we are now setting up a controlled environment. The rheometer has been set up and we have begun optimizing our testing parameters (temperature, capstan diameter) using commercial cheese samples. We anticipate beginning work on objective 2 during calendar year 2000.

APPLICATIONS PROGRAMREPORT

CDR specialty cheese applications program

Personnel

Jim Path, outreach specialist, John Jaeggi, assistant researcher

Funding

Wisconsin Milk Marketing Board

Dates
January 1999–December 1999

Objectives

1. Continue developing the artisan workshops, a module of the Wisconsin Master Cheesemaker® program.

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

3. Manage the Wisconsin Master Cheesemaker® program.

4. Develop a cheese database.

Summary

The third class of certified Wisconsin Master Cheese makers graduated and were honored at the CDR/WCMA ceremony in La Crosse, Wisconsin on April 14, 1999. Eight cheesemakers received this honor during the ceremony, which was widely covered by the media. CDR has featured cheeses by Wisconsin Master Cheesemakers at several meetings and events.

Eight more Wisconsin Master Cheesemakers (class of 2000) were certified by the Master Cheese maker Board on December 9, 1999. They will be officially recognized at the CDR/WCMA ceremony on April 27, 2000. The class of 2001 has completed the second round of cheese sample testing in the apprenticeship phase of the program. The board received 12 new applications and approved 12. These 12 (class of 2002) have completed the oral exam and plant visit phase of the apprenticeship.

A 2nd Cheeses from Mexican/Latin America Cheese Seminar was held on April 22-24, 1999. Instructors from Mexico, CDR and a consultant with experience in Costa Rica and Mexico presented information about the manufacture of Mexican cheeses. Production of cheeses was demonstrated in the dairy plant.

On Feb 22-23,1999 the Wisconsin Process Cheese Course was held as part of the Wisconsin Master Cheese makers curriculum. This unique course, the only course of it's type in the USA, was filled to capacity.

On May 4-5, 1999 the Wisconsin Dairy Plant Water & Waste Management Short course was held at Babcock Hall in Madison. This is a joint effort between CDR and Agricultural Extension.

The CDR World Cheese Exchange is now available on CDR's website. This database lists over 1400 different varieties of cheese and we encourage submissions from cheesemakers as we continue adding photos and data.

chapter 2,section 2

Cheese safety

Microbiological safety of reduced fat and fat free pasteurized process cheese products 81

Safety/quality applications program 83

INTERIM REPORT

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

Personnel

Eric A. Johnson, professor, Kathleen A. Glass, researcher, Food Research Institute, University of Wisconsin-Madison

Funding

Dairy Management, Inc.

Dates

January 1997–December 1999

Objectives

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

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

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

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

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

Summary

Previous research in our laboratory revealed that 5% fat and fat free pasteurized process cheese products delayed toxin production by *Clostridium botulinum* when compared with full fat products with similar moisture, pH, and total salts. The objective of this study was to evaluate the effects of cheese-base type, fat, 0.05% monolaurin, 1.5% Cheddar enzyme modified cheese (EMC), 1.5% sodium lactate, and 3% ß-glucan fat replacer on the botulinal toxin production in pasteurized process cheese products.

To evaluate the effect of type of natural cheese from which it was derived, process cheese products were formulated using full fat Cheddar, 30% reduced fat Cheddar, or skim milk cheese, respectively, and standardized to 59% moisture, pH 5.8, 3 or 4% total salts (sodium chloride+disodium phosphate), and 15-19% fat using anhydrous milk fat. Subsequent trials evaluated the effect of adjunct ingredients in process cheese products formulated to <1, 10, and 20% fat when made with skim cheese, reduced fat and full fat cheese, respectively.

In trials evaluating cheese-base type (fat standardized to 15-19%) botulinal toxin production was delayed several days in 15-19% fat products formulated with skim cheese compared with reduced fat or full fat cheese. However, the effect was not statistically significant ($p>0.05$). When fat levels were not standardized, botulinal toxin production was significantly delayed in products made with skim cheese (<1% fat) compared with reduced fat (10% fat) or full fat (20% fat) cheese. Reducing fat in skim milk-process cheese product formulations from 15 to <1% fat resulted in a 2-week delay for botulinal toxin production.

In a previous reporting period, we formulated fat free process cheese products with high moisture skim milk cheese. The formulation was adjusted to 68% moisture, pH 5.8, and 3% total salts (disodium phosphate+sodium chloride). Inoculated formulations were supplemented with 1% EMC, 1.5% sodium lactate, 0.05% monolaurin or 4% ß-glucan fat-replacer. No difference in time to detectable toxin production was observed among the four treatments and the control without supplement, likely due to the high moisture of the product. We repeated the tests in fat free, reduced fat, and full fat products formulated to 62% moisture, pH 5.7%, 3 or 4% total salts, and 1.5% Cheddar EMC (sample previously shown to inhibit botulinal growth in media), 1.5% sodium lactate, 0.05% monolaurin or 3% ß-glucan fat-replacer.

Sodium lactate significantly delayed toxin production for all cheese types tested, however the fat-replacer did not delay growth. Botulinal toxin production was delayed 11, 14, and 47 d for full fat, reduced fat, and fat free process cheese products formulated with 1.5% sodium lactate, respectively. No delay in toxin production was detected in products formulated with ß-glucan. Monolaurin and EMC significantly delayed toxin production in skim cheese products, but had less effect in reduced fat and full fat products. Monolaurin did not delay time to toxin production in reduced fat and full fat products but the number of toxic samples was fewer. However, botulinal toxin production was delayed two weeks in fat free product formulated with monolaurin. Addition of 1.5% EMC did not delay toxin production in full fat cheese, but delayed toxin production 3 and 67 days for reduced fat and fat free products, respectively. One should use caution in interpreting the effect of EMC because antibotulinal activity may be dependent on the method used to produce the EMC.

These results verify that reduced fat process cheese products manufactured with fat free and reduced fat cheese may exhibit greater stability than full fat products and that safety may be enhanced by using certain adjunct ingredients.

Process cheese and related foods and spreads account for over 2 billion pounds of dairy food in the United States. Traditionally, the microbiological safety of these products relies on formulation to inhibit toxin production by *Clostridium botulinum*. In order to produce organoleptically acceptable reduced fat and fat free process cheese products, microbial control factors such as moisture, salt, and pH are often adjusted to more permissive conditions. This raises safety concerns among the dairy industry and regulators.

Preliminary research at FRI suggests that *C. botulinum* toxin production is delayed in reduced fat and fat free process cheese products compared with full fat products with similar levels of moisture, salt, and pH. However, the mechanism of inhibition is unknown. The objective of our study is to identify factors that control botulinal toxin production in process cheese products which will permit greater flexibility in formulating safe products that appeal to the consumer. This in turn, will increase dairy consumption.

APPLICATIONS PROGRAM REPORT

Safety/quality applications program

Personnel

Marianne Smukowski, coordinator

Funding

Wisconsin Milk Marketing Board

Dates

January 1999–December 1999

Objectives

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

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

3. Assist in executing national safety/quality program

4. Conduct technology development targeted at WI cheese manufacturers

5. Assist the Wisconsin Master Cheesemaker Program®

6. Participate and assist in UW and industry sponsored courses

Summary

The Safety/Quality Applications Program assists Wisconsin dairy manufacturers in the following areas: safety/quality audits, GMP reviews, developing HACCP plans, aid the WI Master Cheesemaker program®, and provide technical support in regulatory matters. A total of 30 plant visits were made this year and numerous phone calls were answered to address S/Q audits, HACCP implementation, and regulatory issues. I am a member of the NCIMS laboratory committee, which addresses the use of FDA 2400 forms and laboratory practices. I reviewed several grade standards and specifications for cheese including Swiss and Muenster. These changes to the grade standards would affect WI manufacturers.

One of the major accomplishments of the Safety/Quality program was the offering of a dairy HACCP workshop. The class enrollment was originally 45 people due to the breakout sessions. However, the enrollment had to be expanded to 55 to accommodate the overwhelming response for this first time workshop.

Publications and presentations

WI cheese grading short course, Italian cheese evaluation (twice a year)

Intercollegiate Dairy Products Evaluation Contest, Lead Butter Judge

WI CIP Workshop, Plant Sanitation Audits

WI Dairy Products Assoc. Cheese and butter evaluation clinic, Overview of butter Grading

GMP presentation, Kerry Ingredients, Vesper, WI

Dairy HACCP Workshop, Phase II, Green county, WI

1999 WI State Fair Judge for butter and cheese products

Intro to Codex, WDATCP All Employee Meeting, WI Dells, WI

Importance of HACCP Implementation, Managing Dairy Food Safety Workshop, Madison, WI

Overview of HACCP, USDA Dairy Division All Employee meeting, Mpls, MNHACCP and the Dairy Industry: An Overview of International and U.S. Experiences by B. Gould, M. Smukowski and J. R. Bishop

chapter 3

Fluid milk

Identification and characterization of components of the proteolytic enzyme system of *Lactobacillus helveticus* which affect bioactive peptide accumulation 87

Application of milk powders in milk chocolate, butter and butter spreads 88

Growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder 89

INTERIM REPORT

Identification and characterization of components of the proteolytic enzyme system of *Lactobacillus helveticus* which affect bioactive peptide accumulation

Personnel

James L. Steele, professor, Jeff Pederson, post-doctoral researcher, Dept of Food Science, UW-Madison, Bart Weimer, associate professor, Jeff Broadbent, assoc. professor, Utah State Univ.

Funding

Dairy Management Inc.

Dates

June 1997–June 2000

Objectives

1. To screen strains of *Lactobacillus helveticus* for the type and level of bioactive peptides/bioactive peptide precursors which accumulate as the result of the organism's growth in milk.

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

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

Summary

Our progress towards objective one involved developing analytical techniques for the rapid identification of peptides. Specifically, progress has been made in the area of coupling capillary chromatography with mass spectroscopy. The coupling of these pieces of equipment should allow us to rapidly screen strains of *Lactobacillus helveticus* for the type and level of bioactive peptides which accumulate as a result of organism growth in milk. Research towards objective two has focused on the cell-envelope proteinase specificity of various *Lactobacillus helveticus* strains. To date, the proteinase specificity of eight strains has been determined using the alpha S_1-casein fragment (f1-23) as a substrate for hydrolysis. However, *Lactobacillus helveticus* has unique cell surface proteinase specificity. The gene encoding a cell-envelope proteinase from *Lactobacillus helveticus* CNRZ32 was cloned by PCR amplification. Primers were designed based on the nucleotide sequence of the proteinase gene from *Lactobacillus delbruickii* subsp. *bulgaricus*. The entire *Lactobacillus helveticus* CNRZ32 proteinase gene (called prtH) has been sequenced. It shares 45% identity at the amino acid level with the proteinases of lactococci and prtH has been classified as a new group, designated group I. A *prtH*-negative CNRZ32 strain has been constructed via gene replacement. Importantly, there still is proteinase activity on the surface the *prtH*-negative CNRZ32 derivative and there is no difference in acidification or growth rate in milk compared to wild-type CNRZ32. This will allow us to examine these isogenic derivatives which differ only in cell surface proteinase activity/specificity for the accumulation of bioactive peptides during growth in milk. Then, we can determine if proteinase specificity has an essential role in the accumulation of bioactive peptides/bioactive peptide precursors from casein during growth of *Lactobacillus helveticus* CNRZ32 in milk.

The intent of this project is to begin developing the knowledge required to select/construct strain of lactic acid bacteria which will enhance the level of casein-derived bioactive peptides produced by digestion of fermented milk products.

Publications/Presentations

Christensen, J.E., E.G. Dudley, and J.A. Pederson, J.L. Steele. 1999. Peptidases and amino acid catabolism in lactic acid bacteria. Antonie van Leeuwenhoek 76:217-246.

Pederson, J.A. and J.L. Steele. 1999. Characterization and physiological role of a cell-envelope associated proteinase from *Lactobacillus helveticus* CNRZ32. J. Bacteriol. 181:4592-4597.

INTERIM REPORT

Application of milk powders in milk chocolate, butter and butter spreads

Personnel
RW Hartel, professor, Baomin Liang, assoc. researcher, Dept of Food Science

Funding
Dairy Management Inc.

Dates
June 1999–June 2000

Objectives
Use of milk powders as specialized ingredients in chocolate and confectionery products can be enhanced through a better understanding of the factors that influence the physical and chemical properties, sensory qualities and storage stability of chocolates. The specific objective is:

1. To compare the effects of free fat and particle structure in milk powders on chocolate quality, processing requirements and storage stability. This will involve measurement of molten chocolate rheology, conditions needed to properly temper the chocolates, measurement of chocolate hardness, sensory characteristics and stability to fat bloom.

Summary

The milk chocolate industry is a major user of milk powders, with the most common ingredient in the US being spray-dried milk powder. However, the chocolate industry prefers roller-dried whole milk powder since it gives better and more economic chocolate. The qualities of a milk powder important to milk chocolate include free fat and particle structure (size, shape, air content, crystallinity, etc.). These properties have a large impact on the economics of milk chocolate production as well as on the physical, sensory and storage characteristics.

In this project, the effects of type of milk powder on milk chocolate qualities will be investigated and documented. A variety of powders will be obtained with a range of free fat and particle structure. Chocolates will be evaluated for a range of physical and chemical, sensory and storage (bloom) properties.

FINAL REPORT

Growth and biocontrol of enterotoxigenic *Bacillus cereus* in infant formula and processed cheese prepared with milk powder

Personnel

Amy C. Lee Wong, associate professor, John B. Luchansky, associate professor, Amy B. Ronner, research specialist, Alan J. Degnan, senior research specialist, Dept. of Food Microbiology and Toxicology; Mark E. Johnson. senior scientist, Center for Dairy Research

Funding

Dairy Management Inc.

Dates

July 1997–June 1999

Objectives

1. Determine the potential for growth of *B. cereus* and enterotoxin production in rehydrated infant formula and processed cheese spread at refrigeration and abuse temperatures.

2. Validate the effectiveness of bacteriocins against *B. cereus* in rehydrated infant formula and in processed cheese spread during storage.

Summary

Five *B. cereus* strains were used for these studies: strains B4-ac, F4433/73, and FM-1 were originally isolated from diarrheal outbreaks and were tested as a three-strain cocktail; strains HRM44 and D1 were isolated from dairy products and were tested individually. Three types of powdered infant formula [low iron ("S"), and iron-fortified with ("GS") and without ("SF") maltodextrin] and steam distilled "infant drinking water" were purchased from a local grocery store chain. Formulas were rehydrated with infant drinking water according to label directions, which was approximately 10 g powder per 60 ml water. Each of the three types of rehydrated infant formula was added to sterile 500 ml glass bottles, inoculated with two target levels of spores, 10 cfu/gram and 1000 cfu/gram powder, and incubated at three refrigeration temperatures (4, 8, and 12°C) and one abuse temperature (25°C). Inoculated formulas were assayed for bacterial numbers immediately after inoculation, at 8 hr, 24 hr, and 2, 3, 5, 7, and 10 days.

Both the three-strain cocktail and strain HRM44 grew readily in the three formulas at 25°C . Cell numbers increased by at least 4 logs by 24 hr. Rehydrated formula containing the three-strain cocktail became overtly spoiled after 48 hr, however formula containing HRM44 did not become noticeably spoiled until 72 hr. Another difference between HRM44 and the three-strain cocktail was the ability of HRM44 to grow at 12°C. HRM44 grew most rapidly in the iron-fortified formula and most slowly in the low-iron formula, however by day 10 all bacterial counts were 10^6/ml or higher. After 10 days at 4 and 8°C, counts of both the three-strain cocktail and HRM44 either remained the same or decreased slightly. Like HRM44 and the three-strain cocktail, strain D1 was unable to grow in any formula at 4°C. However, this strain did grow readily at 12°C in all formulas, and at 8°C in SF and GS formulas by day 5. These data indicate that there are strain differences in the growth characteristics of *B. cereus*. Although none of the strains we examined could grow in rehydrated formula at 4°C, one strain did grow at 8°C and thus could potentially produce enterotoxin even while refrigerated, if stored for long periods. Analysis with an ELISA we developed showed that hemolysin BL, a diarrheal enterotoxin produced by *B. cereus*, could be detected when growth exceeded 10^6 cfu/ml. We also inoculated all three types of formula with a low level of spores (5.9 cfu/gram powder) and incubated the samples at 25°C. Growth to 10^5/ml and higher occurred by 24 hr.

We considered the possibility that consumers might rehydrate infant formula but not use it immediately, or that unused portions of formula might be left unrefrigerated, and outgrowth of

any *B. cereus* spores present in the powder could occur. We conducted pre-incubation experiments using one type of rehydrated infant formula (SF) at a spore inoculum target level of 1000 cfu/gram, and held the inoculated formula at 25°C for 6 hr before storing it at 4, 8, or 12°C. Pre-incubation of the three-strain cocktail allowed outgrowth of the spores to greater than 10^5 cfu/ml after 48 hr of subsequent storage at 12°C, even though growth would not normally occur at this temperature. Similarly, strain HRM44, which grew less than 1 log in 10 days when incubated at 8°C, grew to 10^6 cfu/ml or higher when pre-incubated at 25°C. No growth was observed at 4°C.

We investigated the use of several bacteriocins active against *B. cereus*, and found that nisin was the most widely effective against the strains of *B. cereus* used in our studies. It is also the easiest to use since it is commercially available in a standardized formulation. We examined the effectiveness of a commercial nisin preparation, which contains 2.5% active nisin in milk solids, against 1000 cfu/gram *B. cereus* spores in rehydrated SF formula. We found that 0.05% nisin was sufficient to prevent outgrowth of 10^3 spores of strain HRM44 per gram of formula for 5 days. The three-strain cocktail was slightly more resistant to nisin, and although the spores were not completely inactivated, a level of 0.1% nisin could reduce outgrowth to less than 10^2 cfu/ml at up to 5 days at 25°C. Our data show that the addition of nisin to powdered infant formula can reduce the potential for *B. cereus* growth in rehydrated formulas.

We produced processed cheese spread from a cheese blend containing aged and new Cheddar, unsalted butter, nonfat dry milk, whey powder, and whey protein concentrate. The cheese blend was inoculated with spores of strain HRM44 or the three-strain cocktail, to concentrations of 7.5 x 10^4 cfu/gram to 2.4 x 10^5 cfu/gram, respectively, and heated in a steam-jacketed cooker to a maximum temperature of 88°C. The melted cheese was poured into sterile glass vials in approximately 20 ml portions, tightly capped, and incubated at 8. 12, or 25°C for up to 6 months. At regular intervals the cheese samples were assayed for *B. cereus*. Neither HRM44 nor the three-strain cocktail grew in the processed cheese during 6 months of storage. Because spores of these strains were unable to grow in the product, studies examining the effect of nisin in processed cheese spread were not conducted.