1998

1998 Annual Meeting

Various Authors

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<td>Calcium intake among adolescents in Utah</td>
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<td>Bart C. Weimer</td>
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</table>
Improvement of low-fat Cheddar cheese through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese, Utah State University portion.
PI: Jeff Broadbent

Development of a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in Cheddar cheese.
PI: Charlotte Brennand

Injection of fluids and microorganisms into cheese.
PI: Conly Hansen/Carl Brothersen

Thermal inactivation of milk clotting enzymes.
PI: Marie Walsh

Whitening of skim milk using a continuous-flow, immobilized enzyme reactor.
PI: Marie Walsh

Commercialization of skim milk technology
PI: Carl Brothersen/Robert Fife

Lactococcal nasal vaccine
PI: Bruce Geller

The use of bacteriophage-receptor genes of Lactococcus lactis to develop bacteriophage-resistance in Cheddar cheese starter strains.
PI: Bruce Geller

Influence of milk fat interfacial material and area on the physical and sensory properties of fat-reduced cheese.
PI: Lynn V. Ogden

Improvement of Mozzarella cheese functionality by understanding exopolysaccharide production in thermophilic starter cultures
PI: Jeff Broadbent

Biopolymers: A value added product derived from low value whey and lactose; part 1
PI: Janine Trempy
Western Dairy Center
Activities Summary

The Western Dairy Center is a consortium of researchers devoted to improving the dairy industry in the United States by conducting research in all areas of dairy foods. The Center includes researchers from Utah State University, University of Idaho, Oregon State University, Brigham Young University and Weber State University.

In 1997 a new contract was negotiated between the Western Dairy Center and Dairy Management Inc. (DMI). This new contract brought the following changes to the Western Dairy Center.

- The name of the center was changed from the Western Center for Dairy Protein Research and Technology to the Western Dairy Center.
- The research focus was changed from dairy proteins to the DMI national research plan.
- The Center changed from an academic year to a calendar year. To accomplish this DMI extended Center funding from the end of the academic year on June 30, 1996, to December 31, 1996. Funding under the new contract began January 1, 1997.

This report covers research activities from January 1, 1996 to December 31, 1997, when projects under the new contract began. During this period twenty-two research projects were conducted.

The Center Annual Meeting was held on August 27-28, 1998, Sun Valley, Idaho. A large group representing both dairy producers, processors and researchers attended and provided significant input onto the future direction of the Center.

The Center conducted the 13th Annual Cheese Management Short Course on Feb. 11-13, 1997, at Utah State University.

At the request of industry representatives, the Center established the Swiss Cheese Consortium. The purpose of the consortium is to conduct research and solve problems related to Swiss cheese manufacture and marketing. Currently there are nine industry representatives participating with the Consortium.

Waterford Foods Inc. donated a new temperature controller to the Center for installation on the pasteurizer in the Utah State University pilot plant in February 1997. The controller will facilitate cheese research at the Center by allowing the pasteurizer to be used for processing fluid milk and ice cream mixes
while providing cheese researchers to select the pasteurizer conditions they desire for cheese making.

The Center is in the process of commercializing products and technologies from five projects. These products include three new starter cultures, a new manufacturing procedure for Mozzarella cheese and a new technology for manufacture of skim milk.

Ten new Center projects were approved for funding by DMI in 1997. These projects include work on Cheddar cheese flavor, improvement of skim milk, improvement of Cheddar and Mozzarella cheese manufacturing procedures, improved bacteriophage resistance of Cheddar starter cultures and improved Cheddar and Mozzarella cheese starter and adjunct cultures. Six projects were funded by Center funds.
WESTERN DAIRY CENTER

OPERATIONAL ADVISORY COMMITTEE

Pursuant to the Western Dairy Center proposal and contract with the National Dairy Promotion and Research Board, the voting members of the Operational Advisory Committee are:

Bob Champion
Dairy Management Inc
10255 W. Higgins Road, Suite 900
Rosemont, IL 60018-5616

Gary L. Clawson
Utah Dairy Commission
375 S. 800 W.
Hyrum, UT 84319

Rodney J. Brown, Dean
College of Agriculture
Utah State University
Logan UT 84322-4800

Donald McMahon, Director,
Western Center
Department Nutr. & Food Sciences
Utah State University
Logan UT 84322-8700

James Moran
Kraft, Inc.
Research and Development Div.
801 Waukegan Road
Glenview IL 60025

Kevin Gillies
Marschall Products
P.O. Box 592
Madison WI 53701

Raj G. Narasimmon
Schreiber Foods, Inc.
P.O. Box 19010
Green Bay WI 54305

Mr. Carl E. Zurborg
Swiss Valley Farms
P.O. Box 4493
Davenport IA 52808

Gale Moser
United Dairymen of Idaho
1864 South Hulls Crossing
Preston ID 83263

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Department of Nutr. & Food Sciences
Utah State University
Logan UT 84322-8700

Bruce Geller
Dept. of Microbiology, Nash 220
Oregon State University
Corvallis OR 97331-3804

Lawrence Welch
Western Dairy Farmers Promotion Assoc.
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Thornton, CO 80233-0120

Barney Krueger
Avonmore West, Inc.
1341 Fillmore
Twin Falls, ID 83301

Lance Williams
Tillamook County Creamery Association
P.O. Box 313
Tillamook, OR 98414
OPERATIONAL ADVISORY COMMITTEE
(Continued)

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Gist Brocades
10 W. 300 S.
Millville, UT 84326

Bob Ramsayer
Holmes Cheese, Inc.
9444 State Route 39
Millersbury, OH 44654

Don Jensen
Western Dairymen Coop
P.O. Box 26427
Salt Lake City, UT 84126-0427

Gene Hong
Brewster Dairy, Inc.
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Brewster, OH 44613

Roy Leach
Chr. Hansen’s Laboratory, Inc.
9015 W. Maple St.
Milwaukee, WI 53214

Chuck Ellis
Pearl Valley Cheese Company
54760 T.R. 90
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Kraft Foods Inc.
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Glenview, IL 60025

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Logan, UT 84321

John Rothenbuhler
Hans Rothenbuhler and Son Inc.
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Middlefield, OH 44062-0757

Ted Van Tuyle
Swiss Valley
P.O. Box 38
Luana, IA 52156

Tom Rank
Chr. Hansen’s Laboratory, Inc.
9015 W. Maple St.
Milwaukee, WI 53214
WESTERN CENTER FOR DAIRY PROTEIN RESEARCH AND TECHNOLOGY

PRINCIPAL INVESTIGATORS

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Dept. of Microbiology  
Weber State University  
Ogden, UT 84408

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Moscow ID 83843

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Logan UT 84322-8700

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Dept. of Nutrition & Food Sciences  
Utah State University  
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Logan UT 84322-8700
Ilka Nemere  
Dept. of Nutrition & Food Sciences  
Utah State University  
Logan UT 84322-8700

Frost M. Steele  
Dept of Food Science & Nutrition  
Brigham Young Univeristy  
Provo UT 84602-5140

Tilak R. Dhiman  
Animal Dairy & Veterinary Sciences  
Utah State University  
Logan UT 84322-4815
NATIONAL DAIRY PROMOTION AND RESEARCH BOARD $417,618

REGIONAL/INDUSTRY SUPPORT:

- Utah Dairy Commission $50,000
- United Dairymen of Idaho 50,000
- Western Dairy Farmers' Promotion Association 10,000
- Swiss Valley Farms 26,622
- Department of Defense 115,000
- USDA 50,000
- Kraft General Foods, Inc. 5,000
- Schreiber Foods, Inc. 5,000
- Marshall-Rhone Poulenc, Inc. 5,000
- Tillamook Co. Cream. Assoc 5,000
- Avonmore West, Inc. 5,000
- Waterford Food Products Inc 5,000
- Chr. Hansen's Laboratory Inc. 10,000
- Kraft Foods 5,000
- Great Lakes Cheese 5,000
- Swiss Valley 5,000
- Gossner Foods 5,000
- Holms Cheese 5,000
- Pearl Valley 5,000
- Hans Rothenbuhler & Son 5,000
- Western Dairymen 5,000
- Brewster Dairy 5,000

TOTAL REGIONAL/INDUSTRY SUPPORT $386,622

FY97 TOTAL DAIRY RESEARCH CONTRIBUTIONS $804,240

FY97 COMMITTED RESEARCH FUNDS

- DMI funds ($417,618)
- Western Center funds (340,223)

TOTAL FY96 COMMITTED RESEARCH FUNDS ($757,841)

FY97 BALANCE FORWARD $46,399
Financial Summary of Approved Projects for 1997

<table>
<thead>
<tr>
<th>Project Description</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appetite Suppressing Properties of a Peptide From Milk - Deborah Campbell</td>
<td>$45,455</td>
</tr>
<tr>
<td>Calcium intake among adolescents in Utah - Deborah Campbell</td>
<td>$7,500</td>
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<tr>
<td>Identification and characterization of components of the proteolytic enzyme system of Lactobacillus helveticus which effect bioactive peptide accumulation, Utah State University part - Bart C. Weimer</td>
<td>$18,200</td>
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<tr>
<td>Microbial catabolism of methionine to improve Cheddar cheese flavor - a comparative study of the relative contribution by starter cultures and adjunct bacteria - Bart C. Weimer</td>
<td>$58,800</td>
</tr>
<tr>
<td>Conversion of amino acids to short and branched-chain fatty acids by starter and adjunct bacteria - Bart C. Weimer</td>
<td>$14,000</td>
</tr>
<tr>
<td>Investigate the role of metabolic cross-feeding between starter, brevibacterium and nonstarter cheese bacteria in the production or removal of aromatic off flavor compounds - Bart Weimer</td>
<td>$9,500</td>
</tr>
<tr>
<td>Growth of non-starter lactic acid bacteria in reduced fat Cheddar cheese, Utah State University part - Jeff Broadbent</td>
<td>$23,469</td>
</tr>
<tr>
<td>Improvement of low-fat Cheddar cheese through identification and characterization of microbial enzymes responsible for conversion of aromatic amino acids into off flavor compounds in cheese, Utah State University portion - Jeff Broadbent</td>
<td>$30,593</td>
</tr>
<tr>
<td>Improvement of low-fat Cheddar cheese through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese, Utah State University portion - Jeff Broadbent</td>
<td>$36,710</td>
</tr>
<tr>
<td>Development of a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in Cheddar cheese - Charlotte Brennand</td>
<td>$7,100</td>
</tr>
<tr>
<td>Injection of fluids and microorganisms into cheese - Conly Hansen/Carl Brothersen</td>
<td>$9,650</td>
</tr>
<tr>
<td>Thermal inactivation of milk clotting enzymes - Marie Walsh</td>
<td>$11,418</td>
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</tbody>
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Financial Summary of Approved Projects for 1997 continued

Whitening of skim milk using a continuous-flow, immobilized enzyme reactor - Marie Walsh $16,000

Commercialization of skim milk technology - Carl Brothersen/Robert Fife $16,000

Lactococcal nasal vaccine - Bruce Geller $5,200

The use of bacteriophage-receptor genes of Lactococcus lactis to develop bacteriophage-resistance in Cheddar cheese starter strains - Bruce Geller $71,130

Influence of milk fat interfacial material and area on the physical and sensory properties of fat-reduced cheese - Lynn V. Ogden $47,500

Improvement of Mozzarella cheese functionality by understanding exopolysaccharide production in thermophilic starter cultures - Jeff Broadbent $44,610

Biopolymers: A value added product derived from low value whey and lactose; part 1 - Janine Trempy $10,000

Identification of the causes of splits and cracks in Swiss cheese - Carl Brothersen $40,000

Rapid detection of pathogens - Bart Weimer $115,000

Evaluation of electro heating technology for UHT processing of milk - Donald McMahon $50,000
Western Dairy Center
Project Report
Reporting Period July 1, 1997 — June 30, 1998

Principal Investigators: Deborah R. Campbell
Co-Investigators: 

Project Title: Calcium Intake Among Adolescents in Utah

Institution’s Project #: 98098

Project Completion Date: 7/31/98


Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
The objective of this project is to create a dietary survey that will accurately and reliably assess calcium intake among Caucasian and Hispanic adolescent boys and girls in Utah.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Recent declines in consumption of dairy products in the United States has caused concern among health professionals regarding the future health of the nation. Osteoporosis is a major public health concern, and can be prevented by consuming adequate amounts of dairy foods during the first three decades of life. National survey data show that intake of dairy foods and calcium among adolescents is below recommended intakes. Only 50% of children ages 6-11 years and 26% of children ages 12-19 met the current RDA for calcium intake, which is 1300 mg calcium or 4 servings of dairy foods per day. The primary and best absorbed form of calcium in the American diet is present in dairy foods such as milk, yogurt, and cheese.

The purpose of this project is to create a dietary survey that will accurately and reliably assess dairy food and calcium intake among Caucasian and Hispanic adolescents in Utah. To date, there has not been developed a valid or reliable dietary survey for the measurement of dairy food or calcium intake among adolescents in the U.S. The adolescent age span represents a critical time of bone growth and is an important time for the establishment of dietary and lifestyle patterns. Insights gained about these vulnerable
populations and effective ways to motivate them to change dietary behavior could have wide ranging implications for public health. If future education campaigns can promote appropriate dietary behavior relative to consumption of dairy and calcium-rich food sources among adolescents, future benefits for the dairy industry may arise as the marketability of dairy foods will greatly increase. In addition, this project will yield a tremendous amount of information on two understudied populations - adolescents and Hispanics.

This project will consist of three phases. Phase I will involve the creation of a list of commonly consumed dairy and calcium-containing foods. Phase II will be dietary survey development. Phase III will be dietary survey testing.

Specifically, it is proposed that a unique dietary survey will be optimal for measuring adolescents' intakes, and that estimates of calcium and dairy food intake using the newly created survey will be more accurate.

1. Significant Progress against Objectives:

During the first five months of this project we have accomplished a number of preliminary goals and will begin data collection this summer. First, our primary effort was to decide on appropriate and unique diet assessment methods for adolescents. This was accomplished by discussions with other Nutrition researchers across the country and literature review. Since there has not been much research conducted in this area, discussions with researchers who are actively doing data collection is imperative. A standardized protocol established by the U.S. Department of Agriculture's Continuing Survey of Food Intake In Individuals (CSFII), will be used to measure adolescents' diets. This protocol involves the use of a dietary measurement technique called the "24 hour recall". Second, a sampling scheme based on seasons of the year and urban versus rural residence has been decided upon to ensure representative dietary data collection. Third, contacts have been made among school teachers in Cache Valley, Provo, and Salt Lake City for conducting the 24 hour recalls. Fourth, preliminary Utah State University Institutional Review Board approval has been obtained. Fifth, one graduate student and two undergraduate students have been identified to work on the project. Finally, additional funding for the project has been obtained via a Utah State University New Faculty Research Grant.

2. Significant Conclusions:

NA

3. Anticipated Problems/Delays:

None

Publications:

NA
Theses: NA

Published Abstract: NA

Presentations: NA

Patent/Invention Disclosures: NA

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<th>Technology Transfer Activities</th>
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Visitors Hosted: 0
The purpose of this project is to investigate the effects of a milk protein (caseinomacropeptide, CMP) on the regulation of food intake in humans. It has been suggested that CMP suppresses appetite in humans. If so, the marketability of milk would be greatly improved. Milk, milk products, and other dietary formulations based on milk components, would empirically have a very large market in the United States and the world. National survey data indicate that approximately one-third or 58 million American adults 20 years of age or older are overweight; and the prevalence of overweight continues to increase. The health consequences of overweight and obesity include heart disease, diabetes, high blood pressure, and certain cancers. As a result, obesity is the second leading cause, after cigarette smoking, of preventable deaths in the United States.

The increasing trends in overweight, in addition to higher energy intakes, are occurring despite the growing prevalence of fat replacers and no- and low-fat foods sold in American markets, and the interest of Americans in these products.
Thus, alternative strategies must be developed to decrease weight and obesity among Americans. One way of accomplishing this goal is through the ingestion of naturally-occurring food substances that decrease food intake. CMP may be one of these substances.

The objectives of this project are to determine the short-term effect of CMP on appetite suppression in humans and to identify changes in blood markers that may be associated with satiety following CMP intake.

1. Significant Progress against Objectives:
   A feeding study was completed May 14, 1998, to measure the effect of CMP intake on food intake over a 10 hour period. Fifty-two healthy men and women, ages 18-36 years, who met study selection criteria, were randomized into the study over a two week period. All participants received each of the following four treatments: 1) 0.2% CMP solution, 2) 5.0% CMP solution, 3) vehicle alone, and 4) water containing colorant and clouding agent. The vehicle was a low-calorie beverage sweetened with aspartame and containing colorant and clouding agent.
   Each treatment took approximately two hours during a single day. Participants came to the study center between 11:00 AM and 12 noon, and consumed the test beverage followed by lunch one hour later. The lunch was provided by us and consisted of chicken salad sandwich food units. Participants were instructed to consume the lunch until they were full. Each study day, participants kept records of all foods consumed and completed a questionnaire to measure current feelings of hunger and stomach fullness throughout the day. We are currently in the process of entering and analyzing these dietary and sensory data.
   In addition, a sensory study of test and control beverage vehicles with and without added CMP was conducted in preparation for the aforementioned study. One hundred men and women were recruited from students, faculty, and staff. Participants judged the sensory characteristics of test and control beverages that were used in the feeding study.
   Recruitment for the study on blood markers of satiety will begin Summer, 1998.

2. Significant Conclusions:
   NA

3. Anticipated Problems/Delays:
   NA

Publications:
   NA

Theses:
   NA
Published Abstract:
NA

Presentations:
NA

Patent/Invention Disclosures:
NA

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Visitors Hosted:
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Western Dairy Center

Project Report

Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Bart Weimer
Co-Investigators:

Project Title: Identification and characterization of components of the proteolytic enzyme system of Lactobacillus helveticus which effect bioactive peptide accumulation, Utah State University part.

Institution’s Project #: 97083

Project Completion Date: 12-31-97

Identify and pursue the health and nutritional benefits of milk; to leverage bioactive peptides in milk for positioning or potential positioning; investigate microbial enzymatic activities leading to the formation of bioactive compounds in milk.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to 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 organisms growth in milk.
2. Determine which components of the proteolytic systems of the selected strains of Lb. helveticus are essential for the accumulation of the bioactive peptides/bioactive peptide precursors from milk.
3. Construct strains of Lb. helveticus which accumulate elevated levels of the bioactive peptides/bioactive peptide precursors of interest.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Strains of lactobacilli (60) are being screened for degradation of the alpha-casein 1–23. In Lactobacillus helveticus at least 4 patterns have been found. Specific peptides are unique to strains based on the data to date. The gene sequence is partially determined, which will be combined with the hydrolysis patterns to form a classification system. Accumulation of peptides is being determined as the hydrolysis progresses. To aid in the characterization, analysis of the peptides with mass spectrometry is also in progress after separation with either HPLC or capillary electrophoresis.

1. Significant Progress against Objectives:
All objectives are being completed as listed in the proposal.

2. Significant Conclusions:
Numerous hydrolysis patterns are found in the strains. Confirmation of the gene sequence is underway, and a classification system is being developed based on both sets of information.
3. Anticipated Problems/Delays:
none

Publications:
none

Theses:

Published Abstract:
none

Presentations:
none

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities Technology is available for licensing
For information on licensing contact:

Visitors Hosted:
Western Dairy Center  
Project Report  
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Bart C. Weimer, Utah State University  
Co-Investigators: 

Project Title: Microbial catabolism of Methionine to improve Cheddar cheese flavor - a comparative study of the relative contribution by starter cultures and flavor adjunct bacteria.

Institution's Project #: 79088  
Project Completion Date: 12-31-99

Develop/characterize/modify strains with particular flavor producing capabilities and enzymes linked to actual cheese flavor development.

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
Use bacteria with defined flavor producing capabilities as model systems for the study of enzymes and metabolites linked to the development of 1) desirable and 2) undesirable flavors in Cheddar cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Metabolism in dairy related bacteria is being investigated. Brevibacteria produce more sulfur containing compounds during their metabolism than do lactic acid bacteria. Additionally, the mechanism by which production of methanethiol occurs is different. Purification of the enzyme responsible for methanethiol production in brevibacteria has been isolated to homogeneity and characterized. Studies on the enzyme stability in cheese slurries are underway. Use of these strains in Cheddar cheese production demonstrated that brevibacteria improve the trained cheddar-scores and the consumer acceptance of lower fat cheese.

1. Significant Progress against Objectives:  
All objectives are on time.

2. Significant Conclusions:  
Brevibacteria produce more sulfur compounds from Met than lactic acid bacteria. While a broad range of MTPC occurs in lactococci and lactobacilli, it seems the mechanism of production varies between the two genera. Further work as stated in the objectives is underway.
3. Anticipated Problems/Delays:
none

Publications:

Theses:
none

Published Abstract:

Presentations:

Patent/Invention Disclosures:

Technology Transfer Activities This technology has been licensed
For information on licensing contact:

Visitors Hosted:
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Bart C. Weimer, Utah State University
Co-Investigators:

Project Title: Conversion of amino acids to short and branched-chain-fatty acids by starter and adjunct bacteria.

Institution’s Project #: 97089

Project Completion Date: 12-31-99

Understand how cheese matrix composition influences survival and metabolism of starter and adjunct cultures.

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
To use bacteria with defined flavor producing capabilities as model systems for the study of enzymes and metabolites linked to the development of 1) desirable and 2) undesirable flavors in Cheddar cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Lactic acid and brevibacteria have been screened for fatty acids produced from branch chain amino acids. Each strain produced various amounts of fatty acids from each amino acid, NMR studies with radiolabeled amino acids demonstrated the interconversion occurs in these bacteria. Work is underway to define the conditions, bacteria, and mechanism for these conversions.

1. Significant Progress against Objectives:
All objectives are on schedule as listed in the proposal

2. Significant Conclusions:
Dairy related bacteria convert amino acids to fatty acids that are not found in milk fat. The implications for cheese flavor are being explored.

3. Anticipated Problems/Delays:
none

Publications:
none

Theses:
Published Abstract:
none

Presentations:
none

Patent/Invention Disclosures:

<table>
<thead>
<tr>
<th>Technology Transfer Activities</th>
<th>Technology is available for licensing</th>
</tr>
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<tbody>
<tr>
<td>For information on licensing</td>
<td>contact:</td>
</tr>
<tr>
<td>contact: Bart Weimer (435) 797 3356</td>
<td></td>
</tr>
</tbody>
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Visitors Hosted:
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Bart C. Weimer
Co-Investigators:

Project Title: Investigate the role of metabolic cross-feeding between starter, brevibacterium and nonstarter cheese bacteria in the production or removal of aromatic off flavor compounds.

Institution’s Project #: 97087

Project Completion Date: 12/31/98

Establish knowledge matrices relating flavor and the role of starter and non-starter organisms and adjuncts.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
To investigate the role of metabolic cross-feeding between starter, brevibacterium and nonstarter cheese bacteria in the production or removal of aromatic off flavor compounds.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Cross feeding of intermediates between dairy related bacteria was demonstrated for lactobacilli and brevibacteria. While lactobacilli produce undesirable aromatic off flavors from aromatic amino acids brevibacteria remove those compounds during metabolism to compounds that are flavorless or energy.

1. Significant Progress against Objectives:
All objectives are being finished as preposed.

2. Significant Conclusions:
The total microflora of the cheese is important for the final flavor profile due to cross feeding. Further work to define the microflora beyond NSLAB is needed to determine the full impact of this finding.

3. Anticipated Problems/Delays:
none

Publications:
Broadbent, Jeffery R., Charlotte Brennand, Mark E. Johnson, James L. Steele, Marie Strickland, and Bart C. Weimer. 1997. Contributions by starter and selected adjunct
bacteria to flavor development in reduced-fat cheddar cheese. Dairy Industry Int. 62:35.


**Theses:**
Madhvi Ummadi

**Published Abstract:**


**Presentations:**


**Patent/Invention Disclosures:**

<table>
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<th>Technology Transfer Activities</th>
</tr>
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<td>For information on licensing contact:</td>
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</table>

| Visitors Hosted: |
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Jeff Broadbent, Utah State University
Co-Investigators: Dr. Mark Johnson, Wisconsin Dairy Center
Dr. James Steele, University of Wisconsin

Project Title: Growth of Non-starter Lactic Acid Bacteria in Reduced Fat Cheddar Cheese, Utah State University part.

Institution’s Project #: 97085

Project Completion Date: 12-31-97

Establish knowledge matrices relating cheese composition, flavor and the role of adjunct and nonstarter microorganisms.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
Objective 1 To establish the population dynamics between starter, non-starter, and adjunct bacteria during ripening of 50% reduced fat Cheddar cheese.
Objective 2 To establish the population dynamics between starter, non-starter, and adjunct bacteria during ripening of 50% reduced fat Colby cheese.
Objective 3 To construct derivatives of the adjunct Lactobacillus casei subsp. pseudoplanatarum 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.
Objective 4 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 non-starter bacteria and flavor attributes during aging of the cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
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. Thus, we will investigate 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.
1. Significant Progress against Objectives:
The ability to address population dynamics between starter, non-starter, and adjunct bacteria during cheese ripening requires methodology that will allow us to monitor changes in that population, over time, at the strain level. To accomplish this, we have been investigating the use of random amplified polymorphic DNA (RAPD) fingerprinting by the polymerase chain reaction (PCR). Our results to date indicate that we can obtain unique DNA patterns from different strains of Lactococcus lactis, Lactobacillus casei, and Lactobacillus helveticus.

2. Significant Conclusions:
RAPD-PCR will be an effective method for the analysis of NSLAB strain dominance in Cheddar cheese during ripening.

3. Anticipated Problems/Delays:
none

Publications:
none

Theses:
none

Published Abstract:
none

Presentations:
none

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact:
Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Jeffery R. Broadbent, Utah State University
Co-Investigators:
  Dr. Bart C. Weimer, Utah State University
  Dr. James L. Steele, University of Wisconsin-madison
  Dr. Mark E. Johnson, Center for Dairy Research and Dr.
  Scott A. Rankin, University of Maryland

Project Title: Improvement of low-fat Cheddar cheese through
identification and characterization of microbial
enzymes responsible for the conversion of
aromatic amino acids into off flavor compounds in
cheese.

Institution’s Project #: 97087

Project Completion Date: 12-31-99

Clarify which organisms are responsible for cheese flavor (positive and negative) and
understand how these organisms assert their influence.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
The overall objective of this proposal is to identify and characterize key enzymes and
mechanisms which are primarily responsible for the conversion of aromatic amino
acids into off flavor compounds in low-fat Cheddar cheese.

Objective 1. Investigate the role of metabolic cross-feeding between starter, adjunct, and
nonstarter cheese bacteria in the production or removal of aromatic off flavor
compounds.

Objective 2. Characterize the contribution of key enzymes to the conversion of aromatic
amino acids into off flavor compounds.

Objective 3. Confirm the action of key enzymes in cheese slurries and in low-fat
Cheddar cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Development of off flavors is a significant problem in low-fat Cheddar cheese.
Compounds associated with unclean, medicinal or utensil, and floral or rosy off flavors
may arise via microbial catabolism of aromatic amino acids. Starter, adjunct, and
nonstarter lactic acid bacteria may catabolize aromatic amino acids under conditions found
in Cheddar cheese, and pathways involved in these reactions can facilitate the production of
off flavor compounds. This project will investigate the contribution of metabolic cross-
feeding between starter, adjunct, and nonstarter bacteria to the production or removal of
aromatic off flavor compounds, the specific roles for selected enzymes in the production of
these compounds, and confirm that these enzymes and pathways are functional in low-fat
Cheddar cheese. Results from the project will facilitate industry efforts to understand and
control flavor development in low-fat Cheddar cheese by providing new strategies, based
on enzyme assays, gene probes, or recombinant DNA technology, that can be used to identify or develop starter systems which avoid or reduce development of utensil, medicinal, unclean, putrid, and floral off flavors in low-fat Cheddar cheese.

1. Significant Progress against Objectives:
Research to date has focused primarily on Objective 1. Metabolic cross-feeding studies for Trp, Phe, and Tyr have been completed and those results confirm that these reactions are active under simulated cheese conditions (pH 5.2, 4% NaCl, no carbohydrate, 13-15oC). Experiments will now be performed to isolate genes for key enzymes and create isogenic constructs that will allow us to establish the contribution of each enzyme to aromatic amino acid catabolism and off-flavor production.

2. Significant Conclusions:
Starter, adjunct, and nonstarter lactic acid bacteria are each able to catabolize aromatic amino acids in defined media under conditions found in Cheddar cheese, and pathways involved in these reactions can facilitate the production of off flavor compounds.

3. Anticipated Problems/Delays:
none

Publications:
none

Theses:
none

Published Abstract:
none

Presentations:
none

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact: Jeff Broadbent

Visitors Hosted:
none
Improvement of Cheddar cheese quality through identification and characterization of microbial enzymes responsible for the production or degradation of bitter peptides in cheese.

Institution’s Project #: 97086

Project Completion Date: 12-31-99

Establish knowledge matrices relating flavor and role of starter, adjunct, and nonstarter bacteria to clarify which organisms are responsible for positive and negative attributes of cheese flavor and provide an understanding of how these organisms assert their influence.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
The overall objective of this proposal is to identify and characterize enzymes produced by starters and flavor adjunct bacteria which are responsible for the production or degradation of bitter peptides in Cheddar cheese.

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

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

Objective 3 Determine bitter taste thresholds for b-CN (f193-209) and aS1-CN (f1-9).

Objective 4 Define the contribution of Lactobacillus helveticus CNRZ32 peptidases to degradation of b-CN (f193-209) and aS1-CN (f1-9).

Objective 5 Construct L. lactis SK11 derivatives with enhanced activity of peptidases demonstrated to be important in hydrolysis of b-CN (f193-209) & aS1-CN (f1-9).

Project Summary: (Suitable for inclusion in Center documents released to the public)
Bitterness is a significant problem in Cheddar cheese, and this defect is particularly common in low-fat cheeses. Bitterness has been a problem in cheese for decades, but modern consumer preference for mild-flavored Cheddar has lent greater significance to the impact of bitterness on dairy economics. Bitterness is caused by the accumulation of hydrophobic peptides produced by some starter bacteria and chymosin. Starter proteinase specificity is the primary determinant in whether or not a starter culture produces bitter peptides. Fortunately, bitter peptides produced by chymosin and starter bacteria can be degraded by intracellular peptidases from starters and adjunct bacteria, but the relative contribution of individual peptidases to these reactions remains unknown. This project will identify and characterize microbial enzymes responsible for the production or degradation of bitter peptides in Cheddar cheese.
of bitter peptides in cheese. Results from the study will facilitate industry efforts to understand and control flavor development in Cheddar cheese by providing new strategies to identify or develop starter systems which eliminate or control bitter flavor defect in full and low-fat Cheddar cheese.

1. Significant Progress against Objectives:
Research to date has primarily focused on Objectives 1, 2, and 4. Under objective 1, Dr. Broadbent has successfully cloned the bitter L. lactis S3 proteinase using the PCR-based protocol outlined in the grant. His lab has now begun to construct a series of isogenic L. lactis strains that differ only by the expression of a group a, e, or h (bitter) cell envelope proteinase. Once HPLC analysis confirms that these constructs retain the wild-type proteinase specificity, they will be used in cheesemaking experiments to establish the role of CEP in peptide accumulation and bitterness. Under Objective 2, Dr. Brennand has assembled a trained bitter sensory panel and those individuals are now receiving final training using the cheese-based model system described in the proposal. Once training is complete, the panelists will determine taste thresholds for bitter peptides as outlined under objective 3. Research under objective 4 has been performed by Dr. Steele. That work has clearly shown that the Lactobacillus helveticus CNRZ 32 general aminopeptidase can degrade the bitter peptide b-CN (f 193-209) under cheese pH and salt levels, and HPLC studies are underway to characterize the influence of other enzymes on this peptide and aS1-CN (f 1-9). Once those experiments are complete, Dr. Steele will construct the SK11 peptidase overexpression system outlined under Objective 5.

2. Significant Conclusions:
The most noteworthy observation to date has been that unlike the lactococcal enzymes, the Lactobacillus helveticus CNRZ 32 oligoendopeptidase cannot degrade b-CN (f 193-209), while the CNRZ 32 general aminopeptidase (PepN) can.

3. Anticipated Problems/Delays:
none

Publications:

Theses:
none

Published Abstract:
none

Presentations:

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact: Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Charlotte Brennand, Utah State University

Co-Investigators:

Project Title: Development of a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in Cheddar cheese.

Institution’s Project #: 97086

Project Completion Date: 12/31/98

Establish knowledge matrices relating flavor and role of starter, adjunct, and nonstarter bacteria to clarify which organisms are responsible for positive and negative attributes of cheese flavor and provide an understanding of how these organisms assert their influence.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
Develop a cheese-based test for bitterness in Cheddar cheese and establish factors that influence sensory perception of bitterness in Cheddar cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
A taste panel has been trained to evaluate the bitterness of full-, reduced, and non-fat cheese. Parameters which affect the bitterness of cheese have been identified. This panel will be used to evaluate the effectiveness of methods used to reduce bitter characteristics of Cheddar cheese.

1. Significant Progress against Objectives:
A panel was selected from applicants for their ability to detect bitterness in Cheddar cheese and trained to distinguish bitterness from other flavors in cheese. This trained panel was then used to determine the following param.
2. Significant Conclusions:
The trained panel can be used to evaluate factors that cause bitterness in Cheddar cheese and to determine the effectiveness of methods used to reduce the bitterness in cheese.

3. Anticipated Problems/Delays:
None

Publications:
None

Theses:
None

Published Abstract:
None

Presentations:
None

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:
Western Dairy Center
Project Report
Reporting Period July 1, 1997 - June 30, 1998

Principal Investigators: Dr. Conly Hansen, Utah State University
Co-Investigators: Carl Brothersen, Utah State University

Project Title: INJECTION OF FLUIDS AND MICROORGANISMS INTO CHEESE.
Institution's Project #: 98089

Project Completion Date: June 30, 1998 - extension has been requested

National Research Plan (1997): Priority: Goal: Tactic:

Modifications to Project/Budget: None save an extension request

Project Objectives: (Include any revisions to objectives)

Determine the parameters necessary to efficiently inject polar and non-polar substances into Cheddar cheese. Determine the parameters necessary to efficiently inject polar and non-polar dye into Mozzarella cheese. Determine the survival rate of lactic acid bacteria in the injection system. Determine the survival rate of lactic acid bacteria injected into Cheddar and Mozzarella cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Experiments were performed using high pressure injection (HPI) to inject dye into Cheddar, Mozzarella and a small sample of Muenster cheese and microorganisms suspended in water into Muenster cheese blocks. Injection was accomplished by accelerating liquid through sapphire orifices with pressure. In all trials, a single orifice injection nozzle was used. The parameters considered for the depth of penetration studies were: injection pressure (6,890 KPa and 34,500 Kpa (1000-5000 psi)), orifice diameter (102 μm and 152 μm (4 and 6 thousandths of an inch)), injection duration (1, 5, and 15 s), and cheese temperature (3.5°C and 20 °C (39°F and 69 °F)). Dye type was varied between polar and nonpolar to indicate which type diffused best in cheese. Cheddar cheese was injected to an average of 161 mm (6.3 inches). Temperature and pressure had the greatest influence on depth of injection. Microbial plate counts showed approximately the same number of bacteria before and after injection. The results of the microbial study indicate the bacteria can be very successfully injected into cheese.
1. Significant Progress against Objectives:

**Phase I - Penetration Studies**

Preliminary experiments were done to indicate the parameters out of those considered (injection pressure (pressure, orifice diameter, injection duration, cheese temperature and, polar or non-polar dye) that were significant for penetration into a 40 pound block of Cheddar cheese that was over a month old. Table one gives the correlation coefficients for a line (model) that describes depth of the injectate in the phase I experiments.

<table>
<thead>
<tr>
<th>COEFFICIENTS</th>
<th>SD</th>
<th>P</th>
<th>CONDITION TERM</th>
<th>0 CONSTANTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.3125</td>
<td>-7.27083</td>
<td>0.0001</td>
<td>1.000</td>
<td>1 temp[20-deg]</td>
</tr>
<tr>
<td>4.27083</td>
<td>17.2292</td>
<td>0.0123</td>
<td>1.000</td>
<td>2 dye[nonpolar]</td>
</tr>
<tr>
<td>9.4375</td>
<td>2.8073</td>
<td>0.0018</td>
<td>0.816</td>
<td>3 orifice[.006]</td>
</tr>
<tr>
<td>-13.0625</td>
<td>2.8073</td>
<td>0.0000</td>
<td>0.816</td>
<td>4 pressure[5000]</td>
</tr>
<tr>
<td>6.4375</td>
<td>1.6208</td>
<td>0.0003</td>
<td>1.000</td>
<td>6 BLOCK[3]</td>
</tr>
<tr>
<td>-5.1875</td>
<td>1.6208</td>
<td>0.0029</td>
<td>1.000</td>
<td>7 BLOCK[4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 temp[20-deg]*pressure[5000]</td>
<td>11 dye[nonpolar]*pressure[5000]</td>
</tr>
</tbody>
</table>

N trials = 48, R Squared = 0.868, P=0.0000

These coefficients are multipliers for the condition term in an equation for predicting the depth from the given variables. Terms that showed little significance have been deleted. Even with the best estimated line for the data given, only 86.8% of the variation is explained by this line as is shown by the R squared term. The larger the coefficient, the larger the effect on the depth. Pressure had the largest effect on the depth of the independent variables, but the next most significant coefficient was BLOCK (4). The block variables were ECHIP designated variables that were randomly assigned and did not have actual bearing on the depth. Each block was as similar as ECHIP could make it and all blocks should have had equal effects on the depth. Since they were not equal, it indicates that the data was not consistent across the trials. However, based on these and other observations the pressure and temperature variables seemed to have a relatively greater influence than other variables studied on the penetration depth.

Other data was collected using Muenster cheese. New Muenster caused greater deflection of the injectate and resulted in more even distribution of the injectate (data not shown).

Temperature and pressure had more effect on depth than any other single independent variables, so the procedure for Phase I was modified to compare the changes in the depth to temperature differences at the more effective pressure (34,500 kPa (5000 psi)). Besides effecting penetration depth, it was observed that temperature had effects on the path the injectate took through the cheese block. Cold cheese caused the injectate to deflect and form a zigzag pattern in the cheese while the warm cheese allowed injectate to pass through without deflection. Coupled with the temperature alterations, different injection durations were also studied. Increased injection times resulted in deeper penetration as did warmer cheese. The results are summarized in the tables below.
in the tables below.

Table 2. Cheese Injection Depth Verses Time (n=16).

<table>
<thead>
<tr>
<th>Time</th>
<th>Average Depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Second</td>
<td>91 (3.6 In)</td>
</tr>
<tr>
<td>5 Seconds</td>
<td>128 (5 In)</td>
</tr>
<tr>
<td>15 Seconds</td>
<td>160 (6.3 In)</td>
</tr>
</tbody>
</table>

The depth information for the 15 second category was not completely accurate because the injectate often exited the cheese during these injections. The data does still show that increased time does cause deeper penetration.

**Phase II - Flavor Studies**

A sensory data has not yet been done in the flavor study. In an informal taste test, the researchers determined that Muenster cheese did carry injected flavors well. Because of the relatively small amount of flavors injected, strong, concentrated flavors were more detectible in the cheese. Muenster cheese was selected because of its mild flavor and open texture. New Muenster cheese distributed the injected flavors more evenly than two-week-old Muenster cheese.

**Phase III - Bacterial Survival**

Microbial plate counts showed approximately the same number of bacteria (about $10^6$/ml) in samples taken before passing through the injection system and after being injected into a beaker. The injection into the cheese resulted in an approximately 1/100 dilution of the injectate. Two cheese block samples that were blended and plated in triplicate showed about $1.5 \times 10^5$ bacteria (which correlates to $1.5 \times 10^7$ bacteria/ml injected) surviving in the cheese on day two. Similar counts taken on day seven revealed $1.4 \times 10^4$ bacteria ($1.4 \times 10^6$ bacteria/ml injected) surviving in the cheese. These counts were only slightly reduced on day fourteen to $1.0 \times 10^4$ bacteria ($1.0 \times 10^6$ bacteria/ml injected). This showed that bacteria can not only be injected successfully but if the appropriate type of bacteria is chosen, these microbes can survive at least two weeks in cheese. The results of the microbial study indicate the bacteria can be very successfully injected into cheese.

2. **Significant Conclusions:**
   1. Temperature and pressure had more effect on depth of penetration into cheese than any other single independent variables.
   2. Muenster cheese carries injected flavors well.
3. Bacteria can be injected into cheese with very little mortality. Injected bacteria survived two weeks in cheese.

3. Anticipated Problems/Delays:

None

Publications:

None

Theses:

None

Published Abstract:

None

Presentations:

None

Patent/Invention Disclosures:

High pressure injection to improve characteristics of cheese including flavor, color, moisture and texture. Prepared on May 6, 1998.

Technology Transfer Activities:

For information on licensing contact:

Conly Hansen

Visitors Hosted:
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — June 30, 1998

Principal Investigators: Marie K. Walsh, Ph.D., Utah State University
Co-Investigators: Carl Brothersen, Utah State University

Project Title: Thermal inactivation of Milk Clotting Enzymes in Milk
Institution’s Project #: 98090
Project Completion Date: 6/30/98

National Research Plan (1997): Priority: Goal: Tactic: 

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
1. Develop an assay to measure the activity of milk clotting enzymes in milk.
2. To investigate the thermal inactivation of milk clotting enzymes (Chymax ultra, Maxiren, Sure curd, Fromase, Porcine pepsin) in skim milk, at various temperatures from 120 to 180°F at a hold time of 20 sec.
3. Investigate the rate of whitening of skim milk by the action of milk clotting enzymes (Chymax ultra, Maxiren, Sure curd, Fromase, Porcine pepsin) at various temperatures from 70 to 130°F.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Limited and controlled hydrolysis of k-casein in skim milk can cause an increased whitening and decreased blueness. Preliminary investigations have shown that subsequent pasteurization of skim milk may not denature the enzyme, which is essential to prevent milk coagulation and off-flavors in the final product. Therefore, milk clotting enzymes were characterized with respect to rate activation energies of denaturation in skim milk. The rate constants of denaturation of each enzyme was used to calculate the time needed at each temperature to completely inactivate each enzyme. The activation energies of enzyme denaturation were also calculated from this and used to rank each enzyme from the most thermal labile to the most thermal stable. The order of the enzymes is: Porcine pepsin, chymostatin, fromase, SF-100, chymax II, chymax extra, chymax ultra, chymostart, emporase-EL, thermolase, and chymosin.
Objective Revisions:

Objective 2. This objective was expanded to include additional milk clotting enzymes and temperatures. Measure the thermal inactivation of milk clotting enzymes (Thermolase, Emporase EL, Chymax II, Chymax Ultra, Hannilase XL, Fromase, SF-100, Chymostart, Sure curd, Chymosin, Chymostatin, Porcine pepsin) in skim milk, at various temperatures from 110 to 170 °F.

Objective 3. This objective was expanded to include additional milk clotting enzymes. Investigate the whitening of skim milk by the action of milk clotting enzymes (Thermolase, Emporase EL, Chymax II, Chymax Ultra, Hannilase XL, Fromase, SF-100, Chymostart, Sure curd, Chymosin, Chymostatin, Porcine pepsin).

Methods and Materials:

Milk Clotting Enzymes

The milk clotting enzymes used in this study are listed in table 1. All enzymes were stored at 4°C prior to use.

Enzyme activity measurements

Thermal inactivation of enzymes was measured using κ-casein-agar tubes as described in Holmes et al., 1977. Briefly, 600 mg κ-casein (Sigma Chemical Co.) is dissolved into water (50 ml final volume) containing 600 mg sodium acetate trihydrate (Fisher Scientific) and the pH is adjusted to pH 5.9 with 0.1 N HCl. A separate solution of 6 g sodium acetate trihydrate, 800 mg bacto agar (Defco) at pH 5.9 is brought to 90 ml with water and autoclaved for 10 min. Both solutions are adjusted to 75°C, mixed and dispensed into Wintrobe tubes (Becton Dickinson). Unused tubes were capped in parafilm and stored for no longer than 14 days at 4°C.

All enzymes were adjusted to the same strength (i.e. 1 X) before being assayed. Enzymes were diluted into skim milk for a final dilution factor of 1.98 x 10-5 (10 μl sample) or diluted into water for a final dilution factor of 1.1905 x 10 -5 (10 μl sample) for enzyme activity measurements. Aliquots (5, 10, 15, and 20 μl) of the diluted enzyme solutions were added to the κ-casein-agar tubes and incubated at 37°C for 48 hours. The distance from the top of the meniscus to the bottom of the white precipitate in the tubes is measured and used to draw a standard curve.

Thermal inactivation of enzymes

Enzymes (75 μl of a 1/42 dilution) were added to 9 ml of skim milk. This is approximately the concentration used for cheese making (3.6 ml of a 1/42 dilution of enzyme into 40 lb milk). Enzyme treated skim milk is added to Wintrobe tubes and incubated at 110, 120, 130 and 140°F for 1 hr or at 160 and 170°F for 1 min. Samples,
100 ml, are removed every 10 min or every 30 sec for residual enzyme measurement as described above for enzyme standards. Each sample was tested twice at each temperature in triplicate samples.

Table 1. Milk clotting enzymes.

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Description</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Recombinant Chymosins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymosin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymmax II</td>
<td>15127 Lot 29227</td>
<td>Chris Hansens</td>
</tr>
<tr>
<td>Chymostatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymmax Ultra</td>
<td>P/N 73764 Lot 1111727750</td>
<td>Chris Hansens</td>
</tr>
<tr>
<td>Chymostart</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymax Extra</td>
<td>P/N 73863 Lot 1401830608</td>
<td>Chris Hansens</td>
</tr>
<tr>
<td><strong>B. Microbial Origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emporase-EL 2 X</td>
<td>10540 Lot 0000305967</td>
<td>SKW Biosystems</td>
</tr>
<tr>
<td>Thermolase 3 X</td>
<td>10549 Lot 0000308818</td>
<td>SKW Biosystems</td>
</tr>
<tr>
<td>Hannilase XL</td>
<td>Lot 22097</td>
<td>Chris Hansens</td>
</tr>
<tr>
<td>Sure Curd</td>
<td>7514A Lot 1529724716913</td>
<td>Gist-Brocades</td>
</tr>
<tr>
<td>Fromase</td>
<td>6713 Fromase 5602</td>
<td>Gist-Brocades</td>
</tr>
<tr>
<td><strong>C. Rennets</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-100</td>
<td>110111 Lot 963840312</td>
<td>SKW Biosystems</td>
</tr>
<tr>
<td><strong>C. Porcine Origin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine Pepsin</td>
<td>1112-40-A</td>
<td>SKW Biosystems</td>
</tr>
</tbody>
</table>

Thermal inactivation calculations

The natural log of the percent activity left of the thermally treated enzymes was graphed vs the time for each temperature and enzyme analyzed. The slope of these lines is the k value, or the rate constant for denaturation of the enzyme. This data also allows calculation of the time needed at each temperature to completely inactivate a given enzyme.

A second graph of the natural log of the k values at each temperature vs 1/T (Kelvin) allows calculation of the activation energy (Ea) for enzyme deactivation from the slope of the line (Ea/R). The R value used was 8.3144 J/mol K.

Measuring the whiteness of enzyme treated skim milk

The appropriate amount of enzyme will be added to tempered skim milk. A sample of the milk will be transferred to 24 well micro titer plates, and placed in an Omnispec colorimeter and held at one of the temperatures used. The Hunter L*
and $b^*$ values will be determined at one minute intervals for temperatures of 120 and 130°F and at ten second intervals for temperatures of 160 and 170 °F. Three levels of enzyme concentration can easily be explored for each milk clotting enzymes using this method. This data will be used to compare the increase in sample whiteness over time for each enzyme tested at each temperature and can be used to calculate the activation energies for each milk clotting enzyme in milk.

Taste and stability analysis

Milk clotting enzymes which are thermally inactivated during processing and produce a significantly whiter skim milk will be used to produce 40 lb. of product using standard milk processing times and temperatures. This milk will be tasted for off-flavors by a trained taste panel and analyzed for whiteness using the Omnispec colorimeter on days 1, 5, and 20 after production.

1. Significant Progress against Objectives:

Enzyme activity measurements

The activity of each of the enzymes was tested prior to analyzing the thermal inactivation in skim milk to assess the applicability and sensitivity of the diffusion tube method. Activity values are the slope of the lines generated when the distance (mm) of the precipitate that formed in the agar is graphed vs the volume of enzyme (μl). This data is listed in Table 2 and displayed graphically in Figure 1. All enzymes were diluted to a 1 X concentration which represents 50,000 milk clotting units/ml. This diffusion tube method is capable of detecting enzyme activities as low as 0.6 milk clotting units/ml. The values listed are adjusted for dilution. In general, the activities measured in skim milk are lower than those measured in buffer.

Figure 2, panel A, shows the thermal inactivation of chymax II at each of the 4 temperatures tested. The slopes of these lines are the k values (rate constants of denaturation) which were used to construct the graph in panel B. Similar graphs were constructed for each of the enzymes tested and the k values are given in table 3.

The data generated from the thermal inactivation of milk clotting enzymes was used to calculate the time needed at each temperature to completely inactivate each enzyme. This data is shown in Figure 3. Porcine pepsin the least thermal stable of the enzymes tested, while chymosin appears to be the most stable.

The activation energies of enzyme denaturation are shown in Table 4 and Figure 4. Porcine pepsin is the least thermal stable while chymosin is the most thermal stable.
Table 2. Activities of Milk Clotting Enzymes

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Activity in Buffer (mm/µl)</th>
<th>Activity in Skim Milk (mm/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymosin</td>
<td>~0.5</td>
<td>0.287</td>
</tr>
<tr>
<td>Chymax II</td>
<td>0.482</td>
<td>0.287</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>0.365</td>
<td>0.388</td>
</tr>
<tr>
<td>Chymax Ultra</td>
<td>0.251</td>
<td>0.243</td>
</tr>
<tr>
<td>Chymostart</td>
<td>0.274</td>
<td>0.340</td>
</tr>
<tr>
<td>Chymax Extra</td>
<td>0.493</td>
<td>0.255</td>
</tr>
<tr>
<td>Emporase-EL</td>
<td>0.285</td>
<td>0.150</td>
</tr>
<tr>
<td>Thermolase</td>
<td>0.472</td>
<td>0.356</td>
</tr>
<tr>
<td>Hannilase XL</td>
<td>0.253</td>
<td>0.331</td>
</tr>
<tr>
<td>Sure Curd</td>
<td>0.421</td>
<td>0.255</td>
</tr>
<tr>
<td>Fromase</td>
<td>0.257</td>
<td>0.229</td>
</tr>
<tr>
<td>SF-100</td>
<td>0.384</td>
<td>0.269</td>
</tr>
<tr>
<td>Porcine Pepsin</td>
<td>0.408</td>
<td>0.164</td>
</tr>
</tbody>
</table>

Figure 1. Enzyme Activities

![Enzyme Activities Graph](image)

Panel A: The natural log of the percent activity left of chymax II at each temperature tested allows calculation of the rate constant for denaturation (k) from the slope of the lines. Panel B: The natural log of the rate constants of denaturation at each temperature allows calculation of the activation energy of denaturation (Ea) of chymax II from the slope of the line.

Figure 2. Thermal inactivation of chymax II in skim milk. Panel A: The natural log of the percent activity left of chymax II at each temperature tested allows calculation of the rate constant for denaturation (k) from the slope of the lines. Panel B: The natural log of the rate constants of denaturation at each temperature allows calculation of the activation energy of denaturation (Ea) of chymax II from the slope of the line.
Table 3. Rate constants of denaturation at each temperature (F).

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>k 110</th>
<th>k 120</th>
<th>k 130</th>
<th>k 140</th>
<th>k 160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymosin</td>
<td>.015</td>
<td>.021</td>
<td>.044</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymax II</td>
<td></td>
<td>.035</td>
<td>.098</td>
<td>.241</td>
<td></td>
</tr>
<tr>
<td>Chymostatin</td>
<td>.013</td>
<td>.021</td>
<td>.161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymax Ultra</td>
<td>.037</td>
<td>.041</td>
<td>.137</td>
<td>.341</td>
<td></td>
</tr>
<tr>
<td>Chymostart</td>
<td></td>
<td>.029</td>
<td>.059</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chymax Extra</td>
<td>.024</td>
<td></td>
<td>.077</td>
<td>1.791</td>
<td></td>
</tr>
<tr>
<td>Emporase-EL</td>
<td>.032</td>
<td>.052</td>
<td></td>
<td>.152</td>
<td></td>
</tr>
<tr>
<td>Thermolase</td>
<td>.031</td>
<td>.057</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hannilase XL</td>
<td>.034</td>
<td>.042</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fromase</td>
<td>.021</td>
<td>.055</td>
<td>.220</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-100</td>
<td>.023</td>
<td>.073</td>
<td>.190</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porcine Pepsin</td>
<td>.087</td>
<td>.921</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. The times necessary to inactivate a given enzyme at a particular time. Data generated from the thermal inactivation of each enzyme was used to calculate the times needed to inactivate an enzyme at a given temperature.
Table 4. Ea of enzyme denaturation

<table>
<thead>
<tr>
<th>Enzyme Name</th>
<th>Ea (KJ/gmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymosin</td>
<td>82029.87</td>
</tr>
<tr>
<td>Chymax II</td>
<td>145726.49</td>
</tr>
<tr>
<td>Chymostatin</td>
<td>193234.97</td>
</tr>
<tr>
<td>Chymax Ultra</td>
<td>121794.51</td>
</tr>
<tr>
<td>Chymostart</td>
<td>118976.30</td>
</tr>
<tr>
<td>Chymax Extra</td>
<td>140047.75</td>
</tr>
<tr>
<td>Emporase-EL</td>
<td>113719.16</td>
</tr>
<tr>
<td>Thermolase</td>
<td>101277.71</td>
</tr>
<tr>
<td>Fromase</td>
<td>178037.35</td>
</tr>
<tr>
<td>SF-100</td>
<td>158705.27</td>
</tr>
<tr>
<td>Porcine Pepsin</td>
<td>393528.80</td>
</tr>
</tbody>
</table>

Figure 4. Ea of enzyme denaturation

2. Significant Conclusions:

The thermal inactivation of milk clotting enzymes in milk was determined for thermolase, emporase EL, chymax II, chymax Ultra, fromase, SF-100, chymostart, sure curd, chymosin, chymostatin, porcine pepsin. The κ-casein agar tubes was successfully used to measure the thermal inactivation.

The rate constants of denaturation of each enzyme was used to calculate the time needed at each temperature to completely inactivate each enzyme. The activation energies of enzyme denaturation were also calculated from this and used to rank each enzyme from the most thermal labile to the most thermal stable. The order of the enzymes is: porcine pepsin, chymostatin, fromase, SF-100, chymax II, chymax extra, chymax ultra, chymostart, emporase-EL, thermolase, and chymosin.

References


3. Anticipated Problems/Delays:

None
Publications:

Theses:
None

Published Abstracts:
None

Presentations:
None

Patent/Invention Disclosures:
Filed

Visitors Hosted:
None
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Marie K. Walsh, Assistant Professor, Department of Nutrition and Food Sciences, Utah State University. Paul A. Savello, Associate Professor, Department of Nutrition and Food Sciences, Utah State University.

Co-Investigators:

Project Title: Whitening of skim milk using a continuous-flow, immobilized enzyme reactor.

Institution’s Project #: 97084

Project Completion Date: 6-30-98

Research thermal and mechanical processing methods to improve sensory and color aspects of whiteness, texture and flavor.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
To develop a suitably active immobilized protease for use in skim milk whitening. To characterize the immobilized enzyme catalyst for operational stability and productivity in a fluidized-bed reactor with respect to skim milk whitening.

Project Summary: (Suitable for inclusion in Center documents released to the public)

1. Significant Progress against Objectives:
Milk clotting enzymes (Chymax, Chymosin, and Sure Curd) have been covalently immobilized onto both glass and ceramic beads using glutaraldehyde. The activities of the immobilized catalysts were ten times higher using the glass beads as assayed with a fluorometric substrate. The immobilized catalysts were then used to whiten skim milk in a fluidized-bed reactor.

2. Significant Conclusions:
Covalently immobilized milk clotting enzymes have been used to whiten skim milk. This whitening is, at present, not as efficient as using a soluble enzyme. We are in the process of increasing the level of active immobilized enzyme.

3. Anticipated Problems/Delays:
Enzyme leaching from the matrix has been observed. We are in the process of modifying the immobilization procedure to prevent this occurrence and do not expect any delays.
Publications:
None

Theses:
None

Published Abstract:
None

Presentations:
None

Patent/Invention Disclosures:

<table>
<thead>
<tr>
<th>Technology Transfer Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>For information on licensing contact:</td>
</tr>
</tbody>
</table>

Visitors Hosted:
None
Western Dairy Center
Project Report

Principal Investigators: Carl Brothersen, Utah State University
Co-Investigators: Robert Fife, Utah State University

Project Title: Commercialization of skim milk technology
Institution’s Project #: 98101

Project Completion Date: 6-30-98

National Fluid Milk Research Plan (1997): Priority: 1 Goal: 2 Tactic:
Establish a knowledge matrix relating milk processing parameters to sensory and color attributes.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
1. Optimize the reaction for the enzymatic whitening of skim milk. 2. Develop a procedure to enzymatically whiten skim milk in a commercial fluid milk plant.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Factors such as temperature, enzyme level, and hold time were determined in order to establish the procedures necessary to maximize the whiteness of skim milk in a commercial milk processing plant.

1. Significant Progress against Objectives:
The enzymatic whitening of skim milk is accomplished by adding a milk clotting enzyme such as chymosin to milk, allowing enzyme activity to effect the partial aggregation of the casein micelles, and denaturing the enzyme to prevent coagulation of the milk. This procedure increases the white color of skim milk to that of milk containing 1% fat.
The whiteness of skim milk can also be increased by heating the milk. This reaction is temperature dependent and reversible. That is, the milk becomes more opaque as it is heated and returns to its original color when the temperature is reduced. The enzymatic procedure is not reversible.
The following treatment parameters resulted in the maximum whiteness of
skim milk:
1. Three minute hold time.
2. Enzyme level of 68 to 90 ml enzyme per 1000 pounds of milk.
3. Temperature of 110°F.
A procedure was developed to apply this technique to whiten skim milk in a commercial fluid milk plant and minimize the risk of coagulating the milk in the pasteurizer.

2. Significant Conclusions:
By using the method described, raw skim milk may be enzymatically whitened to the same degree as 1% milk.

3. Anticipated Problems/Delays:
None

Publications:
None

Theses:
None

Published Abstract:
None

Presentations:
Western Dairy Center Annual Meeting

Patent/Invention Disclosures:
Patent/invention disclosures have been filed

Technology Transfer Activities
Licensing this technology is underway
For information on licensing contact:
Steve McMaster, Utah State University, phone: 435-797-9602

Visitors Hosted:
Western Dairy Center
Project Report

Principal Investigators: Bruce L. Geller, Associate Professor of Microbiology, Oregon State University.
Co-Investigators:

Project Title: Lactococcal Nasal Vaccine
Institution’s Project #: US 021
Project Completion Date: 06/30/98

National Research Plan (1997): Priority: Goal: Tactic:

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
1. To express on the surface of Lactococcus an immuno-protective antigen from a bacterial pathogen. This engineered strain will be used in future studies to vaccinate mice and cows. The immune response of the vaccinated animals will be measured to determine if the mucosal immune systems can be stimulated by this live lactococcal vaccine strain.

Project Summary: (Suitable for inclusion in Center documents released to the public)
In collaboration with Siga Pharmaceutical Co., we will construct a strain of Lactococcus lactis to express on its surface the immuno-protective antigen from the causative agent of strep throat (Streptococcus pyogenes). Siga’s interest are to test this vaccine strain first in mice, and then in humans. Concurrently with the construction of the strep vaccine, we plan to begin the construction of a strain of Lactococcus that will express on its surface a conserved surface antigen (fibronectin binding protein) from Staphylococcus aureus (a causative agent of bovine mastitis). In future studies, cows will be vaccinated nasally with the lactococcal vaccine strains, and the immune response will be measured. This will test the feasibility of using Lactococcus as a vaccine delivery vehicle in cows. Ultimately, vaccinated cows will be challenged with an infectious dose of S. aureus to determine if the vaccine against staph prevents mastitis. The response to the challenge will be measured by clinical and laboratory analyses.

1. Significant Progress against Objectives:
We have fulfilled the project’s objective of expressing on the surface of Lactococcus the immuno-protective antigen from Streptococcus pyogenes. We are now quantitating the
expression relative to another strain of Streptococcus that expresses the antigen on its surface. We are also trying to increase the amount of antigen expression.

The lactococcal strain was made by inserting the genetic sequence for the C repeat region of the M6 surface protein from Streptococcus pyogenes into the unique BspEI site of pip. pip is a lactococcal gene that codes for a surface protein, and is not required for viability. The C repeat region was copied from the cloned gene (emm) by PCR, and included BspEI restriction sites on the primers. After ligation into pip, the construct was sequenced to verify the correct insertion. The pip-emm6C fusion was subcloned into a high copy number shuttle vector (pTRKH2) and transformed into Lactococcus lactis subsp. lactis LM2301.

The level of surface expression of the pip-C repeat antigen (pip-M6C) in Lactococcus was measured by slot blot analysis. Cells were adsorbed to a nitrocellulose membrane and probed with monoclonal antibodies to the C repeat region. Quantitatively, the level of expression appears to be 2-fold to 10-fold lower than the amount expressed from Streptococcus gordonii, which is currently being tested in animals as a mucosal vaccine for strep throat. Quantitation of expression using slot blot analysis is variable, and we are trying to reduce this variability by using different substrates for the peroxidase-conjugated secondary antibody. We also plan to use another technique that avoids the use of the nitrocellulose, and is based on the adsorption of radiolabelled protein A to the cells.

The level of expression of Pip-M6C in Lactococcus is being optimized. We have found that about half of the Pip-M6C is expressed on the surface of Lactococcus, and the other half is secreted into the culture medium. The level of expression does not appear to vary with the phase of the culture. Maintaining a neutral pH does not affect the level of expression, even in overnight cultures. We are currently in the process of deleting about half of the coding region of pip in the pip-emm6C construct, and replacing the wild-type pip promoter with a high expression promoter.

Work on constructing an analogous antigen from a surface protein of Staphylococcus aureus (a causative agent of bovine mastitis) has begun. A new graduate student, Jennifer Duncan, joined our group in January, and copied by PCR the appropriate genetic sequence from S. aureus. The PCR product was cloned into an expression vector for E. coli. After Jennifer completes her spring term research obligation in another lab, she plans to continue with purifying the staph antigen so that antisera can be made. The antisera will enable us to measure expression of the staph antigen.

2. Significant Conclusions:
Pip can serve as a vehicle for directing expression of a foreign antigen to the surface of Lactococcus lactis. The C repeat region of the M6 protein of Streptococcus pyogenes can be expressed on the surface of Lactococcus lactis in the context of the Pip protein. About half of the Pip-M6C is associated with the cells, and about half is secreted into the culture medium.
3. Anticipated Problems/Delays:

Publications:
None yet, but a manuscript is in preparation.

Theses:
None

Published Abstract:
None

Presentations:
None

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:
Western Dairy Center
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Bruce L. Geller, Associate Professor of Microbiology, Oregon State University

Co-Investigators:

Project Title: The Use of Bacteriophage-Receptor Genes of Lactococcus lactis to Develop Bacteriophage-Resistance in Cheddar Cheese Starter Strains

Institution’s Project #: 97081

Project Completion Date: 12/31/99

Understand phage-resistance systems of starter cultures and phage counter defense systems to develop longer lasting phage resistance strategies.

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
1. Identify and isolate host genes other than pip that are required for infection by phage of the c2 species. 2. Identify a gene encoding a receptor for a phage of the p335 species. 3. Identify a gene encoding a receptor for a phage of the 936 species. 4. Construct a phage-resistant strain of L. lactis with defective copies of the receptor genes for phages of the c2, 936, and p335 species. 5. Evaluate the effects of receptor gene mutations on phage resistance, growth rate, acidification and coagulation of milk, and other cheese production traits.

Project Summary: (Suitable for inclusion in Center documents released to the public)
The proposed research examines early steps of bacteriophage infection of L. lactis, which include attachment of phage to the surface of cells, and entry of phage DNA into cells. Both of these steps are required for infection by phage. Our strategy of strain improvement is to prevent phage from attaching or entering the host in the first place. To do this requires a knowledge of the host components required for attachment and phage entry. The outcomes of this proposal will enable the construction of new strains with defined mechanisms of phage-resistance. Host genes required for phage infection of L. lactis will be identified and isolated. We have previously isolated one such gene named pip (an acronym for phage infection protein). The protein encoded by pip (Pip) is a receptor for phage attachment and phage DNA entry into the host. We have constructed phage-resistant strains of L. lactis by replacing the pop gene with a defective version. There is evidence that host components in addition to pip are required for phage attachment and DNA entry. Isolating genes in addition to pip that are required for phage infection will enable the construction of new strains with alterations in two or more different host components. The strategy of combining multiple phage-resistance mechanisms will greatly decrease the chance that the strain will fail after introduction into commercial use. Genes will be isolated that also extend the range of resistance to phages that do not require pip. We propose to
isolate genes that encode host receptors for two different types of small isometric-headed phage (p335 & 936). Together with the phages that required pip, the p335 and 936 species of phage cause nearly all the starter failures in US cheese factories. Phages of the p335 species are particularly troublesome, as they have only recently emerged as a major problem, and less is known about their mechanism of infection. The isolated receptor genes will be inactivated and used to construct a new commercial strain with a combined phage-resistance defined by each of the inactivated genes (including pip). The phage-resistant strain will be evaluated for physiological characteristics important for making cheese.

1. Significant Progress against Objectives:

2. Significant Conclusions:
A phage skl-resistant phenotype that raises the minimum calcium concentration required for phage infection to 20mM may not be complementable. The extra complexity of the calcium concentration-dependency appears to impose a risk that this phenotype may not lead to host factors that are directly related to phage infection. As a consequence of this, different phenotypes are now being complemented.

3. Anticipated Problems/Delays:

Publications:
None

Theses:

Published Abstract:
None

Presentations:
None

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:
Western Dairy Center  
Project Report  
Reporting Period January 1, 1997

Principal Investigators: Lynn V. Ogden, Associate Professor, Department of Food Science and Nutrition, Brigham Young University.  
Co-Investigators: David M. Carr, Graduate student, Department of Food Science and Nutrition.

Project Title: Influence of milk fat interfacial material and area on the physical and sensory properties of fat-reduced cheese.

Institution’s Project #: 97082  
Project Completion Date: 6/30/98

Adaption and modification of cheese manufacturing technologies to improve flavor.

Modifications to Project/Budget: None

Project Objectives:  
To verify that increase in interfacial area improves the quality of fat-reduced cheese, to study that effect over a range of fat reduction from 50-85% in the cheese, to study the effect of various surfactants present when washed cream is homogenized and to determine if any beneficial effect can be obtained by adding effective surfactants without washing the cream before homogenization.

Added objective:  
To study two other commercial starter/adjoint systems in comparison of current system of Lactococcus lactis ssp. lactis S1 and Brevibacterium linens.

Project Summary:  
Smaller fat globules in milk used to make low-fat Cheddar cheese do not improve quality by making the fat go further. As much as 70% of the fat originally in the milk is lost when making Cheddar cheese. The economics of this loss are devastating to the use of the procedure of making small globules. This fat loss can be avoided, however, if milk protein is left available when processing the milk to produce smaller fat globules. The retention of these smaller fat globules with milk protein available during processing did not significantly improve the cheese quality in our experiment.

Different emulsifiers used in processing to make small fat globules do not appear to make a difference in cheese characteristics. The types of flavor producing bacteria used in this study were not shown in preliminary analysis to provide substantially different Cheddar cheese characteristics.

Improvements in Cheddar cheese quality shown in previous studies with smaller fat globules in cheese did not carry over into the more severely fat-reduced levels in this experiment.
1. Significant Progress against Objectives:

Cheeses were made in two phases of this project. Twenty-four (24) cheeses were manufactured as part of a two factor response surface design in ‘phase 1’. Fat in the cheesemilk and %polysorbate 80 were design factors to study separate homogenization of casein-free cream over a range of fat levels in the cheese and varying amounts of fat globule interfacial area. The fat globule size was controlled by amount of surfactant and a constant homogenization pressure.

Thirty (30) cheeses were manufactured in ‘phase 2’ of the project. Three objectives are identified from these cheeses: 1) a common amount of interfacial area was desired in the cheeses, controlled with differing interfacial material under appropriate homogenization conditions; sodium steroyl-2-lactylate, de-oiled lecithin, sucrose esters and polysorbate 80 were chosen, 2) investigation of the washing regimen prior to homogenization, and 3) comparison of commercial starter/adjunct culture with our present system we are using.

Compositional analysis, microbial enumerations, sensory acceptance panels, and descriptive sensory analysis are completed and have been statistically analyzed. Formal conclusions of phase 2 are being prepared now.

2. Significant Conclusions:

Fat loss from cheesemilk to whey increased quadratically in relation to $d_{32}$ of fat globules. With the smallest of fat globules near 0.5 um, fat lost into the whey was 60-70%. We found that nearly all fat could be recovered from cheesemilk with casein present on the interface, however, regardless of fat globule size. Thus, the process of washing seems disadvantageous to economics and practicality to maximize interfacial area in separate homogenization of cream. Flavor problems may be associated after ageing with homogenized casein protein on the interface of fat globules, however.

The amount of interfacial area did not improve the attributes of the cheeses. The phase 1 objective is difficult to judge in light of sever fat losses and the constraints of interpretation with RSM contour plots. The severe fat loss was unexpected.

The type of interfacial material did not prove to be an effect and cheeses with altered interface were not significant from control cheeses, except for lecithin which was a significant loser in the design. Lecithin had associated flavor and texture problems.

Culture systems did not show significantly different effects.

3. Anticipated Problems/Delays:

None

Publications:

None

Theses:

Related Published Abstract:

Related Presentations:


Patent/Invention Disclosures:
None

Technology Transfer Activities
None

Visitors Hosted:
Rex Infanger, Chr. Hansen’s Lab, Inc.
Dr. Gene L. Hong & Larry Johnson, Brewster Dairy, Inc.
Project Report
Reporting Period January 1, 1997 — December 31, 1997

Principal Investigators: Dr. Jeffery Broadbent, Utah State University
Co-Investigators: Donald J. McMahon, Utah State University
                     Dr. Craig Oberg, Weber State University

Project Title: Improvement of Mozzarella Cheese Functionality by Understanding Exopolysaccharide Production in Thermophilic Starter Cultures

Institution’s Project #: 97079
Project Completion Date: 12-31-99

Understand the impact of polysaccharide production by starter cultures in cheese quality and functionality.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
Understand the influence of microbial exopolysaccharides on Mozzarella cheese moisture status and the relationship of moisture to cheese composition and functionality.
Objective 1: Characterize the structure, molecular weight, and polymer properties of the exopolysaccharide produced by S. thermophilus 10JC.
Objective 2: Isolate and characterize the S. thermophilus 10JC gene cluster for exopolysaccharide production.
Objective 3: Transform EPS-Mozzarella starter cultures with the cloned gene cluster and evaluate the influence of these constructs on moisture level and functional properties of low-fat Mozzarella cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Mozzarella cheese functionality is significantly affected by cheese moisture level, and increasing moisture content has been used to improve the melting properties of low-fat Mozzarella cheese. Previous work by our group has shown that exopolysaccharide (EPS)-producing starter cultures can be used to significantly increase the moisture content of lower fat Mozzarella cheese. We have identified a culture, Streptococcus thermophilus 10JC, that produces a large capsular EPS, and have shown that this capsule is involved in cheese moisture retention. Experiments are now underway to characterize the structure of the 10JC EPS and isolate the genes that encode its biosynthesis. This project will provide basic information needed to understand the influence of EPS on cheese quality and functionality.

1. Significant Progress against Objectives:
Research to date has addressed objectives 1 and 2. Compositional analysis of the 10JC EPS using GC-mass spectroscopy indicate this polymer has a novel octomeric repeating unit composed of galactose, rhamnose and fucose in a 5:2:1 ratio. Methylation studies
have been used to obtain a preliminary structure that will now be confirmed by NMR. With respect to objective 2, efforts are underway to isolate and sequence the 10JC EPS gene cluster. The nucleotide sequence for the 6.5-kb region encoding epsA-F is nearly completed, and we have cloned and begun to sequence a 5-kb region that we believe encodes epsF-J. As that sequence is determined we will continue our efforts to isolate the rest of the 10JC EPS genes, and then use the nucleotide sequence to identify a useful strategy to clone the entire gene cluster. Once that is achieved, we will transform the cluster into EPS-Mozzarella starter cultures and evaluate the influence of these constructs on moisture level and functional properties of low-fat Mozzarella cheese.

2. Significant Conclusions:
Our data demonstrate that the S. thermophilus 10JC EPS is a novel polymer with useful commercial applications.

3. Anticipated Problems/Delays:
none

Publications:
none

Theses:
none

Published Abstract:

Presentations:

Broadbent, J.R. EPS production in Streptococcus thermophilus: physiology, biochemistry, and genetics. Invited oral presentation for the Marschall/Rhône-Poulenc Italian and Specialty Cheese Conference, Sept. 17-19, Madison, WI.

Patent/Invention Disclosures:
No patents/invention disclosures filed

Technology Transfer Activities  Licensing this technology is underway
For information on licensing contact:
Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center
Project Report

Principal Investigators: Janine E. Trempy, Oregon State University
Co-Investigators:

Project Title: Biopolymers: a value added product derived from low value whey and lactose: Phase I. REVISED

Institution’s Project #: 89100

Project Completion Date: 12/31/98

Increase the number of alternative uses for lactose.

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
Further define the media conditions for optimal expression of the desirable ropy biopolymer. Establish and pursue a collaboration with Dr. Jeff Ahlgren at the USDA to characterize the composition of the biopolymer produced by this Ropy isolate. Further the sensory evaluation studies outlined original DMI proposal.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Biopolymers (microbial exopolysaccharides) have diverse applications with significant application as natural thickening, stabilizing and emulsifying agents in bakery, dairy (especially low fat/no-fat products), snacks, beverages and nutraceuticals/health products for human consumption. The demand, and thus market for biopolymers enjoys enormous profits with an estimated world market value at about US$ 10 billion dollars in 1993. To date, very little progress in the US has been made in identifying and developing new biopolymers from GRAS (generally regarded as safe) microbes such as the Lactic Acid bacteria (LAB). This research program has identified a natural lactococcal isolate, a Lactococcus lactis ssp. cremoris (referred to as
Ropy), which produces a biopolymer with commercial application. Preliminary studies demonstrated that this natural lactococcal Ropy isolate expressed a very ropy biopolymer (>20 mm in length) when grown on whey- or lactose- based media. This ropy biopolymer was not produced in glucose based media. Preliminary data from consumer based panels suggested that this ropy biopolymer produced a viscous, fermented milk product with a very smooth body and excellent texture. This study seeks to extend this analysis, with a 1) comprehensive examination of the culture conditions necessary for expression of this desirable ropy biopolymer, 2) initiation of the chemical characterization of the ropy biopolymer in collaboration with Dr. Jeff Ahlgren at the USDA, and 3) additional sensory characterization of products fermented by this natural lactococcal Ropy isolate. Data generated from this study will test the hypothesis that the production of the commercially applicable ropy biopolymer by the natural lactococcal isolate appears to require culturing in whey- or lactose-based media thus providing applications for whey and lactose.

1. Significant Progress against Objectives:
   Collaboration with Dr. Ahlgren established
   Preliminary data suggest a novel structure for the ropy biopolymer

2. Significant Conclusions:

3. Anticipated Problems/Delays:
Because of delay in funding, project completion date extended to December 31, 1998.

Publications:

Theses:
Eric Knoshaug, thesis anticipated 7/98
Title: Exopolysaccharide biothntheses by a natural lactococcal ropy isolate.

Published Abstract:
1998. Mutational analysis defining ropy exopolysaccharide expression on
Lactococcus. ADSA National meeting Denver CO.

Presentations:

**Patent/Invention Disclosures:**
Oregon State University

**Technology Transfer Activities**
For information on licensing contact:

Visitors Hosted:
Confidential - For Center Director's Use Only
(Release of this information requires prior approval by principal investigator)

Provide summary of significant advances in project that you wish to remain confidential at this time. For example, new methodology that you do not wish to release until the current work is completed; information on invention disclosures or patents filed; activities conducted to transfer the technology to industry, or licensing agreements that are underway.

This information is important for meeting the contractual agreement under which your research was funded.

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries: