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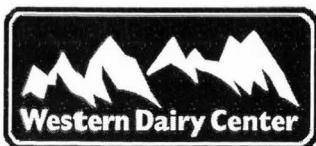
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# Western Dairy Center

## Annual Report

2001



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**Western Dairy Center  
Annual Meeting  
Sun Valley, Idaho  
August 6, 2002**

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## Western Dairy Center Annual Meeting Sun Valley, Idaho August 6 , 2002

### OAC Meeting

- |       |       |                           |
|-------|-------|---------------------------|
| 1:00  | Walsh | Welcome and introductions |
| 1:10  | Walsh | Status of the Center      |
| 1:30: | Walsh | Center Business           |

### Project reports – current projects

- 1:45 Walsh - Characterization of textured whey protein used as meat extender
- 2:00 Hansen - Controlling Chemical Composition and Functionality of Cheese
- 2:15 Weimer - Importance of glutamic acid and a-keto acids in cheese flavor development
- 2:30 Weimer - Rapid detection of *Listeria* in dairy products
- 2:45 Weimer - The proteome of lactic acid bacteria
- 3:00 Break
- 3:15 Broadbent - New starter systems for accelerated ripened Cheddar cheese
- 3:30 Broadbent - Effects of microbial exopolysaccharide on functionality in high moisture cheese
- Understanding capsule production in *Streptococcus thermophilus* MR-1C
- 3:45 Broadbent - Production of intensely flavored Cheddar-type cheeses by adjunct cultures
- 4:00 Cornforth - Dried whey minerals as an antioxidant in processed meats
- Applications of Skim Milk Powder in Processed Meats
- Dried Milk mineral as a antioxidant
- 4:15 Brothersen - Development of vitamin fortified cheese using high pressure injection technology

Effect of size, hydrophobicity and temperature on the diffusion of molecules within the Cheddar cheese matrix.

- 4:30 Swanson - Nonthermal attenuation of *Lactobacilli* to accelerate cheese ripening
- 4:45 Torres - Shredded Cheddar cheese: accelerating shreddability by moderate hydrostatic pressure
- 5:00 Break

**Western Dairy Center  
Project reports – old projects**

- 5:15 McMahon - Characterization of proteolytic enzymes from thermophilic lactic acid bacteria and their influence on Mozzarella cheese functional properties
- Understand the role of proteolysis on functional properties of Mozzarella cheese
- 5:30 Geller - The use of bacteriophage-receptor genes of *Lactococcus lactis* to develop bacteriophage-resistance in cheddar cheese starter strains
- 5:45 McGuire - Effect of butter and margarine intake on human milk CLA and fat concentrations in lactating women
- 6:00 Weimer – Cheese flavor projects.
- Identification and characterization of components of the proteolytic enzyme system of *Lactobacillus helveticus* which effect bioactive peptide accumulation, Utah State University part
- Microbial catabolism of methionine to improve Cheddar cheese flavor - a comparative study of the relative contribution by starter cultures and flavor adjunct bacteria
- Conversion of amino acids to short and branched-chain fatty acids by starter and adjunct bacteria
- Debittering with Brevi Protease
- 7:00 Dinner (on the lawn by the swan pond)

# Western Dairy Center Activities Summary 2001

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, Washington State University and Weber State University. This report summarizes the research activities from January 1, 2001 through December 31, 2001.

Following our Center Annual Meeting on August 6<sup>th</sup> we will be conducting our 15<sup>th</sup> Biennial Cheese Industry Conference in Sun Valley Idaho. We have changed the location of this meeting to coincide with the Idaho Milk Processors Association Annual Meeting, being held August 8-9. Our Biennial Cheese and Whey conference included attendees from both dairy producers, processors and researchers and should be an interesting day of discussion.

Of the technologies highlighted at this year's Dairy Management Booth at the annual Institute of Food Technologists meeting in Anaheim, CA, was Carl Brothersen's flavor injected cheese. This was the last year of funding for the Center of Dairy Technology Commercialization. Carl, in conjunction with Conly Hansen and Robert Fife, designed and tested a high pressure injection system for adding colors and flavors to cheese. They screened over 300 flavors and developed several suitable flavors, such as blueberry, green apple, grape, garlic, smoke, pizza, dill, sage, and coffee. He is now looking for partners in the dairy industry to produce and market the product.

The Center conducted the 17th Annual Cheese Making Short Course February 6-8, 2001, at Utah State University, with 12 attendees. We limit the number of attendees to ensure a "hands on" learning experience. Our short course was able to use our new cheese making facilities, specifically two automated Scherping cheese vats. Our Scherping cheese vats have a 1500 lbm capacity and we also have a new finishing table with a 1500 lbm capacity.

The Center for Microbial Physiology and Rapid Detection, directed by Bart Weimer, continued their activities in 2001. The center is developing methods to detect pathogens in dairy products, other food products, water and air. This center was successful in licensing one of their technologies and developed a new company, BioMatrix Solutions, to pursue the commercialization of a rapid microbe detection instrument.

In 2001, competitive grants awarded by Dairy Management resulted in \$321,689 research dollars and grants awarded by the Western Dairy Center resulted in 42,000 research dollars. Project progress reports of all research projects active in 2000 are included in this report.

## 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:

Eric Bastian  
Avonmore West  
1341 Fillmore  
Idaho Falls, ID 83301

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

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Jeff Kondo  
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9015 West Maple St.  
Milwaukee, WI 53214

Marie Walsh  
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Von T. Mendenhall, Head  
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425 Pine Street  
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Swiss Valley Farms  
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Davenport, IA 52808

Dennis Romero  
Rhodia Inc.  
2802 Walton Commons West  
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## WESTERN DAIRY CENTER PRINCIPAL INVESTIGATORS

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Conly Hansen  
Dept of Nutrition & Food Sciences  
Utah State University  
Logan, UT 84322-8700

## Financial Summary of Approved Projects for 2001

### Projects Funded by DMI

Production of intensely flavored Cheddar-type cheeses by adjunct cultures PI: Jeff Broadbent.....	\$29,100
New starter systems for accelerated ripened Cheddar cheese PI: Jeff Broadbent.....	\$53,054
Effects of microbial exopolysaccharide on functionality in high moisture Cheese PI: Jeff Broadbent.....	\$29,435
Development of vitamin fortified cheese using high pressure injection Technology PI: Carl Brothersen.....	\$17,950
Dried milk mineral as an antioxidant in processed meats. PI: Daren Cornforth.....	\$15,700
Controlling chemical composition and functionality of cheese PI: Conly Hansen.....	\$55,610
Nonthermal attenuation of <i>Lactobacilli</i> to accelerate cheese ripening PI: Barry Swanson.....	\$28,000
Shredded Cheddar cheese: accelerating shreddability by moderate hydrostatic pressure (MHP) PI: J.A. Torres.....	\$35,039
Characterization of textured whey protein used as a meat extender PI: Marie K. Walsh.....	\$35,380
Importance of glutamic acid and $\alpha$ -keto acids in cheese flavor Development PI: Bart Weimer.....	\$28,340
Rapid detection of <i>Listeria</i> in dairy products PI: Bart Weimer.....	\$32,700

Projects funded by WDC

Understanding capsule production in <i>Streptococcus thermophilus</i> MR-1C	
PI: Jeff Broadbent.....	\$11,000
Dried milk mineral as an antioxidant in various processed meats	
PI: Daren Cornforth.....	\$20,000
Rehydration and structure of reconstituted casein micelles.	
PI: Donald McMahon.....	\$10,000
The proteome of lactic acid bacteria	
PI: Bart Weimer.....	\$1,000





# Western Dairy Center

## Project Report

Reporting Period April 1, 2000 — December 31, 2001

**Principal Investigators:** Jeff Broadbent  
**Co-Investigators:** Drs. James Steele and Bill Wendorff, University of Wisconsin-Madison

**Project Title:** Production of intensely flavored Cheddar-type cheeses by adjunct cultures.

**Institution's Project #:** 00116

**Project Completion Date:** 12/31/02

**National Research Plan Priority:** cheese **Goal:** 3.3

**Modifications to Project/Budget:**  
none

**Project Objectives:** (Include any revisions to objectives)

0. The construction of strains of *Lactobacillus casei* which produce elevated levels of diacetyl.
1. Construction of strains of *Lactobacillus casei* which over-express a bacterial lipase known to enhance cheese flavor.
2. Manufacture processed cheese from Cheddar cheese having significantly elevated levels of free fatty acids or furanones and pyrazines.

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

Use of cheese as an ingredient is in part dependent on the impact of the cheese on final product flavor. Process cheese is a significant cheese group and an excellent model system to study carry through of specific flavor compounds into natural cheese-derived products. This project seeks to utilize flavor adjunct lactic acid bacteria to produce elevated levels of specific flavor compounds in natural cheese and then determine the impact of those flavor compounds in processed cheese.

### 1. Significant Progress against Objectives:

Dr. Broadbent's part of this project is focused on Objective 1, and entails construction of *Lb. casei* mutants which lack the ability to produce pyruvate formate lyase (Pfl). To accomplish this, we first needed to clone the *pfl* gene from *Lb. casei*, so primers for polymerase chain reaction (PCR) were designed from consensus regions in *pfl* genes in other microorganisms. This approach allowed us to isolate an internal fragment of the *Lb. casei pfl* gene, and then inverse PCR was used to collect approximately 1-kb of sequence upstream and

downstream of the *pfl* gene. We then constructed a deletion derivative of the *pfl* gene that lacks the region encoding the enzyme's catalytic center, and are now in the process of creating a *Lb. casei* mutant that lacks *pfl* activity by gene replacement. However, the development of a gene replacement procedure for *Lb. casei* proved to be more problematic than anticipated. Efforts to employ temperature-sensitive plasmid vectors proved unsuccessful because plasmid replication at high temperature was not impeded in *L. casei*. However, recent work in Dr. Steele's laboratory has demonstrated that gene inactivation in this species can be achieved by single crossover with a suicide vector. We have since transferred our *pfl* deletion construct into the suicide vector and transformation experiments to introduce it into *L. casei* are underway.

Dr. Steele's laboratory has been working on objective 2, and they have successfully adapted a flood plate screen in *Escherichia coli* to identify lipase/esterase genes from *Lb. casei* Lila. This assay has allowed them to identify genes encoding four distinct lipase/esterases from this organism, and these enzymes have been characterized in significant detail. Unfortunately, none of these enzymes are active on milk triacylglycerides; therefore, they are unlikely to have a role in the formation of free fatty acids in cheese. Subsequently an enzyme with similarity to a clostridial lipase was observed in the genome sequence of *Lactobacillus helveticus* CNRZ32. This enzyme was characterized and was also determined to not have activity on milk triacylglycerides. As noted above, Dr. Steele's lab has also directed its efforts toward developing a gene replacement method for *Lb. casei*. A procedure has been developed which allows for high-frequency electroporation of a number of strains of *Lb. casei*, and this protocol has recently been used to achieve gene disruption with a suicide vector.

## **2. Significant Conclusions:**

see progress, above

## **3. Anticipated Problems/Delays:**

The difficulties in developing a gene replacement method for *Lb. casei* were unexpected and have delayed progress. However, recently significant progress has been obtained towards the development of this component of the project. Therefore, it appears likely that objective one will be accomplished in the near term. Attempts to complete Objective 2 have been abandoned. The focus of this project for the remainder of the time will be to construct a *pfl* deficient derivative of *Lb. casei* and examine its effect on the production of cheese flavor compounds in a model system.

## **Publications:**

none

## **Theses:**

none

**Published Abstract:**

none

**Presentations:**

none

**Patent/Invention Disclosures:**

none

**Technology Transfer Activities**

For information on licensing contact:

Jeff Broadbent

**Visitors Hosted:**

none

**Invention Disclosures: (Title, Date)**

none

**Patents: (Title, Date, #)**

none

**Licensing Activities:**

none

**Discoveries:**

see above

# Western Dairy Center

## Project Report

Reporting Period January 1, 2000 — December 31, 2001

**Principal Investigators:** Jeff Broadbent  
**Co-Investigators:** Dr. Charlotte Brennand, Utah State University  
Drs. James Steele and Mark Johnson,  
University of Wisconsin-Madison  
**Project Title:** New starter systems for accelerated ripened  
Cheddar cheese  
**Institution's Project #:** 00114  
**Project Completion Date:** 12/31/02

**National Research Plan (1997): Priority:** milk/whey powder **Goal:** 2 **Tactic:** 2

**Modifications to Project/Budget:**  
none

**Project Objectives:** (Include any revisions to objectives)

- 0I Determine bitter taste thresholds for casein derived peptides in a cheese model system.
- 1I Define the contribution of *Lactobacillus helveticus* CNRZ32 peptidases to the hydrolysis of casein derived bitter peptides.
- 2I Construct food-grade *Lactococcus lactis* S2 derivatives with enhanced activity of peptidases demonstrated to be important in hydrolysis of bitter peptides.
- 3I Develop a food-grade, genetic system for proteinase gene exchange in industrial strain of *Lactococcus lactis*.

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

Bitterness is a significant concern in accelerated ripened cheese. It is our hypothesis that the most effective strategy to control bitterness and over-ripening by proteolysis in accelerated ripened cheese is to develop a starter system that combines a low propensity for the production bitter peptides with high debittering peptidase activity. A key advantage to this approach is that it will not only help to retard over-ripening by proteolysis (via control over bitterness), it will also boost (via the production of free amino acids) levels of cheese flavor precursors in the curd matrix. Our group has shown that the starter proteinase is a key determinant in the production of bitter peptides, and that we can increase the activity of enzymes that degrade bitter peptides up to 1000-fold using a starter-based enzyme delivery system. In this project, we will expand on our previous work in a manner that provides industry with the information and technology transfer tools to develop food grade starter systems that control bitterness and over ripening by proteolysis in accelerated ripened cheese.

## 1. Significant Progress against Objectives:

Research at Utah State University is addressing objectives 1 and 4, while objectives 2 and 3 are being pursued at the University of Wisconsin-Madison. In objective 1, we have synthesized and purified several peptides at large scale for sensory analysis with our model cheese system. Model cheese for this system, which is prepared using a proteinase-negative and autolysis-resistant starter culture, was also prepared and frozen at  $-80^{\circ}\text{C}$ . Dr. Brennan has recently completed training for our expert bitterness sensory panel, and sensory studies with peptides will be performed before the end of May. To pursue objective 4, we determined the complete nucleotide sequence of *L. lactis* genes for the group h (bitter) and b (nonbitter) cell envelope proteinases (CEP). Those sequences were then utilized to design and test PCR primers that can discriminate between each of these genes, and to identify a region of the group b *priP* gene for gene replacement experiments. As part of this effort, we also determined optimal transformation parameters for several industrial strains of *L. lactis* that contain the group h enzyme, and confirmed that our temperature-sensitive vector was functional in each strain. The group b gene fragment selected for gene replacement was recently cloned into the vector, and gene replacement experiments to convert bitter group h CEP into group b enzyme in our industrial isolates are now underway.

Work on objective 3 at the UW-Madison has led to the identification of a new *Lb. helveticus* post-prolyl endopeptidase, designated PepO2, that appears to have a major role in hydrolysis of the bitter peptide  $\beta$ -CN (f193-209). Because proline is a common constituent of bitter peptides, we believe PepO2 may be one of the most important de-bittering enzymes in *Lb. helveticus*. It was not been possible to construct an isogenic derivative of *Lb. helveticus* CNRZ32 lacking the post-proline endopeptidase (PepO2) by gene replacement. The most likely explanation for this observation is that PepO2 has an essential function for the growth of CNRZ32. Therefore, we have constructed food-grade strains of *Lc. lactis* that over-express PepO2 and PepN. Cell-free extracts prepared from these derivatives will be utilized to examine the effect of these enzymes on the degradation of  $\beta$ -CN(f193-209) and  $\alpha_{s1}$ -CN(f1-9).

Food-grade vectors based on dominant and complementation markers have been constructed. Complementation markers are dependent on a mutation in the host strain and can be developed only for a specific vector-host combination. Dominant markers on the other hand are widely applicable. A food-grade vector based on the  $\alpha$ -galactosidase, a dominant marker, from *Bifidobacterium longum* has been successfully used in the construction of a food-grade vector utilizing a theta replicon from lactococci. Selection for this vector is based upon the vector encoding the ability of lactococcal strains expressing the  $\alpha$ -galactosidase to grow on melibiose.

## 2. Significant Conclusions:

see progress, above

**3. Anticipated Problems/Delays:**

We regret to report that Dr. Marie Strickland is on short-term disability due to serious health complications. D. Strickland is our expert on peptide analysis in cheese, and we have opted to wait and see if she can regain her health before seeking a replacement. Whatever the outcome, this situation will unquestionably delay our progress on this project.

**Publications:**

Broadbent, J. R., M. Barnes, C. Brennand, M. Strickland, K. Houck, M. E. Johnson, and J. L. Steele. 2002. Contribution of *Lactococcus lactis* cell envelope proteinase specificity to peptide accumulation and bitterness in reduced-fat Cheddar cheese. *Appl. Environ. Microbiol.* 68:1778-1785.

Strickland, M., M. E. Johnson, and J. R. Broadbent. 2001. Qualitative and quantitative analysis of proteins and peptides in milk products by capillary electrophoresis. *Electrophoresis* 22:1510-1517.

**Theses:**

None

**Published Abstract:**

None

**Presentations:**

Sridhar, V.R., V.V. Smeianov and J.L. Steele. 2002. Construction and evaluation of food-grade vectors for *Lactococcus lactis* using aspartate aminotransferase and alpha-galactosidase as selectable markers. 2002 ADSA annual meeting abstract submitted.

Brennand, C. P., and J. R. Broadbent. 2001. Use of sensory methods in a molecular-genetic study of bitterness in Cheddar cheese. Invited oral presentation for the Symposium on Sensory Evaluation of Dairy Flavors at the Ann. Mtg of the Institute for Food Technologists. June 23-27, New Orleans, LA.

Chen, Y. C., J. E. Christensen, M. Strickland, and J. L. Steele. 2001. Identification and characterization of endopeptidase O2 from *Lactobacillus helveticus* CNRZ32, an enzyme involved in the hydrolysis of a  $\kappa$ -casein derived bitter peptide. Oral presentation at the Ann. Mtg of the Institute for Food Technologists. June 23-27, New Orleans, LA.

**Patent/Invention Disclosures:**

None

**Technology Transfer Activities**

For information on licensing contact:  
Jeff Broadbent

**Visitors Hosted:**

none

**Invention Disclosures: (Title, Date)**

none

**Patents: (Title, Date, #)**

none

**Licensing Activities:**

none

**Discoveries:**

See above



# Western Dairy Center

## Project Report

Reporting Period January 1, 2000 — December 31, 2001

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

**Project Title:** Effects of microbial exopolysaccharide on functionality in high moisture cheese.

**Institution's Project #:** 00115

**Project Completion Date:** 12/31/01

**National Research Plan Priority:** cheese **Goal:** 10

**Modifications to Project/Budget:**  
none

**Project Objectives:** (Include any revisions to objectives)

1. To characterize the effect of a capsular exopolysaccharide on the firmness of increased moisture stirred curd cheese.
2. To determine whether functional properties of shredded cheese are maintained when cheese is produced with a capsular exopolysaccharide-producing starter culture.

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

The objectives of this study were to determine if the addition of *Streptococcus thermophilus* MR-1C, a strain that produces a large capsular exopolysaccharide (EPS), to cheese can be used to improve shreddability, as measured by firmness, in high moisture American style cheese. Functional attributes of cheese such as shreddability and melt are dramatically affected by cheese moisture level. Previous work at Utah State University has demonstrated that: i) cheese moisture content is related to the water binding capacity of the curd matrix; ii) addition of MR-1C to cheese results in a significant increase in cheese moisture level; iii) this effect is due exclusively to the water-binding properties of the MR-1C capsular exopolysaccharide; and iv) use of MR-1C in cheese manufacture does not have a detrimental effect on whey viscosity. To determine whether the MR-1C EPS can be used to improve cheese firmness, we manufactured stirred curd cheeses with equivalent moisture levels (42%) using either the *S. thermophilus* MR-1C or a non EPS-producing adjunct. The suitability of individual cheeses for shredding was determined using rheometry and texture profile analysis. The firmness of each cheese was determined after 1, 3, and 6 wks of aging, then the cheese was shredded, 3% (wt/wt) powdered cellulose was added to prevent caking, then the

cheese was packaged with a nitrogen gas flush, and melt properties were measured periodically over 24 wks.

### 1. Significant Progress against Objectives:

After manufacturing numerous vats of stirred curd cheese using either the *S. thermophilus* MR-1C or a non EPS-producing adjunct culture, enough lots of cheese with equivalent moisture levels (42%) were obtained. When examined using rheometry and a meltability test, there were no consistent significant differences in the cheeses based on whether an EPS-positive or EPS-negative culture can be used. Cheese made using DM10 was slightly more adhesive than cheese made using MR1C ( $P=0.095$ ), while MR1C-cheese was softer at week one, harder at week three, and softer again at week six. Using an anticaking agent prevented any clumps from forming after the cheese was shredded.

A second experiment was run to determine the validity of the adhesiveness and hardness results calculated in the first experiment and to compare the quality of shreds obtained from cheese using either the *S. thermophilus* MR-1C or the DM10 non EPS-producing adjunct. The quality of the shreds was determined by shredding small, equally sized blocks of cheese. The shredder was weighed before and after shredding to determine the gumminess of the cheese. The shredded cheese was then sifted through six sieves sized for 5 min. Each sieve was then weighed. Three percent (wt/wt) powdered cellulose was added to the cheese shreds, and the shreds were then run through the sieves again for 5 min. The stickiness of the cheese was determined as the difference between the total weight of the shreds in the bottom pan of the sifter with or without the added anticaking agent. The data has not yet been statistically analyzed. It does not appear, however, that using MR-1C may provide some improvement to shreddability of cheese.

If it is found that using the EPS-positive culture does not improve shreddability, the data will be pooled and analyzed to determine the effect moisture content has on shreddability.

### 2. Significant Conclusions:

see progress, above

### 3. Anticipated Problems/Delays:

none

### Publications:

- Broadbent, J. R., D. J. McMahon, C. J. Oberg, and D. L. Welker. 2001. Use of exopolysaccharide-producing cultures to improve the functionality of low fat cheese. *Int. Dairy J.* 11:433-439
- Petersen, B. L., R. I. Dave, D. J. McMahon, C. J. Oberg, and J. R. Broadbent. 2000. Influence of capsular and ropy exopolysaccharide-producing *Streptococcus thermophilus* on Mozzarella cheese and cheese whey. *J. Dairy Sci.* 83:1952-1956.

**Theses:**

none

**Published Abstract:**

Singleton, T. J., D. J. McMahon and J. R. Broadbent. 2001. Effect of microbial exopolysaccharide on functionality in high moisture cheese. 96th American Dairy Science Association Meeting, J. Dairy Sci. 84(Supp. 1):147

**Presentations:**

none

**Patent/Invention Disclosures:**

none

**Technology Transfer Activities**

For information on licensing contact:  
Jeff Broadbent or Donald McMahon

**Visitors Hosted:**

none

**Invention Disclosures: (Title, Date)**

none

**Patents: (Title, Date, #)**

none

**Licensing Activities:**

none

**Discoveries:**

see above

# Western Dairy Center

## Project Report

Reporting Period January 1, 2001 — December 31, 2001

**Principal Investigators:** Carl Brothersen, Utah State University

**Co-Investigators:**

**Project Title:** Development of vitamin fortified cheese using high pressure injection technology

**Institution's Project #:** 01128

**Project Completion Date:** December 31, 2001

**National Research Plan (1997): Priority:** milk/whey powder **Goal:** 2 **Tactic:** 2

### Modifications to Project/Budget:

### Project Objectives: (Include any revisions to objectives)

High pressure injection technology can be used to improve the nutritional quality of cheese.

Objective 1: Data to show the effect of fortifying cheese with vitamins D, B<sub>6</sub>, E and folic acid on the flavor of the cheese

Objective 2: Data to show the stability of added vitamins D, B<sub>6</sub>, E and folic acid in cheese over time.

Objective 3: Data on how the addition of vitamins D, B<sub>6</sub>, E and folic acid effects the dynamics of starter and non-starter bacteria in the cheese.

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

**1. Significant Progress against Objectives:**

One day old Cheddar and Mozzarella cheese was injected with vitamins D, B<sub>6</sub>, and folic acid. Samples for vitamin analysis were collected and frozen at -80 C. Samples collected and analyzed for starter and non starter organisms. Samples were aged at 4 C for flavor analysis to be conducted when the cheese is six months old. The sensory panel has been trained and are waiting until the cheese has aged before analysis.

**2. Significant Conclusions:**

No data or conclusions will be available until the cheese has aged and has been analyzed.

**3. Anticipated Problems/Delays:**

The cooler containing the original samples malfunctioned resulting in the loss of all samples. New samples were prepared, delaying the completion of the project by six months.

**Publications:**

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

[file:///G4/Carl's drop box/WDC Annual meeting/WDC annual meeting reports/Cornforth mineral 326 12%2F01](file:///G4/Carl's%20drop%20box/WDC%20Annual%20meeting/WDC%20annual%20meeting%20reports/Cornforth%20mineral%20326%2012%20F01)

# Western Dairy Center

## Project Report

Reporting Period January 1, 2001 — December 31, 2002

**Principal Investigators:** Dr. Daren Cornforth, Utah State University

**Co-Investigators:**

**Project Title:** Dried milk mineral as an antioxidant in processed meats.

**Institution's Project #:** 01126

**Project Completion Date:** December 31, 2002

<b>National Research Plan (1997): Priority:</b> milk/whey powder <b>Goal:</b> 2 <b>Tactic:</b> 2
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**Modifications to Project/Budget:**

None

**Project Objectives:** (Include any revisions to objectives)

- Objective 1: Determine the effectiveness of dried whey mineral (WM) as inhibitor of rancidity in fresh pork sausage (an uncured sausage cooked immediately before serving). Rancidity will be measured by a chemical method (Thiobarbituric acid or TBA test) and by trained panel sensory evaluation.
- Objective 2: Determine the effectiveness of dried WM as an inhibitor of rancidity in Italian sausage (an uncured, precooked sausage used as a pizza topping). Rancidity will be measured by the TBA test and by trained panel sensory evaluation.
- Objective 3: Determine the effectiveness of dried WM as an inhibitor of rancidity in Summer Sausage (a nitrite-cured, pre-cooked sausage). Rancidity will be measured by the TBA test and by trained panel sensory evaluation.
- Objective 4: Determine the optimum use levels and economic viability of using dried WM as an antioxidant in processed meats (fresh pork sausage, Italian sausage, summer sausage).
- Objective 5: It appears likely that the insoluble calcium phosphate particles in WM bind iron released from meat pigments during cooking, preventing iron catalyzed lipid oxidation. To test this hypothesis, it is proposed to directly measure soluble ionic iron levels in fresh pork sausage, Italian sausage, and summer sausage before and after cooking

and at various intervals during storage in samples with or without added WM. Whey mineral will be added at the optimum levels determined in objective 4 above.

Objective 6: Compare the effectiveness of WM to other known antioxidants (Rosemary, BHT, sodium nitrite) in a cooked ground beef model system.

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

The effect of 0.5–2.0 % MM was tested as an antioxidant in raw and cooked ground pork stored at -20 C for 4 months, compared to 0.01 % (fat basis) butylated hydroxytoluene (BHT) and 0.5% sodium tripolyphosphate (STP). TBA numbers were low (<0.45) and not significantly different ( $p>0.05$ ) between raw meat treatments. TBA number was significantly lower ( $p<0.01$ ) for all treatments with MM or STP after 4 months frozen storage, compared to controls or treatments with BHT. The mean TBA numbers for all MM and STP treatments were < 1.0 at 4 months storage, compared to 2.3 and 2.1 for control and BHT treatments, respectively.

Sensory testing was also done to determine at what level of TBA number do consumers reject the sample as too rancid. Preference test was chosen as an appropriate test. The patties were cooked and stored at 2 degrees C for 1, 2 and 3 days to get TBA numbers of 1.48, 3.37 and 3.94 respectively. Freshly cooked patties (TBA number = 0.409) and patties treated with 0.5% STP (TBA number=0.199) were used for comparisons. Panelists preferred ( $p<0.001$ ) patties with TBA number 0.4 or less over patties with TBA numbers  $\geq$  1.4.

In another study TBA numbers were measured on cooked pork patties as affected by 30, 60, 90 or 120 min of warm storage (71°C). TBA number increased from 0.32 at 0 min to 1.17 at 120 min. TBA numbers after 120 and 90 min were significantly higher than after 0 to 60 minutes. Thus patties could be kept warm for up to 60 minutes after cooking without significantly increasing TBA number.

### **1. Significant Progress against Objectives:**

Milk mineral at 1-2% of raw meat weight was found to significantly reduce rancidity development during frozen storage of cooked pork patties. Milk mineral was a more effective antioxidant than BHT.

### **2. Significant Conclusions:**

Milk Mineral significantly reduced lipid oxidation during frozen storage of cooked pork patties. Sensory panelists detected rancid flavor in samples with TBA number of 1.4 or greater. Cooked pork patties could be held warm for 1 hr before serving without significant increase in thiobarbituric acid (TBA) number.

### **3. Anticipated Problems/Delays:**

None



**Publications:**

Cornforth, D. P. and West E. M. 2002. Evaluation of the antioxidant effects of dried milk mineral in cooked beef, pork, and turkey. J. Food Sci. 67:615-618.

**Theses:**

Preetha Jayasingh, in preparation.

**Published Abstract:**

Jayasingh, P. and Cornforth, D. P. Antioxidant effect of dried milk mineral in fresh and cooked ground pork. 2001. Reciprocal Meat Conf. Proc. 54:198.

**Presentations:**

**Patent/Invention Disclosures:**

Dried Milk Mineral Fraction as an Antioxidant  
U.S. Patent Application No. 09/604,622  
Filing Date: June 27, 2000

**Technology Transfer Activities**

For information on licensing contact: Mr. Russell Price, Technology Park, Utah State University, Logan, Utah 84322-8700
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**Visitors Hosted:** None

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

Dried Milk Mineral Fraction as an Antioxidant  
U.S. Patent Application No. 09/604,622  
Filing Date: June 27, 2000

**Licensing Activities:**

None at present

**Discoveries:**

Milk Mineral significantly reduced lipid oxidation during frozen storage of cooked pork patties. Sensory panelists detected rancid flavor in any samples with TBA number of 1.4 or greater. Cooked pork patties could be held warm for 1 hr before serving without significant increase in thiobarbaturic acid (TBA) number.

# Western Dairy Center

## Project Report

Reporting Period January 1, 1997 – December 31, 2001

**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:** June 30, 2002

**National Research Plan (1997): Priority:** Goal: 4.2 **Tactic:** 1,3,6  
Understand phage-resistance systems of starter cultures and phage counter  
defense systems to develop longer lasting phage resistance strategies.

**Modifications to Project/Budget:**

One year no-cost extension to 12/31/00

**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 the 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 the 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 to phage from attaching or entering the host in the first place. To do this requires 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 pip 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-head phage (p335 and 936). Together with the phages that required pip, the p335 and 936 species of phage cause nearly all the starter failures in U.S. 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:**

We have cloned by complementation, lactococcal genes required for infection of phage sk1, which is a phage of the species 936. An unusual, spontaneous, phage sk1-resistant mutant (RMSK1/1) of *Lactococcus lactis* C2 apparently blocks phage DNA entry into the host. Although no visible plaques formed on RMSK1/1, this host propagated phage at a reduced efficiency. This was evident from center-of-infection experiments, which showed that 21% of infected RMSK1/1 formed plaques when plated on its phage-sensitive parental strain C2. Moreover, viable cell count 0 and 4 hours after infection was not significantly different than an uninfected culture. Further characterization showed that phage adsorption was normal, but burst size was reduced 5-fold and latent period was increased from 28.5 to 36 min. RMSK1/1 was resistant to other, but not all, similar phages. Phage sensitivity was restored to RMSK1/1 by transformation with a cloned DNA fragment from a genomic library of a phage-sensitive strain. Characterization of the DNA that restored phage-sensitivity revealed an open

reading frame with similarity to lysozymes ( $\beta$ -1,4-N-acetylmuramidase) and lysins from various bacteria, a fungus, and phages of *Lactobacillus* and *Streptococcus*, DNA homologous to non-coding sequences of temperate phage of *Lactococcus lactis*, DNA similar to a region of phage sk1, a gene with similarity to tRNA genes, a prophage attachment site, and open reading frames with similarities to *sun*, phosphoprotein phosphatases, and protein kinases. Mutational analyses of the cloned DNA showed that the region of homology with lactococcal temperate phage was responsible for restoring the phage-sensitive phenotype. The region of homology with lactococcal temperate phage DNA was similar to DNA from a previously characterized lactococcal phage that suppresses an abortive infection mechanism of phage resistance. The region of homology with lactococcal temperate phage was deleted from a phage-sensitive strain, but the strain was not phage-resistant. The results suggest that the cloned DNA with homology to lactococcal temperate phage was not mutated in the phage-resistant strain. The cloned DNA apparently suppressed the mechanism of resistance, and may do so by mimicking a region of phage DNA that interacts with components of the resistance mechanism.

A membrane-associated receptor for the small isometric-headed phage sk1 was identified on the plasma membrane of *Lactococcus lactis*. Purified plasma membrane from *L. lactis* sub. *lactis* LM2301 bound and inactivated phage sk1 *in vitro*. The rate of inactivation was proportional to the concentration of membranes. However, the membrane was saturated at about 6 to 12 PFU/ fmoles membrane phosphate. Plasma membrane from *E. coli* did not bind or inactivate phage sk1. Boiling, washing in 2 M KCl, 8M urea or 0.1 M  $\text{Na}_2\text{CO}_3$ /pH 11, or treating the membranes with proteases did not reduce binding or inactivation of phage. Binding and inactivation were significantly reduced by including  $\alpha$ -glycerol phosphate, EGTA, or high concentrations of NaCl in the mixture of phage and membrane. Glycerol,  $\text{NaHPO}_4$  or other components of the plasma membrane did not inhibit binding or inactivation. The phage-inactivating substance was solubilized by a non-ionic detergent and remained in the supernatant after centrifugation. The phage-inactivating and binding activity was completely destroyed by treating the membrane with a muramidase. Cell wall was purified, but the binding and inactivation of phage sk1 was not inhibited by  $\alpha$ -glycerol phosphate, EGTA, or high concentrations of NaCl. One of 5 other small isometric-headed phages tested competed with phage sk1 for the binding site to membrane. Phages that did not bind to the membrane did not affect the inactivation of phage sk1. The results suggest that phage sk1 binds to the glycerol phosphate backbone of membrane lipoteichoic acid, but also requires the participation of the peptidoglycan.

The genetic approach to identifying 936- and p335-phage receptor genes has not been productive. We have tried cloning by complementation and by insertional inactivation with pGhost::ISS1, but neither has yielded anything promising. The PhD student who was working on this approach, Rizwana Ahktar, took maternity leave in July, 2001, and has not returned to school. She was unable to clone genes from any of her insertional inactivated, phage sk1-resistant mutants. The MS candidate, Hang Ngo, found no clones in a genomic library that complemented two separate, chemically-mutagenized, phage sk1-resistant mutants. Hang has focused her efforts on identifying the phage adhesin which binds to Pip (see below).

Recent data has identified a phage c2 tail protein that binds to Pip. We have found that purified Pip binds to a phage protein with a size of about 70 kDa. This size corresponds roughly to three phage c2 proteins: *l10*, *l12*, and *l14*, with sizes of 75, 60, and 73 kDa, respectively. The *l12* protein is a terminase, and is unlikely to be packaged into mature phage particles. The functions of the other two proteins are unknown, because they do not resemble any proteins with known functions. We will identify the Pip adhesin using a combination of biochemical and genetic tactics.

## 2. Significant Conclusions:

A phage sk1-resistant mutant of *L. lactis* strain C2 was restored to phage-sensitivity by complementation with cloned host DNA. Characterization of the complementing DNA revealed a gene that encodes a protein related to lysins of phage of *Lactobacillus* spp and *Streptococcus pneumoniae*. Distal to this gene is DNA homologous to phage sk1 and non-coding sequences of temperate phage of *Lactococcus lactis*. These similarities suggest that this phage-sensitivity region may have originated as part of a prophage. The DNA with similarities to non-coding regions of temperate phage and phage sk1 was responsible for complementation.

Phage sk1 appears to bind to lipoteichoic acid on the cytoplasmic membrane. This binding also requires the participation of the cell wall (peptidoglycan). The membrane receptor for phage sk1 is not a protein.

Phage c2 binds to Pip by the products of either late gene *l10* or *l14*.

## 3. Anticipated Problems/Delays:

### Publications:

Kraus, J. and B. L. Geller. 2001. Cloning of Genomic DNA of *Lactococcus lactis* that restores phage sensitivity to an unusual bacteriophage sk1-resistant mutant. *Appl. Environ. Microbiol.* 67:791-798.

Dai, G. P. Su, G. E. Allison, B. L. Geller, P. Zhu, W. S. Kim, and N. W. Dunn. 2001. Molecular Characterization of a New Abortive Infection System (AbiU) from *Lactococcus lactis* LL 51-1. *Appl. Environ. Microbiol.* 67:5225-5232.

### Theses:

None

### Published Abstract:

None

**Presentations:**

None

**Patent/Invention Disclosures:**

None

**Technology Transfer Activities**

For information on licensing contact:

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**Visitors Hosted:**

None

**Invention Disclosures: (Title, Date)**

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**Patents: (Title, Date, #)**

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**Licensing Activities:**

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**Discoveries:**

# Western Dairy Center

## Project Report

Reporting Period January 1, 1999 – December 31, 2001

Principal Investigators: Conly Hansen, Utah State University  
Co-Investigators: Donald J. McMahon, Utah State University

Project Title: Controlling Chemical Composition and Functionality of Cheese

Institutions Project #: 99104

Project Completion Date: 12/31/02

National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1

### Modifications to Project/Budget:

None

Project Objectives: (Include any revisions to objectives)

To determine the influence of pH, calcium, salt and moisture content of cheese on shredability and meltability.

Objective 1: To develop a high-pressure injection system for modifying the chemical composition of cheese.

Objective 2: To modify pH, calcium, and salt contents of cheese while keeping all other parameters constant, and determine their influence on functionality.

Objective 3: To determine the combinations of calcium, salt, and pH required for optimum shredding and melting of cheese.

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

### Progress

2. Modifying cheese chemical composition by injecting ionic solutions into cheese
  - A. Effect of water and calcium injection on structure-function attributes of Mozzarella cheese

## Introduction

In cheese, calcium content is associated with differences in structure-function attributes. However, the specific way in which calcium influences such structure-function attributes of cheese requires further elucidation. Our objective was to determine how injection of calcium solutions into cheese affects cheese microstructure and to relate changes in structure to changes in hardness and melting of cheese.

## Materials and Methods

Mozzarella cheese (48, 49, and 53% moisture, and 22% fat) was made by a direct-acid, stirred-curd procedure. Cheese was cut into 0.3 to 0.4-kg blocks, vacuum packaged and stored for 10 d at 4°C. Cheese blocks were then high-pressure injected (1 to 5 times) with either water or a 40% calcium chloride solution. Thus, ten treatments were defined, corresponding to five water and five calcium injection levels. A control, uninjected cheese block was also considered. Injections were performed in two opposite sides of the block, and according to a 1 x 1 cm pattern, with successive injections performed 24 h apart. Pressure of injection was set as 1400 psig, and burst injection time as 1 s. After 42 d of storage at 4°C, cheese blocks were analyzed for structural and functional attributes. Scanning electron micrographs, (1500 X magnification, from two different fields) were uploaded into Adobe Photoshop® 4.0 and their gray-scale values analyzed. Dark areas (corresponding to fat/whey pockets) were differentiated from light areas (corresponding to protein matrix) by applying a threshold function, and the proportion of pixels associated with dark and light areas determined. Texture profile analysis was performed using a two-bite compression test run on an Instron 5542 (Canton, MA). Samples, 20 mm by 16 mm diameter, were taken from the cheese immediately after removal from the refrigerator and tested at ~5°C.

## Results and Discussion

Results of the statistical analysis are presented in Table 1. When water was injected, a slight increase in weight was observed. In contrast, when calcium was injected, the cheese lost weight and considerable serum was expelled from the cheese. Moisture content increased with water injection, and decreased with calcium injection. The control (uninjected) cheese had the typical structure of a stirred/pressed-curd cheese, with protein matrix interspersed with areas containing fat and/or serum. Injecting water increased the area occupied by the protein matrix (by 14% after 5 injections) as shown by an increase in the proportion of light pixels in the micrographs. Increasing the calcium content of the cheese (from 0.3% to 1.8% after 5 injections) decreased the area occupied by the protein matrix (by 17%). This represents a contraction of the protein matrix and concomitant release of serum entrapped within the protein matrix. A decrease in cheese pH occurred upon injection of calcium, but it had been



previously observed that pH did not affect cheese microstructure unless it was accompanied by a change in calcium content. Water injection decreased cheese hardness but did not affect any other functional attribute. Hardness increased when calcium was injected, but cohesiveness decreased. Adhesiveness and springiness were unaffected. Meltability of the cheese was inversely proportional to calcium content.

Table 1. Pr>F for the ANOVA sources of variation and specified contrasts by variable of interest.

Variable	Calcium	Moisture	pH	Weight	Meltin g	Hardnes s
Source						
Model	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Block	< 0.0001	< 0.0001	< 0.0001	0.0634	0.0005	< 0.0001
Treatment	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Determination	0.6707	0.9705	0.8773		0.9841	0.7387
Contrast						
Control-Calcium <sup>1</sup>	< 0.0001	< 0.0001	< 0.0001	< 0.0001	<0.0001	< 0.0001
Control-Water <sup>2</sup>	0.6225	0.0253	0.0105	0.1418	0.9455	0.0254

<sup>1</sup>. Contrast of control (uninjected cheese) against all calcium levels.

<sup>2</sup>. Contrast of control (uninjected cheese) against all water levels.

## Conclusions

Increasing calcium content of cheese alters how proteins in the cheese matrix interact. It appears that calcium promotes protein-to-protein interactions, probably through calcium bridging and charge neutralization. Such increased interactions between proteins cause contraction of the protein matrix and expulsion of serum from the matrix. More energy must also be applied to overcome these interactions and allow proteins to flow when heated. Thus,

cheese hardness is increased and meltability decreased when the calcium content of the cheese is increased.

### Presentations

- 04/2000. Utah Academy of Sciences, Arts & Letters. Southern Utah University. Cedar City, UT.
- 06/2000. Institute of Food Technologists. 2000 Annual Meeting. Dallas, TX.

### Publications

Pastorino, A.J., N.P. Ricks, C. Hansen, and D.J. McMahon. 2002. Effect of calcium and water injection on structure-function relationships of cheese. *J. Dairy Sci.* (Accepted for publication)

Pastorino, A.J., N.P. Ricks, C. Hansen, and D.J. McMahon. 2000. Effect of water and calcium injection on structure-function attributes of Mozzarella cheese.

2000 IFT Annual Meeting Book of Abstracts: 86.

B. Effect of salt on structure-function relationships of cheese

### Objectives

Our objective was to determine the effect of salt on structural and functional properties of cheese.

### Materials and Methods

Unsalted, commercial Muenster cheese (41% moisture, 29% fat, 0.7% calcium) was obtained on 1 d and cut into 0.5 to 0.6-kg blocks that were vacuum packaged and stored for 14 d at 4°C. Cheese blocks were then high-pressure injected 1, 3, or 5 times, with a 20% (wt/wt) sodium chloride solution. Successive injections were performed 24 h apart. After 40 d of storage at 4°C, cheese blocks were analyzed for chemical, structural, and functional attributes.

### Results

Injecting sodium chloride increased the salt content of cheese. After 5 injections, the salt content was 2.7% compared to 0.1% in the control, uninjected cheese. At the highest levels, salt injection promoted syneresis, and residual moisture was observed inside cheese packages. After 3 injections the moisture content decreased from 41% to 39%. However, the increased salt content resulted in a net weight gain of 1.9% after 5 injections. Cheese pH, soluble nitrogen, and total and soluble calcium content was unaffected. Although only significant at  $P < 0.1$ , cheese injected 5 times had a 4% increased area of cheese matrix occupied by protein compared to uninjected cheese.

Cheese hardness, adhesiveness, and initial rate of cheese flowing increased upon salt injection. However, the final extent of cheese flow was unaffected.

### Conclusions

Adding salt to cheese alters protein interactions, such that the protein matrix becomes more hydrated and expands. However, increasing the salt content of cheese did not cause an exchange of calcium with sodium. Therefore, calcium-induced protein interactions would remain the limiting factor controlling cheese functionality.

### Presentation

1. none (To be presented 06/2002. Institute of Food Technologists. 2002 Annual Meeting. Anaheim, CA. )
2. C. Effect of pH on structure-function relationships of cheese

### Objectives

Our objective was to determine the effect of pH on chemical and functional properties of cheese.

### Materials and Methods

Commercial Cheddar cheese (34% moisture, 30% fat, 1.7% salt, 0.8% calcium) was obtained on 1 d and cut into 0.4 to 0.5-kg blocks that were vacuum packaged and stored for 14 d at 4°C. Cheese blocks were then high-pressure injected 1, 3, or 5 times, with a 20% (wt/wt) glucono-delta-lactone solution. Successive injections were performed 24 h apart. After 40 d of storage at 4°C, cheese blocks were analyzed for chemical and functional attributes.

### Results

Injection of glucono-delta-lactone solution decreased cheese pH. After 5 injections, cheese pH was 4.7 compared to 5.3 in the control, uninjected cheese. Decreased pH increased the content of soluble calcium and decreased the total calcium content of cheese. At the highest level, injection of acid promoted syneresis, and residual moisture was observed inside cheese packages. Thus, after 5 injections the moisture content of cheese decreased from 34% to 31%. This resulted in decreased cheese weight, 2.5% after 5 injections. Injecting acid decreased cheese hardness, and at the highest levels of injection, decreased pH and moisture content caused the cheese to become brittle. Thus, the cheese lost structural cohesion, fracturing during testing. When heated, the initial rate of cheese flow increased when pH was lowered from 5.3 to 5.0. However, lowering cheese pH to 4.7 caused decreased flowing rate. Also, the final extent of cheese

flow was unaffected by lowering pH to 5.0, but it decreased when cheese pH was lowered to 4.7.

### **Conclusions**

Adding an acid solution to cheese alters protein interactions. At low levels, acid injection decreases interactions between proteins as calcium is solubilized. In contrast, at high levels, acid injection promotes protein-to-protein interactions as the proteins approach their isoelectric point. Hence, the acid precipitation of proteins overcomes the opposing effect caused by increased calcium solubilization. Therefore, calcium content would direct cheese functionality when the pH of cheese is above 5.0.

### **Presentation**

- none (To be presented 07/2002. American Dairy Association. 2002 Annual Meeting. Québec, Canada.)

# Western Dairy Center

## Final Report

Reporting Period January 1, 1998 — December 31, 2001

**Principal Investigators:** Donald McMahon\_Utah State University  
Joseph Irudayaraj, Penn State University

**Co-Investigators:**

**Project Title:** Process technology to improve the favor of heated milk

**Institution's Project #:** 98101

**Project Completion Date:** December 31, 2001

**National Fluid Milk Research Plan (1997): Priority: 3 Goal: 3 Tactic:**  
Fluid Milk 3.3 : Exploration of alternate processes for specific products and development of ingredients and beverages\_\_\_\_\_

**Modifications to Project/Budget:**

\_\_\_\_\_

**Project Objectives:** (Include any revisions to objectives)

- 1) Determine the effect of electroheating on flavor and sensory attributes
- 2) Compare the flavor characteristics to conventional UHT processes

### Project Summary:

A novel Ultra High Temperature (UHT) processing technique called Electroheating™ was investigated for producing UHT milk. Two studies were conducted six months apart to determine if there was any seasonal influence of the raw milk on either the protein denaturation due to processing or its sensory characteristics. Protein denaturation in the UHT milk produced by Electroheating™ was lower than that found in conventionally processed UHT milk. For Electroheated milk processed at 145°C with a holding time of 1 min the descriptive scores were significantly lower for metallic intensity in both studies and lower for both metallic and cardboard for study 1. Sensory scores by consumer panels rated the Electroheated™ milk at a processing temperature of 145°C with 1 sec holding time acceptable while the protein denaturation for this treatment was minimal.

### Introduction

Ohmic heating is based on the principle of passing electric current through a food product, which causes the food to heat. The most distinct advantages of

ohmic heating are: accurate control of the product temperature, uniform heating of liquids, fast heating rates, no fouling and scorching of product on the walls, low maintenance, no residual heat after the current is shut off, and very low heat losses. The major benefit of the process is that heating occurs volumetrically and the product does not undergo large temperature variations.

The Electroheating™ technology of Raztek Corporation is a unique form of ohmic heating using electrical mains frequency in conjunction with non-dissolving electrodes. The Raztek Electroheater™ used in these tests consisted of three electrodes machined from pure graphite and encased in 316 stainless steel housings. These electrode assemblies were connected together using tubes made of ULTEM 1000 plastic or food grade Coors AD995 ceramic. The column is mounted in a vertical or near vertical position with the flow of product in an upward direction. Power to the heater is provided by a main step-up transformer with the power being controlled on the primary side of the transformer using a feed-forward temperature control system. In order to fully exploit the inherent advantages of a process such as Electroheating™, the quality of the processed product becomes important. The focus of this work is to examine the sensory characteristics and protein denaturation electroheated milk.

Pasteurized milk typically has a 14-day shelf life at refrigeration temperatures while ultra-high temperature (UHT) processing allows milk to be stored 1 to 2 years at room temperature (Burton, 1988). However, higher temperatures used in UHT processing may cause cooked flavors that are objectionable to many U.S. consumers (Blake et al., 1995). Specific taste and flavor attributes and their intensities traditionally been determined using descriptive analysis (Quinnes et al., 1997). This is the process of detecting and describing both the qualitative and quantitative sensory aspects of a product by a trained panel of 5 to 10 subjects (Meilgaard et al, 1999). These techniques allow for a complete sensory description of the product, as well as, identifying which sensory attributes are important to consumer acceptance (Lawless and Heymann, 1999). Consumer acceptability of products is primarily determined by its' sensory characteristics, as well as, its' nutritional value (Claassen and Lawless, 1992). Consumer acceptance is also critical to assess the feasibility and marketability of processing treatments.

## Methods and Materials

### Heating System

The Electroheating™ system specifications include: Flow rate (250 liters/hr), temperature rise (85°C – from 70 to 155°C), heat requirement (21,520 kcal), power (24.7 kW), number of sections (2), voltage 8000 volts, current (1.55 amps/leg), area of electrode (5.6 cm<sup>2</sup>), current density (0.28 Amp/cm<sup>2</sup>), resistance of each leg (5161 ohm), specific resistance (100 ohm/cm), volume of each tube (8cc), temperature rise/sec (386°C/sec), holding tube length (20cm), holding tube diameter (0.7cm).

## Sample treatments and preparation

Milk with a 2% fat content was pasteurized at three different temperatures, as well as, two holding times using the Electroheating™ method. Overall, there were four Electroheating™ treatments: treatment 1 was processed at 155°C with a 1 sec holding time, treatment 2 was processed at 145°C with a 1 sec holding time, treatment 3 was processed at 145°C for 4 sec and treatment 4 was processed at 135°C with a 4 sec holding time. Two controls were used for the protein denaturing experiments: commercially pasteurized 2% milk and a commercially processed 2% UHT milk. Sensory evaluation utilized only one control, the commercially available 2% UHT milk.

Electroheated milk were processed and filled into sterile 1 L screw-top glass containers and sealed with a screw cap. Commercial samples of 2% UHT milk were also obtained (San Jose, CA). All milk samples were then packed into cardboard boxes to prevent light exposure and shipped overnight to Penn State University for sensory evaluation. The milk samples were held at 4° without any exposure to light. Both descriptive and consumer panels evaluated samples within one week of processing.

Two studies were conducted six months apart to determine if there were any seasonal differences detected by the descriptive or consumer panel or if there was any difference in the amount of protein denaturation in the milk samples.

## Protein Denaturation

Whey protein denaturation was measured as follows: 10 ml milk samples were diluted with 40 ml distilled water. Twenty-five milliliter of this diluted solution was adjusted to a pH of 4.6 by drop wise addition of 0.1N HCl and filtered. From each filtrate, 10 ml was used to determine whey protein nitrogen (WPN) content by a semi-micro Kjeldahl procedure (Manji and Kakuda, 1987) using a Kjeltac Auto 1030 Analyzer (Tecator AB, Hoganas, Sweden). Percent whey protein denaturation was calculated using: % Denaturation =  $100 \times (WPN_{\text{raw milk}} - WPN_{\text{heated milk}}) / WPN_{\text{raw milk}}$

## Sensory Analysis

### *Descriptive Panel*

Methodologies are the same for studies 1 and 2, except where noted. Sensory characteristics of UHT milk samples were evaluated using descriptive analysis (Lawless and Heymann, 1998). For study 1, trained panelists recruited from the Department of Food Science (n=9) performed the evaluations. Six training sessions were conducted to identify attribute terms and define those terms using reference standards. For study 2, evaluations were performed by 8 trained panelists with previous experience evaluating UHT milk.

Milk samples were served at 13°C in covered 5 oz. plastic cups coded with 3-digit codes using a random order of presentation. Panelists were instructed to swirl the sample in their mouth three times before sample assessment. Unsalted crackers, filtered water and club soda were provided for cleansing the palate between samples. During each of the 4 sessions the panelists evaluated all five experimental treatments totaling 20 samples (5 treatments x 2 production

replications x 2 duplicates). Attribute intensities were scored using a 15 cm scale anchored with 0 = none and 15 = very strong.

#### Consumer evaluation

Consumers, recruited from faculty, staff and students from the University community, were screened for drinking 2% milk. Consumers (n=79) rated the overall acceptability of a commercial 2% UHT milk and four treatments of the Electroheated™ milk samples. In addition, consumers were asked to indicate whether the samples were better than, equal to, or worse than the milk they normally drank.

Four containers of each sample were pooled in stainless steel containers and mixed thoroughly. Each panelist was served the five processed milk treatments in random order in 5 oz. cups coded with three digit codes. The serving temperature was 9°C. In the first study, 40 consumers were served samples from production replication 1 and 39 consumers were served samples from production replication 2. In the second study, 36 consumers were served samples from production replication 1 and 42 consumers were served samples from production replication 2.

## Results

### Protein Denaturation :

A comparison of protein denaturation of electroheated milk with a commercial variety is given in Table 1. The electroheated milk processed at 145°C with minimum holding time had the least denaturation. Results were consistent with all experiments.

Table 1 : Denatured protein results for fresh fluid 2% milk, a commercial 2% UHT milk, and four time temperature treatments of Electroheated™ milk.

Treatment	Study 1 % Denaturation	Study 2 % Denaturation
Pasteurized 2% milk	0.0 f	0.0 f
Commercial 2% UHT milk	70.5 a	65.3 a
Treatment1 (155°C, 1 sec)	23.7 d	28.6 d
Treatment2 (145°C, 1 sec)	21.0 e	21.1 e
Treatment3 (145°C, 4 sec)	35.3 b	36.9 b
Treatment4 (135°C, 4 sec)	27.4 c	30.2 c

*Means followed by different letters were significantly different ( $p < 0.05$ ).*



## Sensory Analysis

Results of the descriptive panel are presented in Table 2. In study 1, the four treatments of Electroheated™ milk had significantly higher sweet scores than the control. The trend was the same in study 2. Treatment 2 had a significantly lower score for metallic when compared to the control in both studies. Treatment 2 had a significantly lower score for bitter in study 2 when compared to the control sample. Collins et al., 1993 found a strong correlation between the extent of proteolysis and bitterness scores in UHT milk. Horner et al. (1980) noted that psychrotrophic bacteria in raw milk may survive thermal processing and cause the development of a bitter flavor. This might account for the fact that bitter notes were detected in study 2, milk processed in the spring while not detected in study 1, milk processed in the fall. Although there was not a significant difference in cardboard in study 2, all but treatment 3 had significantly lower scores in study 1 when compared to the control sample. In both studies, the butter scores were significantly lower for the treatments when compared to the control.

Table 2. Mean scores of descriptive analysis attributes of commercially processed 2% UHT milk as compared to four treatments of Electroheated™ milk.

Study	Milk	Sweet	Sour	Salt	Bitter	Dairy
#1	Control	0.86 b <sup>2</sup>	0.36 a	0.41 a	0.19 a	5.53 a
	TR 1	1.67 a	0.25 a	0.30 a	0.11 a	6.00 a
	TR 2	1.62 a	0.28 a	0.44 a	0.13 a	5.79 a
	TR 3	1.58 a	0.34 a	0.41 a	0.15 a	6.11 a
	TR 4	1.52 a	0.25 a	0.52 a	0.13 a	5.86 a
#2	Control	0.97 b	0.39 a	0.34 a	0.18 a	6.18 a
	TR 1	1.55 a	0.38 a	0.40 a	0.13 ab	5.98 a
	TR 2	1.43 a	0.35 a	0.43 a	0.09 b	6.07 a
	TR 3	1.18 ab	0.28 a	0.30 a	0.11 ab	6.18 a
	TR 4	1.31 ab	0.29 a	0.36 a	0.16 ab	6.32 a

Study	Milk	Cooked	Metallic	Cardboard	Butter
#1	Control	1.57 a	1.82 a	1.36 a	1.20 a
	TR 1	1.93 a	1.25 ab	0.72 b	0.42 b
	TR 2	1.88 a	1.06 b	0.64 b	0.49 b
	TR 3	2.38 a	1.34 ab	0.96 ab	0.50 b
	TR 4	2.38 a	1.34 ab	0.74 b	0.36 b
#2	Control	2.11 a	2.01 a	0.77 a	1.31 a
	TR 1	2.44 a	1.41 ab	0.37 a	0.69 b
	TR 2	1.87 a	1.12 b	0.34 a	0.61 b
	TR 3	1.76 a	1.07 b	0.41 a	0.79 b
	TR 4	1.72 a	1.30 ab	0.59 a	0.64 b

1 TR denotes treatment. Control=commercially processed 2% UHT milk; TR1=Electroheated™ 2% milk at 155°C, 1 min; TR2=Electroheated™ 2% milk at 145°C, 1 sec; TR3=Electroheated™ at 145°C, 4 sec; TR4=Electroheated™ 2% milk at 135°C, 4 sec.

2 Means followed by the same letter within a study and attribute were not significantly different ( $\alpha=0.05$ ).

Blake et al. (1995) found that trained panelists perceived a cooked flavor to be the dominant flavor change associated with increasing temperatures from 120°C to 128°C during processing. However, they also noted that other off flavors were more noticeable at processing below 128°C than at processing temperatures above 132°C. Since our processing temperatures were higher than these, it might explain why there were no great differences between the treatments. The metallic scores for treatments 1 and 2 were significantly lower when compared to the control. From these data, treatment 2 looks to be the best processing treatment in order to minimize any perceivable off-flavors.

Consumer testing indicated that the Electroheated™ milk was more acceptable than the control UHT sample (Table 3) which corresponds to consumer responses regarding how the milk compares to milk they usually drink (Table 4). The percentage of consumers who thought the Electroheated™ milk was equal or better than milk they usually drink was greater than for the control sample. This is in agreement to the previous work of Blake et al. (1995).

Table 3. Mean hedonic scores<sup>1</sup> of four treatments of Electroheated™ 2% milk as compared to commercially processed 2% UHT milk.

Treatment	Overall Liking <sup>2</sup>	
	Study 1	Study 2
Minimum Significant Difference	0.693	0.71
Control	4.2 b <sup>4</sup>	3.8 b
Treatment 1	5.7 a	5.9 a
Treatment 2	6.1 a	6.0 a
Treatment 3	5.6 a	6.0 a
Treatment 4	5.6 a	6.2 a

1 n=79 for study 1 (40 consumers evaluated production replication 1 and 39 consumers evaluated production replication 2). n=78 for study 2 (36 consumers evaluated production replication 1 and 42 consumers evaluated production replication 2).

2 Hedonic scores based on a nine point scale with 9=Like Extremely, 8=Like Very Much, 7=Like Moderately, 6=Like Slightly, 5=Neither Like nor Dislike, 4=Dislike Slightly, 3=Dislike Moderately, 2=Dislike Very Much, 1=Dislike Extremely

3 Minimum Significant Difference

4 Means having the same letter within a study are not significantly different ( $\alpha=0.05$ ).

Table 4. Summary of consumer responses to the question; "In comparison to milk you normally drink, this milk sample was:"

Study	Response	Response to each category (%)				
		Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Study 1	Better than	13.9 %	13.9 %	15.1 %	13.9 %	13.9 %
	Equal to	13.9 %	37.9 %	51.8 %	40.5 %	45.5 %
	Worse than	72.1 %	48.1 %	32.9 %	45.5 %	40.5 %
Study 2	Better than	3.8 %	13.9 %	12.7 %	16.5 %	15.2 %
	Equal to	16.5 %	40.5 %	46.8 %	41.8 %	57.0 %
	Worse than	79.7 %	45.6 %	40.5 %	41.8 %	27.8 %

## Gas Chromatography :

Concentrations of key volatile compounds recovered from Electroheated and control were determined. Significant differences in the volatile compounds (2-pentanone, 2-hexanone, 2-heptanone, and dimethylsulphide) between the control and electroheated milk could not be determined. We are in the process of refining this technique for effective detection of these compounds. Regardless of the treatment level combination, none of the electroheated samples showed identifiable differences in profiles of volatile compounds. Comparing the average electroheated data to the control sample, a total of thirteen volatile compounds were identified as having a potential sensory significance. The carbonyl compounds 2-heptanone, 2-nonanone, and nonanal were approximately 9-fold higher in the electroheated samples. Typically considered products of oxidation of unsaturated fatty acids, they are characterized as having green grass-like odors (Moio, et al, 1993). In contrast to the carbonyl compounds, the presence of two heat process-derived compounds, 3-furanmethanol and tetrahydro-2-furanmethanol, were identified only in the control samples. These compounds are associated with the development of heated flavors in products such as baked breads and caramel and are reported to have a roasted aroma. No significant differences were found between the concentrations of either phenolic or acidic compounds, however, these compounds are potent aroma-imparting compounds and may play some role in the overall background flavor of the milks. Finally, the control sample had a significantly higher amount of total esters, namely butyl acetate, than the electroheated milks.

## Conclusions

Electroheated™ milk was evaluated for protein denaturation and sensory attributes using both descriptive and consumer panels. Results of this study found that Electroheated™ milk had decreased amounts of protein denaturation and higher consumer acceptability scores when compared with a commercial 2% UHT milk product. Descriptive data showed all four treatments of Electroheated™ milk had significantly higher scores for sweetness when compared to the control sample. Data from these studies also indicate that Electroheated™ milk processed at a temperature of 145°C with a holding time of 1 sec had the least amount of protein denaturation while maintaining acceptable sensory characteristics.

## Summary

A novel Ultra High Temperature (UHT) processing technique called Electroheating™ was investigated for producing UHT milk. Two studies were conducted six months apart to determine if there was any seasonal influence of the raw milk on either the protein denaturation due to processing or its sensory characteristics. Protein denaturation in the UHT milk produced by Electroheating™ was lower than that found in conventionally processed UHT milk. For Electroheated milk processed at 145°C with a holding time of 1 min the

descriptive scores were significantly lower for metallic intensity in both studies and lower for both metallic and cardboard for study 1. Sensory scores by consumer panels rated the Electroheated™ milk at a processing temperature of 145°C with 1 sec holding time acceptable while the protein denaturation for this treatment was minimal.

**Anticipated Problems/Delays:**

**Publications:**

FORMTEXT \_\_\_none\_\_

**Theses:**

FORMTEXT \_\_\_none\_\_\_

**Published Abstract:**

FORMTEXT \_\_\_none\_\_\_

**Presentations:**

Irudayaraj, J., McMahon, D. J., and D. Reznik, 2000. Ohmic heating for UHT milk. Annual Meeting, Institute of Food Technologists, Dallas, Texas, June 12-14, 2000 (Abstract 86A-12)

**Patent/Invention Disclosures:**

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Technology Transfer Activities FORMDROPDOWN

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**Visitors Hosted:**

\_\_\_none\_\_

# Western Dairy Center

## Project Final Report

Reporting Period January 1, 1998 – December 31, 2001

**Principal Investigators:** Donald J. McMahon, Utah State University

**Co-Investigators:** Jeffery Broadbent

Craig Oberg

Bonnie Oommen

**Project Title:** Characterization of proteolytic enzymes from thermophilic lactic acid bacteria and their influence on Mozzarella cheese functional properties

**Institution's Project #:** 98092

**Project Completion Date:** December 31, 2001

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

Understand role of moisture and proteolysis on physical and functional properties of cheese.

**Modifications to Project/Budget:**

None

**Project Objectives:** (Include any revisions to objectives)

Overall Objective: Understand the influence of microbial proteolytic systems which have variable effects on the rheological properties of Mozzarella cheese.

Objective 1: Characterize proteolytic enzymes in thermophilic Mozzarella cheese starter cultures.

Objective 2: Investigate the influence of different distinct proteolytic enzyme systems on Mozzarella cheese functionality.

### Project Summary:

#### Objective 1

The purpose of this research was to investigate the diversity in specificity of cell-bound extracellular proteinases in *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. HPLC analysis of whole cell preparations of 14 *L. delbrueckii* subsp. *bulgaricus* and eight *L. helveticus* strains incubated with  $\alpha$ -S1-casein (f 1-23) detected at least six distinct proteolytic patterns. Differences between groups were found in both the primary and secondary specificity toward  $\alpha$ -S1-casein (f 1-23) and its breakdown products. No correlation was found between the *o*-phthalaldehyde (OPA) general proteolysis analysis and  $\alpha$ -S1-casein (f 1-23) cleavage profiles. Utilizing the  $\alpha$ -S1-CN (f 1-23) method, six patterns of proteolysis were found in the dairy lactobacilli tested. Understanding the

influence of *Lactobacillus* proteinase specificity on casein degradation should facilitate efforts to develop starter cultures that predictably improve the functional properties of Mozzarella cheese.

#### Objective 2.

Part-skim Mozzarella cheeses were manufactured from 2%-fat milk and aged for 21 d. Cheese was made with three different strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* in combination with a single strain of *Streptococcus thermophilus*. Although total proteolysis, as indicated by the o-phthalaldehyde analysis, was similar in each of the three strains of *L. bulgaricus*, exhibited different proteolytic specificities towards the peptide,  $\alpha_{s1}$ -CN (f 1-23). On the basis of their  $\alpha_{s1}$ -CN (f 1-23) cleavage patterns and a previously described classification these strains were assigned to the groups I, III, and V. The objective of this study was to investigate the influence of lactobacilli proteolytic systems, based on specificity towards  $\alpha_{s1}$ -CN (f 1-23), on functionality of part-skim Mozzarella cheese. Significant differences were observed in functional properties between cheeses manufactured using Group III and Group V strains. Cheeses made using Group I and Group III strains were similar in their meltability, hardness, cohesiveness, melt strength, and stretch quality. Meltability and cohesiveness increased with age while melt strength and stretch quality decreased with age for all cheeses. Additionally, HPLC showed total peak areas of water-soluble peptides derived from cleavage of  $\alpha_{s1}$ -CN (f 1-23) by different strains of lactobacilli could be highly correlated to meltability and stretch characteristics of cheeses made with those strains.

#### Methods:

##### Objective 1.

Strains of *Lactobacillus helveticus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus* were obtained from the American Type Culture Collection and commercial starter houses. Cell morphology, carbohydrate fermentation profiles, ability to produce D(-), L(+), or D/L lactic acid, and membrane fatty acid analysis were performed to confirm the species designation. Strains of *L. helveticus* and *L. delbrueckii* subsp. *bulgaricus* were screened for diversity in proteinase specificity by incubation of whole cells with the 1-23 fragment of  $\alpha_{s1}$ -CN in 4% (wt/vol) NaCl at pH 5.2. The reaction mixes were incubated at 30°C and a 25  $\mu$ l sample aliquot removed from each after 1 h and 3 h. As soon as samples were removed from the reaction tube, they were briefly spun in a microcentrifuge and the supernatant collected then heated at 75°C for 10 min to inactivate any residual enzymes. Samples were analyzed for small peptides by HPLC with purified peptides were used as standards for peak identification. When needed, capillary zone electrophoresis (CZE) was used to resolve small peptides that co-migrated on HPLC chromatographs. in (f 1-23) digestion patterns for the strains examined.

### Objective 2.

A weakly proteolytic strain of *Streptococcus thermophilus* direct-to-vat starter culture was combined with one of the three different strains of *L. delbrueckii* subsp. *bulgaricus* that were selected based on their specificity toward  $\alpha_{s1}$ -CN (f 1-23) for part-skim Mozzarella cheese manufacture. The three strains belonged to groups I, III, and V of the classification of Oberg et al., (2001). Milk was standardized to 2% fat so that casein-to-fat ratio was approximately 1.2, then pasteurized (78°C for 29 s), cooled (5°C), and stored before being used for cheese making. Milk (12 kg) was assigned randomly to four stainless steel vats, warmed to 34.5°C, then calcium chloride (0.02%) was added to the milk. One vat (control) was made without addition of any *lactobacillus* starter. The *S. thermophilus* culture was added at the rate of 0.01% to the three experimental vats and at 0.02% to the control vat. A higher inoculum was used in the control vat to compensate for the absence of lactobacilli starter. Starter cultures from Group I, III, and V were added to the experimental vats at an inoculum level of 1.25%. Curd produced was salted at a rate of 1% then stretched multi-directionally by hand for 4 min in a 5% brine solution maintained at 75°C, then molded into a ball, immersed in 2°C water at for 1 h, then cut into three blocks, vacuum packaged, and stored at 4°C for 21 d. The three cheeses made using Groups I, III, and V lactobacilli strains were designated as CI, CIII, and CV, respectively.

Meltability of cheeses was measured using a UW Meltmeter and expressed as the percentage change in height of cheese sample (7 mm thickness and 30 mm diameter) after 10 s at a constant force of 0.33 N, when heated to 65°C. Hardness and cohesiveness of the cheeses were measured by texture profile analysis using a two-bite test to 80% compression. A force-distance curve was obtained using an Instron universal testing machine with a crosshead speed of 50 mm/min and a 500 N load cell. The cheese samples tested were 1.6 cm in diameter and 2 cm high. Cheeses were tested for stretchability with the USU Stretch Test utilizing an Instron universal testing machine. A cylindrical cheese sample, 30 mm diameter and 30 g in weight, was placed in a stainless steel cup and tempered in a water bath to 65°C. It was then placed in a hot-water jacketed sample-holder mounted on the Instron and maintained at 65°C. A three-pronged probe was lowered into the melted cheese sample and the sample cup rotated into position. A tensile test was performed by pulling the probe vertically at a crosshead speed of 1000 mm/min until all the strands broke or the beam stroke reached the maximum and a force-distance graph was plotted. Three parameters, melt strength, stretch length, and stretch quality were used to characterize cheese stretchability. Melt strength was the maximum load obtained and is related to melted cheese viscosity. Stretch length was the distance from the maximum melt strength until strand failure or the maximum beam stroke. Stretch quality was calculated as the mean value of the load exerted as the strand elongates from 7 to 15 s and describes the ability of the cheese strands during pulling to remain as a cohesive mass.



## Significant Conclusions:

### Objective 1.

Forty-one lactobacilli strains were characterized for cell morphology, Gram stain reaction and sugar utilization (API) and 27 strains were also confirmed by membrane fatty acid analysis. All strains tested were confirmed to be either *L. helveticus* or *L. delbrueckii* subsp. *bulgaricus*. Twenty-two lactobacilli (eight strains of *L. helveticus* and 14 strains of *L. delbrueckii* subsp. *bulgaricus*) were characterized for proteolysis using the  $_{-s1}$ -CN (f 1-23) method (Table 1). Clustering of these patterns within each species was apparent, but overlaps were also noted. Differences were apparent in both the primary and secondary specificity toward  $_{-s1}$ -CN (f 1-23) and its breakdown products. Most groups produced  $_{-s1}$ -CN (f 1-9). Some strains produced it as a primary product while other groups as a secondary product. In addition, some strains exhibited identical specificity, but much higher or lower relative affinities toward individual peptide bonds. Only one group (III) produced  $_{-s1}$ -CN (f 1-17) while a number of groups produced  $_{-s1}$ -CN (f 1-9),  $_{-s1}$ -CN (f 1-13), and  $_{-s1}$ -CN (f 1-16) with their accompanying partner fragments. Group V cleaved  $_{-s1}$ -CN (f 1-16) to  $_{-s1}$ -CN (f 1-6) and  $_{-s1}$ -CN (f 7-13), but no  $_{-s1}$ -CN (f 14-16) was detected.

Experiments with synthetic peptide standards showed that both  $_{-s1}$ -CN (f 1-7) and  $_{-s1}$ -CN (f 1-8) comigrate with  $_{-s1}$ -CN (f 1-9) on HPLC chromatographs. CZE analysis of HPLC fractions containing  $_{-s1}$ -CN (f 1-9) from selected strains from each group showed that  $_{-s1}$ -CN (f 1-7) was also produced by strains in group IV, and  $_{-s1}$ -CN (f 1-8) and  $_{-s1}$ -CN (f 9-23) were produced by group VI. CZE analysis of two *L. delbrueckii* subsp. *bulgaricus* strains, 39 and 40, showed only  $_{-s1}$ -CN (f 1-7) with no accumulation of either  $_{-s1}$ -CN (f 1-8) or  $_{-s1}$ -CN (f 1-9) (data not shown). These strains hydrolyzed the  $_{-s1}$ -CN (f 1-23) so rapidly that intermediate peptides were not detected by HPLC analysis. Although data was incomplete for these two strains, the finding of only  $_{-s1}$ -CN (f 1-7) indicates that an additional grouping may be warranted.

Utilizing the  $_{-s1}$ -CN (f 1-23) method, six patterns of proteolysis were found in the dairy lactobacilli tested. Variations between groups implies variations in types and/or specificities of cell surface proteinases. This research shows that although overall proteolysis measurements may be the same for two strains of dairy lactobacilli, their proteinase specificity can be markedly different. An improved understanding of the influence of *Lactobacillus* proteinase specificity on casein degradation and cheese functionality should facilitate efforts to develop starter cultures that improve the physical and functional properties of Mozzarella cheese. Differences in preferential cleavage patterns of the  $_{-s1}$ -casein (f 1-23) for various strains can be used for characterization and cleavage patterns for unique strains are being developed.

### Objective 2.

Total lactic acid bacteria were similar in all cheeses ( $6.5 \pm 1.5 \times 10^8$  cfu/g). No colonies were observed on Rogosa agar plates of control cheese through 21 d of storage, while the lactobacillal counts ranged between  $1.6 \times 10^6$  and  $1.1 \times 10^8$

cfu/g for the experimental cheeses. From the HPLC analysis of water-soluble peptides, the major peaks in the control cheese, at d7 were identified as  $\alpha_{s1}$ -CN (f 1-23) and  $\beta$ -CN (f 193-209) produced by the hydrolysis of  $\alpha_{s1}$ -CN and  $\beta$ -CN by the coagulant. At 14 d,  $\alpha_{s1}$ -CN (f 1-23) peak area increased reflecting continued proteolysis of intact casein. Several smaller peptides also appeared by 14 d of age. Hydrolysis of f 1-23 by bacterial enzymes was not significant since *S. thermophilus* is only weakly proteolytic. Higher amounts of  $\alpha_{s1}$ -CN (f 1-23) and  $\beta$ -CN (f 193-209) were seen in CI compared to the control cheese. This would be due to the faster hydrolysis of intact casein by the coagulant because of higher MNFS content. Other peptide peaks such as f 1-9, f 1-13, and a small amount of f 1-16 were also present at 7 d. Over time, there was little detectable change in the CI peaks.

In CIII, the peptide profile was very similar to CI except for a smaller peak area for f 1-23 peptide at 7 d of storage. This suggests that f 1-23 is being rapidly hydrolyzed into smaller peptides especially f 1-13 peptide. Over time, similar peaks were seen as in CI, with the exception of the peaks eluted at 50-min range. For both CI and CIII, major cleavage sites of f 1-23 were apparent at Gln<sub>13</sub>-Glu<sub>14</sub> bond. In vitro, strong and faster cleavage sites were observed at Gln<sub>13</sub>-Glu<sub>14</sub> and Leu<sub>16</sub>-Asn<sub>17</sub> bonds for both Group I and Group III lactobacilli (Figure 1). In CIII, the f 1-9 peptide peak was slightly larger than CI. This corresponds to a strong and faster (primary) cleavage site of Gln<sub>9</sub>-Gly<sub>10</sub> for Group III lactobacilli and a slower and weaker (secondary) hydrolysis for Group I lactobacilli as seen in vitro.

Cheeses made with Group V lactobacilli was characterized by the presence of a larger f 1-23 peak when compared to CI and CIII and the peptide, f 1-13, was present only at a low level. This corresponds to the specificity for Group V lactobacilli where the major cleavage products are f 1-9 and f 1-16, peaks present in CV. A similar profile was seen at both 14 d and 21 d of storage. Overall, the profile of the water-soluble peptides of the cheeses stayed relatively constant between 7 d and 21 d and could be related to the proteinase specificity of the lactobacilli portion of the starter cultures. Total water-soluble peptides were similar in CI, CIII, and CV and increased ( $P \leq 0.05$ ) with age. Although CV had higher lactobacilli counts than the two other treatment cheeses ( $10^8$  vs.  $10^6$ ), this had no apparent effect on the total water-soluble peptides that were produced.

Among treatment cheeses, CI and CIII had similar values for meltability (73.2 and 74.2%) while meltability of CV (81.9%) was highest ( $P \leq 0.05$ ). This difference was seen at 7, 14, and 21 d of age. Meltability of cheese increased ( $P \leq 0.05$ ) with age. This aging effect may be attributed to breakdown of the casein matrix, release of calcium, or to increased casein hydration that occurs in cheese with proteolysis. When comparing the effect of proteolysis on melting, it is not just a simple matter of the cheese with the most proteolysis having the most melt. In this experiment, the cheese with the highest melt was CV but the cheese with the highest level of total water-soluble peptide was CIII. Both CIII and CI had equivalent melts but the extent of proteolysis was significantly lower in CI than CIII.

The three cheeses made using lactobacilli (CI, CIII, and CV) were all softer than the control cheese. At 7 d, there were no differences between the experimental cheeses, but by 21 d, there were some significant differences and CIII increased in hardness while no change (or slight decrease) occurred in CV. There were some minor differences in cohesiveness of the cheeses, with CV having the lowest cohesiveness. Cohesiveness of all cheeses increased from an average of 0.270 at 7 d, to 0.359 N at 21 d. The differences in hardness and cohesiveness may be a function of the different protein hydrolysis profiles of the cheese.

Melt strength was highest ( $P \leq 0.05$ ) for the control cheese and lowest ( $P \leq 0.05$ ) for CV. The control cheese was expected to have a higher melt strength because of its lower moisture. The lower melt strength of CV corresponds to its lower hardness. Melt strength of the experimental cheeses decreased ( $P \leq 0.05$ ) during storage. Melt strength remained constant ( $P > 0.05$ ) in the control cheese with age and can be related to the limited proteolysis that occurred due to the lack of lactobacillal component in the starter culture. Similarly, stretch quality was highest ( $P \leq 0.05$ ) for the control cheese (0.644 N) and lowest ( $P \leq 0.05$ ) for CV (0.211 N). There were no significant differences in stretch quality between CI and CIII. Stretch quality decreased ( $P \leq 0.05$ ) with age from 0.616 N at 7 d to 0.260 N at 21 d for all cheeses. Stretch quality describes the ability of the cheese strands to remain together as a cohesive mass while being pulled. A decrease in stretch quality coupled with a reduction in viscosity of cheese during storage is typical of Mozzarella cheese. All of the cheeses stretched the full length of the test (30 cm) implying the stretch lengths were similar. Overall, melt strength and stretch quality were both negatively correlated with the percent water-soluble peptide content of the cheeses with  $R^2$  values of 0.55 and 0.64 respectively.

Lactobacilli with different proteolytic specificities can be used to make cheese with different functional properties even when the amount of total proteolysis is similar. Cheese made with the Group V culture had lower melt strength, lower stretch quality and high meltability than the other cheeses even though the amount of water soluble peptides in the cheeses were similar. These are, therefore, specific aspects of proteolysis that help predict functional properties of Mozzarella cheese. Thus, the pattern of protein hydrolysis in cheese, which can be linked to the diversity of proteinases among lactobacilli, should be considered an important indicator of functional qualities such as stretch, meltability and melt strength of these cheeses. It must also be acknowledged that the differences in the functional properties among the treatment cheeses may be a result of unobserved hydrolysis occurring elsewhere on the protein molecule.

#### **Publications:**

Oberg, C. J., J. R. Broadbent, M. Strickland, and D. J. McMahon. 2000. Diversity in specificity of the extracellular proteinases in *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. Letters Appl. Microbiol. (Submitted).

Oommen, B. S., D. J. McMahon, C. J. Oberg, J. R. Broadbent, and M. Strickland.  
2001. Proteolytic specificity of *Lactobacillus delbrueckii* subsp. *bulgaricus*  
influences functional properties of Mozzarella cheese. J. Dairy Sci. (Submitted).

**Theses:**

None

**Published Abstracts:**

Oberg, C. J., J. R. Broadbent, M. Strickland, and D. J. McMahon. 2000. Diversity in  
specificity of the extracellular proteinases in *Lactobacillus helveticus* and  
*Lactobacillus delbrueckii* subsp. *bulgaricus*. ASM Annual Meeting Program.

**Presentations:**

Oommen, B. S., D. J. McMahon, J. R. Broadbent and C. J. Oberg. 2001. Influence  
of proteolytic enzymes from thermophilic lactic acid bacteria on the  
functional properties of mozzarella cheese. 96th American Dairy Science  
Association Meeting, J. Dairy Sci. 84(Supp. 1):6

Oberg, C. J., J. R. Broadbent, M. Strickland, and D. J. McMahon. 2000. Diversity in  
specificity of the extracellular proteinases in *Lactobacillus helveticus* and  
*Lactobacillus delbrueckii* subsp. *bulgaricus*. ASM Annual meeting. Los Angeles,  
CA. May 22-25, 2000.

**Patent/Invention Disclosures:**

**Technology Transfer Activities:**

For information on licensing contact:

**Visitors Hosted:**

None

**Invention Disclosures: (Title, Date)**

None

**Patents: (Title, Date, #)**

None

**Licensing Activities:**

None

**Discoveries:**

# Western Dairy Center

## Final Report

Reporting Period January 1, 1998 —December 31 2001

**Principal Investigators:** Donald J. McMahon, Utah State University  
**Co-Investigators:** Craig J. Oberg, Weber State University  
Jeff Broadbent, Utah State University  
Rajiv Dave, South Dakota State University

**Project Title:** Understand the role of proteolysis on functional properties of Mozzarella cheese

**Institution's Project #:** 89093

**Project Completion Date:** December 31, 2001

**National Cheese Research Plan (1997): Priority: 2 Goal: 2 Tactic: 1**  
Establish knowledge matrices relating the effects of processing parameters on cheese functionality by 12/31/01: Understanding role of moisture and proteolysis on functional properties of cheese.

### Modifications to Project/Budget:

Increasing beta-casein hydrolysis in cheese was not successful using added plasmin so an additional experiment was conducted in which *Cryphonectria parasitica* coagulant was compared to chymosin coagulant.

### Project Objectives: (Include any revisions to objectives)

1. Determine the contribution of alphas1-casein hydrolysis on melting properties of mozzarella cheese.
2. Determine the contribution of beta-casein hydrolysis on melting properties of mozzarella cheese.

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

Non-fat (0% fat), reduced-fat (11% fat) and a control (19% fat) mozzarella cheeses were made using direct acidification to test the influence of three levels (0.25X, 1X and 4X) of coagulant concentration on proteolysis, meltability and rheological properties of cheeses during 60 d storage at 5°C. Changes in meltability, level of intact  $\alpha_{s1}$ -casein and  $\beta$ -casein (by capillary electrophoresis), 12.5% TCA-soluble nitrogen, and complex modulus were measured. Coagulant level had only a small effect on initial modulus. Cheeses became softer during storage and the decrease in modulus was influenced by the level of coagulant. At 0.25X, there was very little decrease in modulus after 60 d, while at 1X and 4X coagulant levels the softening of the cheese was more evident. The influence of coagulant level and fat content on cheese melting was similar to their effects on modulus. In general, higher fat contents promoted more melting and so did higher coagulant levels. Melting increased during storage although very little change was observed in the nonfat cheese.

Low moisture part skim mozzarella cheese was made using *Streptococcus thermophilus* alone, or in combination with *Lactobacillus helveticus* starter cultures. Two different coagulants, chymosin and *Cryphonectria parasitica* (sure-curd) coagulant at 1X and 6X the normal levels. Cheeses made with chymosin had more hydrolysis of alphaS1-casein while cheese made with sure-curd had more hydrolysis of beta-casein. Meltability of cheeses measured using a modified Schrieber test was significantly different in cheeses made with the different coagulants, but not different when measured by a creep test. Thus, the cheeses softened to similar extents but had different levels of flowability (measured by the Schrieber test). Extent of flow of the melted cheese correlated best with hydrolysis of beta-casein and soluble nitrogen. There was no correlation with hydrolysis of alpha-casein.

## 1. Methods:

### Objective 1.

#### Cheese manufacture.

Nine batches of mozzarella cheese were made in triplicate by direct acidification using glucono- $\delta$ -lactone. A 3 x 3 factorial design was used with three fat levels [nonfat cheese (0% target fat), reduced-fat cheese (10% target fat) and a control cheese (20% target fat)], and three levels of coagulant [0.25X, 1X, and 4X the normal level used for cheesemaking]. Skim milk was fortified with 2% (wt/wt) of nonfat dry milk and then mixed with cream to give casein-to-fat ratios of 1.2 for the control cheese and 2.4 for the reduced-fat cheese. The nine vats of cheese from each replicate were made simultaneously on each of three days.

Approximately 45 kg of each standardized milk, at 4 C, was acidified by adding 45 g of citric acid, and a further reduction to pH 5.7 was achieved by addition of 10% (w/w) glacial acetic acid. The acidified milk was then warmed to approximately 37°C, and each standardized milk was divided into three lots of 15 kg in small rectangular stainless steel containers for cheese manufacture using the three coagulant levels of 0.25X, 1X and 4X. Milk for the different coagulant levels was then temperature adjusted to 40 C (0.25X), 38 C (1X) and 35 C (4X), so as to facilitate setting milk at different coagulant levels in a timely manner. Double strength chymosin was diluted 20-fold with cold water, then 7.5 ml, 30 ml or 120 ml of diluted coagulant was added to each 15-kg batch of milk to manufacture cheese with coagulant concentrations of 0.25X, 1X and 4X, respectively.

The curd was cut when firm (approximately 50, 30, and 10 min for 0.25X, 1X, and 4X coagulant levels, respectively) and healed for 10 to 15 min and gently stirred to avoid fusion of freshly cut curd cubes and facilitate whey expulsion. All nine cheese vats were then adjusted to a temperature of 37 C. Half of the whey was drained and 40 g of glucono- $\delta$ -lactone was added into each vat and the temperature was gradually raised to 43 to 44 C over 20 min. Another fourth of the original whey volume was removed and an additional 25 g of glucono- $\delta$ -lactone was added into each cheese vat. The curd and whey mixtures were maintained at 43 to 44 C for at least 1 h until the pH of whey dropped to 4.4,

which produced a final cheese pH of approximately 5.4, and then the whey was completely drained.

The curd was salted with 1.5 g of NaCl per 100 g of curd and left for 20 to 30 min with intermittent mixing. Salted curd from each of the nine vats was then stretched by hand in 5% (w/w) brine at 75 C in a randomized order. The stretched cheeses were placed into molds and immersed in ice water for 1 h. After cooling, each batch of cheese was cut into four pieces, individually vacuum packaged, and stored at 4 C.

Proteolysis in the cheese was monitored using capillary electrophoresis by measuring peak areas for each eluting fraction to calculate extent of hydrolysis of intact  $\alpha_1$ -casein and  $\beta$ -casein (A1 and A2 combined), and presence of  $\alpha_2$ -casein (f(24-199)) peptide. The peak areas at d 1 for the 0.25X cheeses were designated as 100%. The times required to hydrolyze 50% and 90% of intact  $\alpha_1$ -casein, and 10% and 50% of intact  $\beta$ -casein were then estimated. Functionality of cheese was measured using a tube-melt test and a rheometer to measure complex modulus as a function of stress at a constant frequency. Frequency sweeps were also performed to help determine the linear viscoelastic region so that cheeses could be compared.

#### Objective 2.

A total of eight different low moisture part skim mozzarella cheeses were made using a 2x2x2 factorized randomized complete block design with four replicates. Cheese was made in small vats containing 15 kg of milk. The eight vats of cheese for each replicate were made over two days. Two types of starter cultures were used: single culture (SCC)– *Streptococcus thermophilus* (R13V), and a mixed culture (MC) – *Lactobacillus helveticus* (DS134) and *S. thermophilus* (R13V). Two different coagulating enzymes were used: chymosin (chymax) and *Cryphonectria parasitica* coagulant (sure-curd) at 1X and 6X the standard level of use. Cheese was analyzed on days 1, 7, 15, and 30 using capillary electrophoresis, TCA-soluble nitrogen, modified Schreiber test, Creep test and by dynamic rheology. Pasteurized milk was warmed to 32 C and the starter culture (SC or MC) was added and the milk ripened for 30 min. Coagulant was added and the curd cut, and then cooked to 40 C over 45 min. One third of the whey was drained and the curds stirred until pH 6.0 when all the whey was drained. The cheese was then cheddared to a target of pH 5.1 to 5.3. After milling, the curd was salted (2% w/w) and then stretched in hot 5% brine (77 C). Cheese blocks were then cooled in chilled water for 30 min, cut into 4 pieces, vacuum packaged and stored at 4 C.

Capillary electrophoresis was used to monitor the hydrolysis of  $\alpha_1$ -casein and  $\beta$ -casein during storage. Meltability of the cheese was tested using a modified Schrieber test and a creep test. In the modified Schrieber test, the cheese block was cut into slices 7-mm thick and 28.5-mm diameter with the weight of the cheese being 5.0 g. After equilibrating the cheese at 5 C, the cheese slices were placed on an aluminum plate a heated in an air convective oven at 125 C. Cheese samples were removed after heating and the area of the cheese measured using image analysis software. A creep test was performed using a UW-meltmeter with the same size sample (7 mm x 28.5 mm diameter). The

cheese sample was heated to 60 C and fall in height after 10, 20 and 30 sec was measured under a constant force of 0.36 N. Dynamic rheology was performed on 3.6-mm thick x 20.4-mm diameter slices of cheese in a parallel plate fixture on a Haake CV20. The system was oscillating at a constant frequency of 1 Hz and constant strain of 0.5%. The cheese was tempered to 50 C and measurements taken every 3 s for a period of 30 s to obtain data on storage modulus and loss tangent.

## 2. Significant Conclusions:

### Objective 1.

Extent of overall proteolysis (as measured by 12%TCA-soluble N) that occurred during storage was proportional to the level of coagulant used during cheesemaking. Disappearance of intact proteins ( $\text{--}_{\text{S1}}$ -casein and  $\text{--}$ -casein) followed a similar trend. Hydrolysis of  $\text{--}_{\text{S1}}$ -casein was more rapid than hydrolysis of  $\text{--}$ -casein. During the first 15 d of storage, 20% to 100% of intact  $\text{--}_{\text{S1}}$ -casein was hydrolyzed depending on the coagulant level and the %MNFS of the cheese. The relative order of hydrolysis rate was control > reduced-fat > nonfat cheese which corresponds to %MNFS content of the cheeses. When 4X coagulant was used, 40% of the intact  $\text{--}_{\text{S1}}$ -casein had been lost by d 1. As intact  $\text{--}_{\text{S1}}$ -casein was lost, a peak corresponding to  $\text{--}_{\text{S1}}$ -casein (f 24-199) was observed in the electropherograms. At the lower coagulant levels (0.25X and 1X), the amount of  $\text{--}_{\text{S1}}$ -casein (f 24-199) increased throughout storage whereas for 4X coagulant, the rate of secondary hydrolysis of  $\text{--}_{\text{S1}}$ -casein (f 24-199) into smaller peptides exceeded the rate of formation of  $\text{--}_{\text{S1}}$ -casein (f 24-199) and the quantity of this peptide in the cheese decreased after 15 d of storage. This coincides with the large increase in %TCA-soluble N that is formed in the 4X-coagulant cheeses during storage. Appearance of other peptides, such as  $\text{--}_{\text{S1}}$ -casein (f 1-23) could also be observed in the electropherograms as the intact proteins were hydrolyzed.

Hydrolysis of  $\text{--}$ -casein occurred gradually throughout storage and by 60 d only about 20% of the intact  $\text{--}$ -casein had been lost for the cheeses made with either 0.25X or 1X coagulant levels. When 4X coagulant was used the loss of  $\text{--}$ -casein increased to 50%. Hydrolysis of  $\text{--}$ -casein was not as dependent on %MNFS of the cheese as was hydrolysis of  $\text{--}_{\text{S1}}$ -casein.

Complex modulus of the cheeses appeared to be dependent on both fat content and %MNFS of the cheeses. There was a decrease in  $G^*$  during storage but increased proteolysis did not fully compensate for the increased  $G^*$  that resulted from removing fat from the cheeses. Meltability of the cheeses increased during storage and with increased coagulant level. Increases in meltability during storage appeared to be related more to secondary hydrolysis of the proteins than initial hydrolysis. Melting did not correspond to loss of intact  $\text{--}_{\text{S1}}$ -casein but instead followed the trend lines for %TCA-soluble N and loss of intact  $\text{--}$ -casein. One explanation for this is that  $\text{--}_{\text{S1}}$ -casein (f 24-199) peptide imparts the same structural and functional properties to the cheese protein matrix as does the intact  $\text{--}_{\text{S1}}$ -casein molecule. The f 1-23 peptide that is removed is positively



charged and of similar hydrophobicity to the rest of the protein. However, all of the phosphoserine groups are in the f 24-199 portion of  $\alpha_{S1}$ -casein which suggests that it is the interaction of the phosphoserine groups, probably via calcium bridging, that has a controlling influence on cheese melting. Further investigations that separate  $\alpha_{S1}$ -casein hydrolysis from  $\beta$ -casein hydrolysis are needed to elucidate the roles of these proteins in cheese texture and melting.

#### Objective 2.

The average moisture of cheese made using the single culture was lower than that made with the mixed culture (43.2% compared to 45.6%) as a consequence of the *S. thermophilus* losing acid drive during cheesemaking at about pH 5.5. Consequently the SC cheese had a longer make time than the MC cheeses. Differences in moisture content between the two coagulants and the levels of coagulants were only minor. The lower moisture of the SC cheeses may have influenced the functionality properties of the cheese, although the extent to which it did so was not determined. There was also a difference in cheese pH with the SC cheese having a pH of 5.45 and the MC cheese a pH of 5.27, however, there were no differences in salt and calcium contents of the cheese and this was thought to be a more controlling factor in cheese meltability.

There was a decrease in levels of both  $\alpha_{S1}$ -casein and  $\beta$ -casein during storage of the cheese. As shown below, there was more hydrolysis of  $\alpha_{S1}$ -casein in the cheese made using chymosin (Figure 1) but less hydrolysis of  $\beta$ -casein (Figure 2). Acid-soluble protein increased during storage with only a slight difference between the coagulants: at 30-day storage the chymosin cheese had slightly more acid-soluble protein than the sure-curd cheese.

Meltability of all cheeses increased during storage. There was no significant difference in softness of the melted cheese measured using the creep test between the two different coagulants (Figure 3). There was a difference in flowability as measured using the modified Schrieber test (Figure 4) that was correlated with loss of intact  $\beta$ -casein ( $r = 0.85$ ) and acid-soluble nitrogen ( $r = 0.87$ ). There was only a low correlation with loss of intact  $\alpha_{S1}$ -casein ( $r = 0.60$ ). This indicates that softening and flow of melted cheese while probably interdependent, are two different aspects of cheese. The cheeses made with rennet were equally soft, but were less flowable than the cheeses made using the *C. parasitica* coagulant. During storage of the cheese, there was a decrease in both the storage modulus and loss modulus. There were no consistent differences in modulus in the cheeses made using the different coagulants.

Figure 1: Hydrolysis of alphaS1-casein

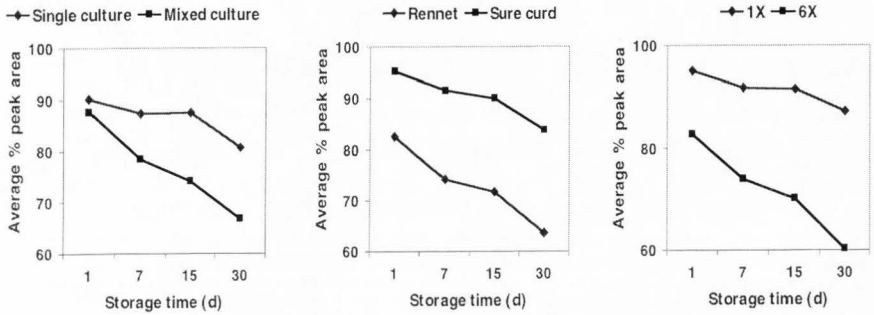


Figure 2. Hydrolysis of beta-casein

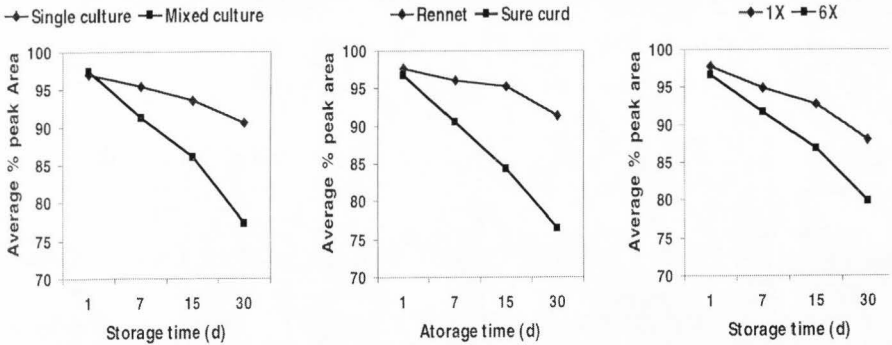


Figure 3. Percent decrease in height (creep test)

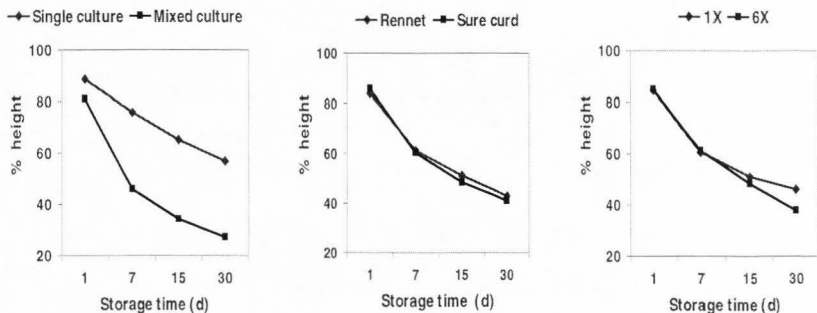
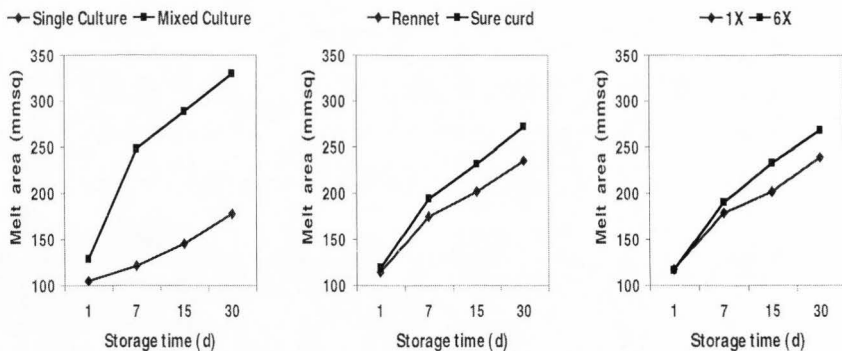


Figure 4 Increase in melt area (modified Schrieber test)



**Publications:**

Dave, R. I., D. J. McMahon, C. J. Oberg, and J. R. Broadbent. 2001. Influence of coagulant level on proteolysis and functionality of mozzarella cheeses made using direct acidification. *J. Dairy Sci.* (Submitted)

**Theses:**

Sharma, P. 2001. Melt and rheological characteristics of low moisture part skim mozzarella cheese as affected by the starter culture and coagulating enzymes, M.S. thesis, South Dakota State University

**Published Abstract:**

Dave, R. I., McMahon, D. J., Oberg, C. J, and J. R. Broadbent. 1999. Influence of coagulant concentration on proteolysis, meltability and rheology of 0, 10 and 20% fat containing Mozzarella cheese made using direct acidification. 94th American Dairy Science Association Meeting, *J. Dairy Sci.* 82(Supp. 1):D59.

**Presentations:**

Dave, R. I., McMahon, D. J., Oberg, C. J, and J. R. Broadbent. 1999. Influence of coagulant concentration on proteolysis, meltability and rheology of 0, 10 and 20% fat containing Mozzarella cheese made using direct acidification. 94th American Dairy Science Association Meeting, *J. Dairy Sci.* 82(Supp. 1):D59.

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

For information on licensing contact:  
Not applicable at this time.

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

\_\_\_\_\_

**Patents: (Title, Date, #)**

\_\_\_\_\_

**Licensing Activities:**

\_\_\_\_\_

**Discoveries:**

\_\_\_\_\_

# Western Dairy Center

## Project Report

Reporting Period January 1, 2001 — December 31, 2001

**Principal Investigators:** Barry Swanson, WSU  
**Co-Investigators:** Joseph Powers, WSU  
Stephanie Clark, WSU  
Lloyd Luedecke, WSU

**Project Title:** Nonthermal attenuation of *Lactobacilli* to accelerate cheese ripening

**Institution's Project #:** 01125

**Project Completion Date:** December 31, 2002

**National Research Plan (1997):** Priority: milk/whey powder **Goal:** 2 **Tactic:** 2

**Modifications to Project/Budget:** n/a

**Project Objectives:** (Include any revisions to objectives) (*Revised 02.28.02*)

1. Determine the relative increase in protease, aminopeptidase and flavor development in full and low fat Cheddar cheese resulting from high hydrostatic pressure (HHP) attenuation of *Lactobacillus* adjunct cultures; and
2. Determine the potential for HHP attenuation of adjunct *Lactobacilli* to accelerate proteolysis and intensify the flavor of full fat and low fat Cheddar cheese during reasonable aging times.

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

The ultra high pressure attenuation of a selected *Lactobacillus sp.* reduces acid forming activity and provides viable cells which are resistant to autolysis in a pseudocurd system, yet provide enhanced protease and aminopeptidase activity. Cheddar cheeses containing adjunct UHP treated attenuated cultures to accelerate ripening will be made within the next three weeks.

### 3. Significant Progress against Objectives:

A. To choose the most appropriate *Lactobacilli* strain, we conducted experiments using several selected *Lactobacilli* strains (*L. helveticus* WSU19, *L. helveticus* W240R and *L. helveticus* W260R), and evaluated aminopeptidase activity in a buffer system and rate of autolysis in a 'pseudocurd' system.

Table 1. Aminopeptidase activity in Cell Free Extract (CFE) after growing cells in Reconstituted Skim Milk (10%) at 37°C for 16 hours

Substrate	Ala-pNa	Leu-pNa	Lys-pNa	ArgPro-pNa
Strain	specific activity (nanomoles/mg/mL)			
WSU19	813	1046	1183	760
W240R	463	541.5	973.2	361
W260R	716	767.6	1143	546

Table 2. Viable cells of selected *Lactobacilli* strains during ripening in pseudocurd system. Incubation at 8°C. Pseudocurd: sterilized milk contains 15 g/L CaCl<sub>2</sub> at pH = 5.0 (adjusted with glucono-δ-lactone) and *Lactobacilli*

Strain	log viable cells/mL						
	0d	4d	8d	12d	16d	20d	24d
WSU 19	7.81	7.91	7.38	6.97	6.28	6.11	4.52
W240R	8.21	8.21	8.01	7.49	7.29	7.34	5.40
W260R	9.00	8.86	8.79	8.63	8.34	8.21	8.10

Results from both experiments demonstrate that *L. helveticus* WSU 19 exhibits the greatest aminopeptidase activity, however the strain lyses readily in the pseudocurd environment relatively similar to the cheese system.

*L. helveticus* W260R exhibits equivalent aminopeptidase activity (except for PepX) and exhibits the lowest rate of autolysis in the pseudocurd. W260R is apparently the most suitable for HHP attenuation.

B. HHP treatment of *L. helveticus* W260R before inoculation of the cells into the pseudocurd system. Pressures of 275 MPa, 410MPa, and 550 MPa were chosen for treatment of W260R.

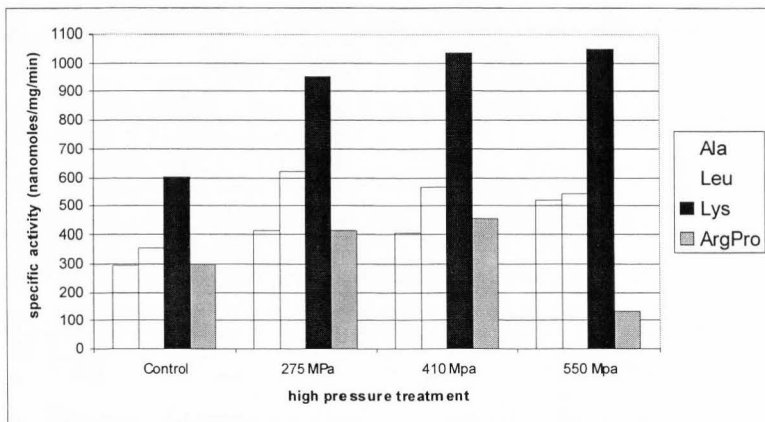


Figure 1. Aminopeptidase activity of CFE prepared directly after HHP treatment of *L. helveticus* W260R.

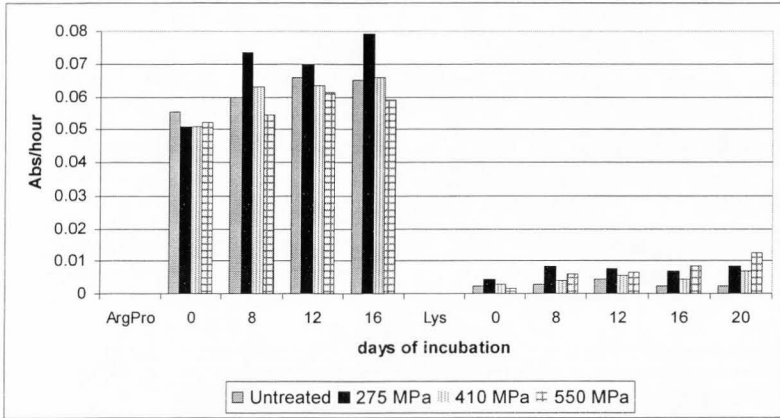


Figure 2. Aminopeptidase activity of *L. helveticus* W260R from pseudocurd system incubated at 8°C on ArgPro p-Na and Lys-pNa substrate



Future Experiments

In the week of March 18 - 25, 2002, we will be making full fat and low fat Cheddar cheese with 410 MPa treated *L. helveticus* W260R as an adjunct culture. The primary culture will be *Lactococcus lactis* 105.

The experimental design will be:

No.	Culture	Fat Content	Replication	Note
1	Primary	Full	1	As negative control
2			2	
3		Low	1	
4			2	
5	Primary + Untreated W260R	Full	1	
6			2	
7		Low	1	
8			2	
9	Primary + Treated W260R	Full	1	
10			2	
11		Low	1	
12			2	
13	Primary + WSU 19	Full	1	As positive control
14			2	
15		Low	1	
16			2	

**1. Significant Conclusions:**

1) *Lactobacillus helveticus* W260R is the most appropriate culture for ultra high pressure (UHP) attenuation with respect to reduced acid production, autolysis in the pseudocurd *system*, and enhanced protease and aminopeptidase activity;

An ultra high pressure (UHP) of 410 MPa was determined to be most effective, and will be utilized for attenuation of adjunct cultures.

3.) Anticipated Problems/Delays: Washington State University has not received an extension of the research project end date until December 31, 2002 nor the second year budget request. Delay in purchasing materials and providing research assistantship may delay experimentation.

**Publications:**

Lin, S. "Daniel", S. Clark, J.R. Powers, L.O. Luedecke and B.G. Swanson. 2001.  
Thermal, ultra high pressure and pulsed electric field attenuation of *Lactobacillus*.  
Part 1. AGROfoodINDUSTRY hi-tech, November/December, Anno 12, N. 6, 19-  
23.

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

For information on licensing contact: n/a

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Project Report

Reporting Period January 1, 2001 — December 31, 2001

**Principal Investigators:** J.A. Torres, OSU  
**Co-Investigators:** D.F. Farkas, OSU  
G. Velázquez, OSU, Mexico  
J. Salas, CICATA, Mexico  
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E. Morales, U. Austral, Chile  
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J. Serrano, OSU & U.A. Querétaro, México  
R. Alfaro, OSU & I.T.E.S. de Monterrey, México

**Project Title:** Shredded Cheddar cheese: accelerating shreddability by moderate hydrostatic pressure (MHP)

**Institution's Project #:** 01121

**Project Completion Date:** December 31, 2002

<b>National Research Plan (1997):</b> Priority: milk/whey powder <b>Goal:</b> 2 <b>Tactic:</b> 2
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### Modifications to Project/Budget:

**Project Objectives:** (Include any revisions to objectives)

Objective 1: Determine the influence of moderate hydrostatic pressure (MHP) treatments on the mechanical properties of Cheddar cheese including shreddability.

HYPOTHESIS/TASK: MHP treatments under 60,000 psi and 120s applied to fresh curd yields immediately a microstructure similar to aged Cheddar cheese. This can shorten the storage needed before shredding Cheddar cheese, an opportunity that we are uniquely qualified to evaluate using our large 20-liter pressure vessel.

Mechanical properties of Cheddar cheese for shredding: We will determine the MHP effect on the mechanical properties of Cheddar cheese produced for shredding. A Dmi-funded project is currently looking at the rheological and structural aspects of cheese to develop a model that defines physical properties of cheese in terms of rheology and relate them to industrial-type measurements such as melt and stretch. This will also include some microstructural work as well to aid in development of the model. This new information will be of great assistance in our work and we hope to be strong collaborators but our work will emphasize shreddability and its dependence on the mechanical properties of a cheese block.

Characterization of shredded Cheddar cheese: MHP-treated, control and commercial shredded cheese will be characterized by particle size/shape and meltability indicators.

Objective 2: Sensory analysis of shredded Cheddar cheese

HYPOTHESIS/TASK: Objective measurements of shreddability must be combined with sensory parameters to generate a full characterization of shredded Cheddar cheese as affected by MHP treatments. In this project we will use a trained descriptive analysis panel to characterize the appearance and meltability of pressure treated, control and commercial shredded cheese samples. Pressure effects on flavor are expected to be none or positive and this will be confirmed experimentally using flavor descriptors.

Objective 3: Recommend MHP treatments to reduce the production costs of shredded Cheddar cheese

HYPOTHESIS/TASK: A preliminary survey of shredded Cheddar cheese in U.S. markets showed large differences in the characteristics of the products on the market. A recommendation for MHP treatment requires feedback from industry. Quality assurance managers for commercial shredded cheese producers will receive summary reports on the mechanical properties of cheese for shredding and the objective and sensory characterization of the shredded cheese obtained. Using this feedback we will select up to six MHP treatment options for consumer tests of melted and shredded cheese as is.

**Project Summary:**

US cheese production has increased to ~730 million tons/month due in significant part to the demand for natural shredded cheese. We discovered that moderate pressure treatments (under 60,000 psi and 2 min) applied to fresh curd yields immediately a microstructure similar to aged Cheddar cheese. In this project we are determining if these pressure treatments can shorten the storage needed before shredding Cheddar cheese. We are uniquely qualified to evaluate this opportunity as our pilot plant is equipped with a 20-liter pressure vessel which is ideal to prepare the large number of samples required for sensory and objective measurements.

Relatively little information has been published on the mechanical properties making cheese blocks suitable for shredding. Texture measurement procedures found in the literature differed in test parameters and those had to be evaluated to find the most adequate conditions for our experimental samples. Also missing was a definition of consumer-acceptable shredded cheese. This information has been included in this study. Objective measurements of shreddability include a computerized vision system built by co-PI Salas. This unit can characterize the size/shape of the shredded cheese and includes an area measurement tool used to assess cheese meltability. A trained descriptive analysis panel has examined the appearance of pressure treated, control and commercial shredded Cheddar cheese.

We have completed the first replicate in the experimental design to determine the pressure treatment conditions and the time after pressure treatment that the treated cheese becomes suitable for shredding. Estimates for capital and operational costs for curd processing by MHP and capital and refrigerated warehouse costs for cheese storage will be determined in the second year of this project to confirm the financial advantages of MHP-treated cheese for shredding. Expected savings are 15\$/1000 lb shredded cheese due to reduced storage time. The technology may apply to other cheese types providing further opportunities for marketing milk.

### **1. Significant Progress against Objectives:**

Commercial Cheddar cheese samples were used for the development of experimental procedures required for this project. This development included (1) construction of an optical and sample handling set up used for both the image analysis of shredded cheese particles and the evaluation of cheese melting; (2) training of a descriptive sensory panel and the development of a sample evaluation ballot; and (3) texture measurement and data analysis procedures. These methods were applied for the first run of the experiments that used 3-day old blocks of Cheddar cheese obtained from the Oregon Tillamook County Creamery Association. Cheese blocks were cut into

18x9x3 cm samples using a wire cheese cutter and were packaged and vacuum-sealed at the plant. The samples were placed in a cooler with ice bags during transportation back to the laboratory where they were stored at 34 °F.

Two sets of samples obtained randomly from the same cheese blocks were stored at 34 F. The first set was used for chemical analysis, determination of mechanical properties and evaluation of cheese meltability. The second set was used to prepare shredded samples that were analyzed by a digital image analysis set up and a trained descriptive sensory analysis panel. All measurements (except when noted) were done after 1, 6, 17, and 27 days of storage.

#### Chemical Analysis

Tests included moisture, protein (0 and 27 days), fat (0 and 27 days), salt (0 and 27 days), pH, and proteolysis.

#### Textural Properties

PA analysis using 60% compression in sixtuplicates cubical samples (2x2x2 cm) for each treatment.

Stress-strain characterization in sixtuplicates cubical samples (2x2x2 cm) using uniaxial compression at 50%.

Puncture tests in sixtuplicates cubical samples (2x2x2 cm) using a " cylindrical stainless steel probe and " Spherical stainless steel probe

Cubical samples for textural properties were obtained using the stainless steel wire cheese cutter built specifically for this project (Figure 1).

**Cheese Meltability**  
Measuring the area and maximum diameter of cheese samples heated at

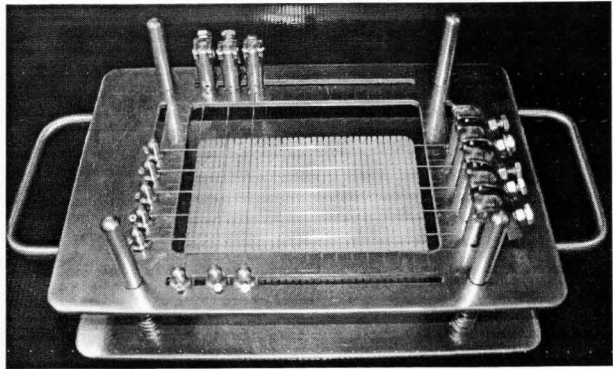


Figure 1. Experimental cheese cutter

90C and 5 min in duplicate. Circular samples 3.5 cm diameter and 2.0 cm high were analyzed using a computerized digital image analysis developed as detailed elsewhere in this report.

1) Measurements on shredded Cheddar cheese

Two shredder plates (3/32" and 3/16") were used on the Hobart AS200T (The Hobart Mfg. Co., Troy, Ohio) to produce thick and thin shredded cheese particles similar to the ones most frequently produced commercially. These were analyzed by instrumental and sensory analysis methods.

a. Size characterization by digital image analysis

The particle size distribution of the shredded cheese were measured using a computer imaging system using two measures of special interest: length and width of each shredded particle. A detailed discussion of the image analysis system developed is presented in Appendix 2.

b. Size characterization by trained sensory panel

Shredded samples were evaluated attributes and find parameters to compare the effect of the different treatments on the cheese properties. A copy of the ballot developed is presented in Appendix 3.

## 2. Significant Conclusions:

The most significant accomplishment in year one was the implementation of analytical techniques, particularly (1) training and development of descriptors for a trained panel for shredded cheese; (2) development of an image analysis set up to characterize objectively shredded cheese particles and cheese melting; and (3) evaluation of testing conditions for determination of textural properties. All this information was not available in the literature and publications are being prepared for dissemination to industrial users. With respect to pressure treatment effects, only one replicate has been completed and thus it is not appropriate to draw conclusions.

## 3. Anticipated Problems/Delays:

The only probable delay is the need to visit with a shredded Cheddar cheese processor in Tulare, California to discuss our preliminary findings. This visit will help us refine conditions for the additional pressure treatments included in the original proposal for this study.

## Publications:

Salas, J., Velázquez, G., and Torres, J.A. 2002. Un algoritmo para la medición en línea de la longitud de tiras flexibles sobrepuestas. Proceedings of the Simposio de Metrología 2002, Querétaro, México.

**Theses:**

Serrano, J. 2002 (expected December 2002). Master of Science, Universidad Autónoma de Querétaro, México.

**Published Abstract:**

Salas, J., Velázquez, G., and Torres, J.A. 2002. Un algoritmo para la medición en línea de la longitud de tiras flexibles sobrepuestas. Simposio de Metrología 2002, Querétaro, México.

**Presentations:**

Salas, J., Velázquez, G., and Torres, J.A. 2002. Un algoritmo para la medición en línea de la longitud de tiras flexibles sobrepuestas. Proceedings of the Simposio de Metrología 2002, Querétaro, México.

**Patent/Invention Disclosures:**

None

**Technology Transfer Activities**

For information on licensing contact: Dr. J. Antonio Torres, Associate Professor of Food Process Engineering, 100 Wiegand Hall, Room 202, Oregon State University, Corvallis, OR 97331-6602, Phone: 541/737-4757; FAX: 541/737-6174, Email: J\_Antonio.Torres@orst.edu

**Visitors Hosted:**

Dr. Joaquin Salas, Research Professor, CICATA-IPN, José Siurob 10. Col. Alameda, Querétaro, México 76040



# Western Dairy Center

## Project Report

Reporting Period November 1, 2000 — December 31 2001

**Principal Investigators:** Marie K. Walsh

**Co-Investigators:** Charles Carpenter

**Project Title:** Characterization of textured whey protein used as a meat extender

**Institution's Project #:** 01127

**Project Completion Date:** December 31, 2001

**National Research Plan (1997): Priority:** milk/whey powder **Goal:** 2 **Tactic:** 2

### Modifications to Project/Budget:

#### Project Objectives: (Include any revisions to objectives)

1. Characterize TWP produced from different sources of WPC and starch. In vitro measures of stability, water holding capacity, thermal stability, pH stability, fat binding, and shelf life will be done.
2. TWP produced and analyzed in Objective 1 which show similar characteristics to our current product will be further characterized in hamburger patties containing 30% TWP (g/g). In situ measures of shelf life, freeze thaw, cook yield and sensory analysis will be done.
3. Determine the nutritional profile, shelf life and market areas for TWP as a meat extender.

**Project Summary:** (Suitable for inclusion in Center documents released to the public)  
We have previously employed thermoplastic extrusion to produce a textured whey protein (TWP) from whey protein concentrate (WPC). The TWP showed significant promise as an extender in ground beef patties. Commercial sources of WPC and starch vary, therefore this research will investigate the influence of various brands of WPC and starch on TWP performance as a meat extender. TWP samples will also be characterized

with respect to thermal and pH stability, water and fat binding, shelf life and freeze thaw stability. The nutritional profile will be developed and sensory evaluation in hamburger patties will be conducted.

### 1. Significant Progress against Objectives:

Seven commercial whey sources (WPC 80) have been extruded at three protein levels (48%, 53% and 64%) as shown in Table 1. The last three sources (Land'O Lakes, Calpro and Plain View Milk) did not produce successful products at any of the three protein levels tested.

Table 1. TWP Blends and WPC Commercial Sources

	2/1 (53% protein)	3/2 (48% protein)	4/1 (64% protein)
Proliant P1.1.1:S1.1.1	3/7/01 ANALYZED fibrous, stable	3/19/01 ANALYZED, lower water fibrous, stable	CURRENT ANALYZEDS fibrous, stable
Century Instant P2.1.1:S1.1.1	4/4/01 ANALYZED some fibrous, stable	4/9/01 ANALYZED, 300 rpm, water = 2.65, fibrous, stable	5/01 Analyzed fibrous, stable
Davisco Foods P3.1.1:S1.1.1	4/16/01 ANALYZED, water 2.3, negative 10-30 psi fibrous, stable	4/19/01 ANALYZED not as fibrous as 2:1, water 2.2, - 10 psi,	6/01 sticky, no fiber formation
Warnambool P4.1.1:S1.1.1	3/12/01: 4/23/01 ANALYZED	4/25/01 ANALYZED, little fibrous texture and sticky	6/12/01 ANALYZED, can produce, sticky, difficult to extrude, different smell
PlainView Milk P5.1.1:S1.1.1	3/21/01 NO ANALYSIS Expanded product	3/28/01 NO ANALYSIS Expanded product	6/15/01 ANALYSIS VERY dark, different smell
Calpro P6.1.1:S1.1.1	4/2/01 NO ANALYSIS, no fibrous texture, sticky	4/3/01 NO ANALYSIS, sticky, not consistent, did not extrude well	6/18/01 NO ANALYSIS, product orange, foamy and non-textured
Land'O Lakes P7.1.1:S1.1.1	4/9/00 NO ANALYSIS, no fibrous texture, sticky	9/00 NO ANALYSIS, sticky, not consistent, did not extrude well	not enough whey sample to evaluate in 4:1 ratio

The first three samples (Proliant, Century Instant and Davisco Foods) did produce suitable TWP as determined visually and Warnambool was successful at the 48% and 53% protein level.

The products produced from the first three commercial sources were analyzed for water holding capacity and % solids lost as compared to a textured soy product (TVP). This

data is shown in the graphs in the appendix. On average there was a higher water holding capacity with an increase in pH and temperature with TVP having a higher water holding capacity.

The % solids lost for each of these samples ranged from 10 to 15% with TVP having values greater than 20%.

This data is being analyzed statistically for the next report.

TWP can have different markets depending on the cost which can be determined by the protein level. With a higher protein level, the TWP is more expensive but may have a different market. In order to determine the maximum and minimum amount of whey protein needed to form a TWP, TWP was extruded at the protein values listed in table 2.

Table 2. Protein Concentrations in TWP\* production

WPC/Starch*	Protein (%)	TWP product
1:1	40	No fibrous texture formed
3:2	48	Fibrous texture formed
2:1	52	Original Product, Fibrous texture formed
3:1	60	Fibrous texture formed
4:1	64	Fibrous texture formed
5:1	66	Very difficult to extrude, Can get fibrous texture
6:1	69	Too difficult to extrude
9:1	72	Too difficult to extrude

\*Proliant WPC 80 and National Melogel used for each sample

## 2. Significant Conclusions:

Whey protein levels from 48 to 64% can lead to the production of a stable TWP.

The water holding capacity and % solids lost of TWP produced at levels from 48-64% was determined and compared to a commercial source of TVP.

Of the 7 different commercial sources of WPC 80, three sources were consistent in the production of TWP at three protein levels.

**3. Anticipated Problems/Delays:**

None

**Publications:**

Theses:

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

This technology is patent pending

**Technology Transfer Activities**

For information on licensing contact:

**Visitors Hosted:**

Appendix 1

Water holding capacity and % solids lost of TWP produced with different commercial sources.

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

patent pending

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Final Report

May 1, 1997 to December 31, 2001

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

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

Institution's Project #: 97083

Project Completion Date: December 31, 2001

National Research Plan (1997): Priority: 5 Goal: 1 Tactic: 4
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### 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 *L. helveticus* are essential for the accumulation of the bioactive peptides/bioactive peptide precursors from milk. 3. Construct strains of *L. 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) and brevbacteria (15) were screened for degradation of the alpha-casein 1-23. In *Lactobacillus helveticus* at least 5 patterns were found. Specific peptides are unique to strains. Each peptide profile is being investigated to determine unique peptides in each strain. Whole caseins are being screened for production of peptides with HPLC/MS. Knockout mutants provided by Jim Steele were screened for peptide production from alpha-casein 1-23, and found that *Lb. helveticus* CNRZ32 produces at least 2 proteases and that the peptide pattern is a product of those enzymes. Pure peptides with bioactivity have been synthesized and were as standards for further analysis of proteolytic patterns. 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.

Hydrolysis of the alpha-casein 1-23 fragment by lactobacilli was complete within 15 minutes of incubation at 37°C. Identity of the resulting peptides were determined for CNRZ32 and protease deficient mutants by HPLC/MS and co-elution of synthetic peptide standards. Multiple peaks were observed with 11 dominate peptides being produced in the wild type. The most noteworthy is the 1-9 fragment that was produced at high levels in all strains tested. It appears that the substrate is hydrolyzed once in various locations that result in two fragments, opposed to hydrolysis of each fragment in multiple locations. For example, one fragment pair was 1-9 and 10-23. In some cases, one part of the pair was not observed. However, since every peak was not sequenced, it is possible that it was present and not identified. Alternatively, these peptides may have been metabolized by cultures during incubation, but this is unlikely considering the short incubation times used in the assay. These data are ready to be used to define a protease classification system.

The proteolytic system of *L. helveticus* is significantly different to that of lactococci. This strain contains at least 2 proteases. The first cloned and sequenced has a different substrate binding site and different substrate specificity to that of the lactococcal proteinase. The new proteinase changed the peptide pattern. In the deletion mutant, 3 bitter peptides were not produced, despite the large amount of 1-9 produced by all strains tested (>65 cultures).

Additional comparisons are in progress to group all the species into distinct classifications based on the peptides produced. This will be completed in the next few months.

### 1. Significant Progress against Objectives:

All objectives are being completed as listed in the proposal. Final analysis of the proteolytic comparisons and strain characteristics are in progress. A significant delay was a change in personnel and additional data comparison. The final analysis is being completed with the utmost urgency.

### 2. Significant Conclusions:

Numerous hydrolysis patterns are found in the strains. At least 2 proteases seems be produced in *L. helveticus* CNRZ32. The gene sequence is finished, and was found to be a new type of enzyme in LAB. *L. helveticus* and *L. casei* produce similar degradation patterns from the 1-23 fragment. Other lactobacilli are significantly different from these two species.

### 3. Anticipated Problems/Delays:

A new student just arrived to finish the project.

### Publications:

Pederson, J. A., G. J. Mileski, B. C. Weimer, J. L. Steele. 1999. Genetic Characterization of a Cell Envelope-Associated Proteinase from *Lactobacillus helveticus* CNRZ32. *J. Bact.* 181:4592-4597.



**Theses:**

Paul Joseph – Ph.D. candidate

**Published Abstract:**

Pederson, J. A., G. J. Mileski, **B. C. Weimer**, J. L. Steele. 1999. Genetic Characterization of a Cell Envelope-Associated Proteinase from *Lactobacillus helveticus* CNRZ32. 6<sup>th</sup> Lactic acid bacteria meetings (Veldhoven, The Netherlands). Sept. '99.

**Presentations:**

**Weimer, B. C.**, P. Joseph, J. Petersen, and J. Steele. 2000. The proteinases of lactobacilli. International Dairy Federation Biennial Cheese Flavor Conference, Banff, Alberta.

**Patent/Invention Disclosures:**

none

**Technology Transfer Activities**

For information on licensing contact:

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435 797 3356

**Visitors Hosted:**

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# Western Dairy Center

## Final Report

Reporting Period July 1, 1997 — December 31, 2001

**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:** December 31, 2001

**National Research Plan (1997): Priority: 4 Goal: 4 Tactic: 1**

Understand how cheese matrix composition influences survival and metabolism of starter and adjunct cultures to produce fatty acids from keto acids.

**Modifications to Project/Budget:**

None

**Project Objectives:** (Include any revisions to objectives)

Hypothesis:

The first catabolic step for amino acids is catalyzed by aminotransferases using keto acids as co-factors (amino donors and acceptors) to form flavor compounds in cheese. The most common co-factor transformation is ketoglutarate to glutamic acid. Therefore, generation of these two co-factors is important in controlling the rate of flavor development in cheese.

1. Determine the diversity of transferase reactions that use ketoglutarate to glutamic acid in LAB.
2. Determine the role of this transformation in production of cellular energy and flavor compound production from each class of amino acids (branched chain, aromatic, acidic, neutral, basic).
3. Determine the rate of product formation with the addition of keto acids in relation to the amino acid precursor concentration. (Aminotransferases are bidirectional enzymes based on the products and reactant concentrations. If this reaction is increased it may lead to a method to accelerate cheese flavor.)
4. Determine the environmental triggers that induce each aminotransferase in lactococci and lactobacilli with transcription analysis.

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

Short-chain fatty acids have a role in Cheddar cheese flavor development. The mechanisms responsible for their production in cheese are not established.

Microbial lipases are involved, however, lactic acid bacteria typically possess

extremely weak lipolytic ability and do not produce improved flavor, even with use of strains that over express the lipase (Holland et al., 1996). Alternatively, short chain fatty acids in Cheddar cheese may arise from microbial catabolism of branched chain amino acids. The aim of this study was to determine the ability of volatile fatty acid (VFA) production in lactococci associated with cheese processing in absence of carbohydrate and in carbohydrate starvation.

All bacteria tested produced VFA in laboratory conditions with diversity in genera and species in complex laboratory media. Strains of lactococci, lactobacilli, and brevibacteria convert amino acids to various VFA. Brevibacteria produced 10–100 times more VFA than the other bacteria tested. Studies linking carbohydrate starvation and amino acid metabolism demonstrated that some strains appeared to die by growth studies. However, brevibacteria and lactococci maintained the ability to produce increasing amounts of VFA in carbohydrate starvation conditions in a chemically defined media (CDM), despite an apparent decrease in viable cells. Isovaleric acid alone was produced by strains in CDM at pH 7, while no fatty acids were detected in CDM at cheese-like conditions. These studies indicate amino acids are converted to VFA by cheese related bacteria. Further, they indicate that the amount of sugar present regulates these metabolic processes.

NMR studies with radiolabeled amino acids demonstrated the interconversion occurs in these bacteria. Brevibacteria produced significantly more FFA from branched chain amino acids than lactococci. Lactococci produced FFA only after the onset of carbohydrate starvation.

Deletion mutants from J. Steele (Wisconsin) and M. Yvon (France) that lacked specific aminotransferase enzymes retained the ability to produce fatty acids from amino acids, but the type of VFA produced was different compared to the wild type. This indicates that multiple metabolic pathways exist for production of VFA in lactococci. This will allow a hypothesis for the catabolic pathway in cheese. Screening for VFAs' production from various amino acid precursors showed that genera involved are not diverse within the species but different between genera.

Another attempt to exclude the aminotransferase reaction was made by using precursor  $\alpha$ -keto acids as substrates for *in vivo* catabolism to VFAs. The keto acids degraded in solution during incubation to VFAs. However, lactococci and lactobacilli produced VFAs above the observed background degradation. They also produced different products from what would be expected from a reaction subsequent to an aminotransferase in the catabolic pathway. This makes the role of an aminotransferase in VFA production more complicated than it would seem to be.

*Lactococcus lactis* ssp. *lactis* ML3 was inoculated into CDM (modified from Gao et al., 1997) at pH 7.2 and 5.2, containing 0.2% lactose. The cells metabolized the small amount of lactose to become carbohydrate starved for 6 months. ML3 entered the non-culturable state within 10 days of inoculation into CDM, but remained metabolically active and contained ATP. They eventually produced

branched-chain fatty acids such as isovaleric and isobutyric acid. The lack of milk fat in CDM and associated fatty acid production during starvation implicates branched-chain amino acid catabolism during starvation as a plausible explanation for branched-chain fatty acid production. Monitoring the activities of aminotransferases in cell-free extracts lead to no patterns correlated patterns with branched-chain fatty acid production. Current work is underway to determine patterns of amino acid utilization and genes expressed associated with the catabolic pathways.

Use of keto acids (added as a reactant in an enzyme assay) was found in all strains tested. Lactococci are being used to determine the exact pathway for this conversion with NMR.

**1. Significant Progress against Objectives:**

All objectives are on schedule as listed in the proposal

**2. Significant Conclusions:**

Dairy related bacteria convert added keto acids to fatty acids that are not found in milk fat. The implications for cheese flavor indicate the starters, adjunct, and NSLAB bacteria produce VFA.

**3. Anticipated Problems/Delays:**

None

**Publications:**

Balasubramanian Ganesan, B.C. Weimer, K. Seefeldt, R. Koka, and B. Dias. 2002. Fatty acid production by lactococci, lactobacilli and brevibacteria. Submitted.

**Dissertations:**

B. Ganesan - In progress

**Published Abstract:**

Weimer, B. C., B. Ganesan, and K. Seefeldt. 2000. Volatile fatty acid production by starter and adjunct bacteria. International Dairy Federation Biennial Cheese Flavor Conference, Banff, Alberta.

**Presentations:**

Balasubramanian Ganesan and Bart Weimer. 2002. Fatty acid production by lactococci during carbohydrate starvation in a chemically defined medium. American Society for Microbiology, Salt Lake City, UT.

**Patent/Invention Disclosures:**

None

**Technology Transfer Activities**

For information on licensing contact:  
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**Visitors Hosted:**

Paul Cihak – IFF

Vaughan Crow – NZDRI

Chakra Wijesundera – Food Science Australia

**Invention Disclosures: (Title, Date)**

\_\_\_\_\_

**Patents: (Title, Date, #)**

\_\_\_\_\_

**Licensing Activities:**

none - too early

**Discoveries:**

\_\_\_\_\_

# Western Dairy Center

## Project Report

January 1, 2001 to December 31, 2001

**Principal Investigators:** Bart Weimer, Utah State University

**Co-Investigators:**

**Project Title:** Importance of glutamic acid and  $\alpha$ -keto acids in cheese flavor development

**Institution's Project #:** 01123

**Project Completion Date:** December 31, 2002

<b>National Research Plan (1997): Priority:</b> milk/whey powder <b>Goal:</b> 2 <b>Tactic:</b> 2
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### Modifications to Project/Budget:

### Project Objectives: (Include any revisions to objectives)

Hypothesis: The first catabolic step for amino acids is catalyzed by aminotransferases using  $\alpha$ -keto acids as co-factors (amino donors and acceptors) to form flavor compounds precursors in cheese (also keto acids). The most common co-factor is important in the rate of flavor development in cheese, but additional keto acids need to be studied in relation to flavor formation potential.

Objective 1: Determine the diversity of transferase reactions that use acids in LAB.

Objective 2: Determine the role of this transformation in production of cellular energy and flavor compound production from each class of amino acids (branched chain, aromatic, acidic, neutral, basic).

Objective 3: Determine the rate of product formation with the addition of keto acids in relation to the amino acid precursor concentration. (Aminotransferases are bidirectional enzymes based on the products and reactant concentrations. If this reaction increases in the forward direction it may lead to a method to

accelerate cheese flavor.)

Objective 4: Determine the environmental triggers that induce each aminotransferase in lactococci and lactobacilli with transcription analysis.

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

### 1. Significant Progress against Objectives:

During this study, \_\_keto acids spontaneously degraded in solution to fatty acids. All bacteria tested produced fatty acids from \_\_keto acids at levels above spontaneous degradation. Brevibacteria catabolized amino acids only in carbohydrate starvation conditions. Lactic acid bacteria utilized amino acids and \_\_keto acids to produce fatty acids above flavor threshold levels. Higher amounts of fatty acids were produced from individual amino acids than a mixture. Addition of amino acids or \_\_keto acids as substrates yielded similar fatty acids, but different quantities during the incubation time. Lactococci, lactobacilli, and an *ilvE* deletion mutant of *Lactococcus lactis* ssp. *lactis* LM0230 utilized precursor amino acids and their corresponding \_\_keto acids differently; yet the *ilvE* deletion mutant retained the ability to produce branched chain fatty acids. These results indicate that bacteria associated with cheese production metabolize amino acids to fatty acid associated with desirable flavor. Production of BCFAs by the *ilvE* mutant indicate that multiple aminotransferases are involved in production of branched chain fatty acids in lactococci.

Lactococci withstand long-term (months) of carbohydrate starvation and remain metabolically active in a chemically defined medium. This metabolic capability is relevant in fermented foods when lactococci are entrapped in a protein-rich matrix with low pH, high NaCl, and a low oxidation/reduction potential. The aim of this work was to determine if these characteristics are possible in different subspecies of lactococci and to examine the length of time needed to induce these metabolic shifts. The present work examined starvation profiles of *Lactococcus lactis* ssp. *cremoris* SK11 and *Lactococcus lactis* ssp. *lactis* IL1403, a lactose and protease deficient strain. IL1403 provides insights into how lactose metabolism is related to starvation and fatty acid production since it lacks the plasmids associated with these functions. Each culture survived during carbohydrate starvation, but varied in their ability to maintained their ability to divide and form colonies. However, they were determined to be viable using staining dyes and they maintained similar levels of ATP as actively growing cultures. They catabolized branched-chain amino acids to branched-chain fatty acids, an activity they do not

conduct in laboratory conditions or with carbohydrate present. The intracellular aminotransferase activity was similar during incubation, but decreased. These data indicate that multiple lactococcal strains are capable of surviving and metabolizing protein substrates during carbohydrate starvation.

## **2. Significant Conclusions:**

A diverse set of keto acid intermediates were found in all bacteria studied. Deletion of a single aminotransferase (*ilvE*) did not significantly reduce the total VFA's produced, but it did change the ratios.

Metabolism of branched chain amino acids can provide energy (ATP) for survival in cheese-like conditions without a carbohydrate source.

## **3. Anticipated Problems/Delays:**

none

### **Publications:**

Balasubramanian Ganesan, B.C. Weimer, K. Seefeldt, R. Koka, and B. Dias. 2002. Fatty acid production by lactococci, lactobacilli and brevibacteria. Submitted.

### **Theses:**

Balasubramanian Ganesan – Ph.D. in progress

### **Published Abstract:**

Volatile fatty acid production by starter and adjunct bacteria. 2000. Bart Weimer, Bala Ganesan, and Kimberly Seefeldt. International Dairy Federation Biennial Cheese Flavor Conference, Banff, Alberta.

### **Presentations:**

Balasubramanian Ganesan and Bart Weimer. 2002. Fatty acid production by lactococci during carbohydrate starvation in a chemically defined medium. American Society for Microbiology, Salt Lake City, UT.

### **Patent/Invention Disclosures:**

none



**Technology Transfer Activities**

For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Project Report

January 1, 2001 to December 31, 2001

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

**Project Title:** Rapid detection of *Listeria* in dairy products

**Institution's Project #:** 01124

**Project Completion Date:** December 31, 2002

National Research Plan (2000): Priority: Goal: safety Tactic: 2  
Combine the patented ImmunoFlow system with PCR to obtain a final result about the presence of *Listeria monocytogenes* in the food product

**Modifications to Project/Budget:**  
None

**Project Objectives:** (Include any revisions to objectives)

Hypothesis:

Contamination of *Listeria* in dairy products is difficult to detect and is responsible for a large proportion of deaths from foodborne pathogens. False positives are a problem in detection of this organism. Rapid detection of *Listeria* is needed to aid in limiting distribution of contaminated milk and dairy products combined with genetic verification will reduce or eliminate false positive tests.

1. Optimize addition of secondary reagents for detection of *Listeria* in raw milk and soft cheese to produce a presumptive test that takes 15 to 30 minutes.
2. Couple the presumptive result to a PCR test to determine the species (and potentially) the strain.
3. Determine the detection limit and total test time to detect *Listeria*.

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

*Listeria monocytogenes* is a pathogenic microbe that causes serious illnesses and even death. Each year, the bacteria cause at least 2,493 cases of listeriosis. Of these, 2,298 persons are hospitalized and 499 persons die. The case fatality rate is high, i.e. 20 deaths per 100 cases of illness. Although HACCP and GMP are increasingly common in food industry companies, product recalls because of the chance of contamination are still the case. *Listeria monocytogenes* remains the primary agent in those recalls (25 out of 55 in 1999). This information and the fact that this organism grows well at refrigeration temperatures, suggest that a rapid detection technique for *Listeria monocytogenes* in food is needed.

A 2-stage test will be created. A preliminary (presumptive) result will be generated quickly (15 minutes). If the presumptive test is positive, the sample will be further processed to extract the DNA from the cells on the bead surface. This will be used in a PCR reaction to confirm the presumptive result and differentiate *L. monocytogenes* from other species. The combined use of the ImmunoFlow system and PCR allows a presumptive and confirmed diagnostic test in a rapid (30 minutes) and sensitive format (~100 cells). The easy use of the system will also be a big advantage.

The basis of this system involves the use of a patented flow-through capture cartridge that binds the pathogen onto the surface for subsequent detection. In the cartridge, a flow through fluidized bed module containing large (3 mm) glass beads was created. The glass beads are coated with covalently-bound antibodies against *Listeria*. By pumping contaminated samples through the bed, bacterial cells are captured and concentrated on the beads. This lead to three advantages: Larger sample volumes of milk can be tested.

No pre-enrichment step is needed.

Lower amounts of *Listeria monocytogenes* in food samples is concentrated on the beads for detection.

The presumptive step is automated.

Subsequent verification with PCR is done in an inhibitor-free environment.

### **1. Significant Progress against Objectives:**

Antibodies were screened for capture and background production. A limited number of Ab were selected to optimize the concentration for the presumptive step, that provides a system with low background and good binding. An indirect sandwich ELISA was found to be a good test format to maximize signal and minimize the background.

Optimization of the covalent binding step is being done by comparing two spacers (poly-Dextran and poly-PEG). Experiments are in progress to test these in static and ImmunoFlow formats.

Cell capture efficiencies have defined a reselection of the antibody system to allow greater cell capture. This is done and the reagent concentrations are being optimized.

Methods to extract the DNA from cells attached to beads are defined to standard methods without organic extraction. Verification of this result in a full ImmunoFlow test is underway. PCR verification for the capture and lysis is underway.

### **2. Significant Conclusions:**

A combination of antibodies in the indirect sandwich ELISA were identified. Preliminary conditions to lyse the cells and DNA extraction will influence the choice of beads.

**3. Anticipated Problems/Delays:**

None

**Publications:**

None

**Theses:**

Wim Lippens - in progress

**Published Abstract:**

none

**Presentations:**

None

**Patent/Invention Disclosures:**

Some is covered in previous patents, but new IP may be generated in the last portion of the work

**Technology Transfer Activities**

For information on licensing contact:

Bart Weimer (435) 797 3356

**Visitors Hosted:**

None

**Invention Disclosures: (Title, Date)**

\_\_\_\_\_

**Patents: (Title, Date, #)**

\_\_\_\_\_

**Licensing Activities:**

none - too early

**Discoveries:**

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# Western Dairy Center

## Final Report

Reporting Period January 1, 1997 to December 31, 2001

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 #: 97088

Project Completion Date: December 31, 2001

National Research Plan (1997): Priority: 1 Goal:1 Tactic: 1 Develop /characterize/modify strains with particular flavor producing capabilities and enzymes linked to actual cheese flavor development.
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### 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)

Met metabolism in dairy related bacteria is under investigation. 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 than lactococci. The enzyme responsible for methanethiol production in brevibacteria was isolated to homogeneity and characterized - methionine gamma-lyase (MGL). Addition of MGL, whole cells of *B. linens* BL2 (commercially available from GB) with either GDL or *L. cremoris* S2 demonstrated that MGL and whole cells of BL2 produced significantly more volatile sulfur compounds (VSC) than S2 alone in slurries. *L. cremoris* S2 produced VSC at levels just above flavor threshold. Addition of MGL or BL2 produced 2 to 5 times more VSC than S2 alone. These increases were associated with the treatments and not contamination from other organisms. The predominant VSC were methanethiol, dimethyldisulfide, and dimethyltrisulfide. As the methanethiol content decreased the dimethyldisulfide content increased. This observation suggested the redox potential was important, but was not measured.

Studies to screen other lactic acid bacteria (LAB) for met utilization indicate LAB produced significantly less VSC than brevibacteria. Whole cells or cell free extracts produce VSC, but whole cells produce less than cell free extracts, suggesting that met transport may be important in the production rate.

Studies are underway to determine the relative amounts of VSC production from organic and inorganic sulfur sources. Initial work was needed to determine a method for measuring total inorganic sulfate in milk. This is done and results indicate that whole and skim milk contain ~45 mg/L of sulfate. Transport of inorganic sulfur (sulfate) by lactococci indicate that they can significantly reduce the sulfate content during log growth and stationary phase. Work is under way to determine if the cells convert sulfate into organic precursors for VSC production.

### 1. Significant Progress against Objectives:

A change in personnel has delayed the completion of the comparison of organic and inorganic sulfur metabolism

### 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. MGL is active in slurries, suggesting that it maybe active in Cheddar cheese curd. Lactococci produce more VSC that expected in slurries, suggesting an unknown mechanism is at play.

The genes associated with MGL are being investigated now. The genetics of brevibacteria are significantly more difficult than those in other bacteria associated with cheese.

### 3. Anticipated Problems/Delays:

A change in personnel (twice) delayed the completion of the comparison of organic and inorganic sulfur metabolism. A person is now in place and making progress to finish this work.

### Publications:

Seefeldt, K. and B. C. Weimer. 2000. Diversity of sulfur compounds in lactic acid bacteria J. Dairy Sci. 83: 2740-2746.

Weimer, B. 1999. Brevibacteria. In Encyclopedia of Food Microbiology, R. K. Robinson et al. (Ed.), Academic Press, London.

Ummadi, M. and B. C. Weimer. 1999. Use of capillary electrophoresis-laser induced fluorescence detection to monitor bacterial growth and amino acid utilization. J. Chrom A. (in press).

Dias, B., and **B. C. Weimer**. 2001. Detection of  $\alpha$ -keto acids with capillary electrophoresis in culture supernatants and cheese. *J Chrom A* (accepted).

Dias, B., and **B. C. Weimer**. 1999. Production of volatile sulfur compounds in Cheddar cheese slurries. *International Dairy Journal* 9:605-611.

**Weimer, B.**, K. Seefeldt, and B. Dias. 1999. Sulfur metabolism in bacteria associated with cheese. *Antonie van Leeuwenhoek* 76:247-261

Steele, J.L., M.E. Johnson, J.R. Broadbent, and **B.C. Weimer**. 1998. Starter culture attributes which affect cheese flavor development, pp. 157-170. *In*, Proc. LACTIC '97 conference, Which strains? For which products?

M. E. Johnson, J. L. Steele, J. Broadbent, and **B. C. Weimer**. 1998. Manufacture of gouda and flavour development in reduced-fat cheddar cheese. *Aust. J. Dairy Tech.* 53:67.

Dias, B., and **Bart Weimer**. 1998. Conversion of methionine to thiols by lactococci, lactobacilli, and brevibacteria. *Appl. Environ. Microbiol.* 64:3320.

Dias, B., and **Bart Weimer**. 1998. Purification and characterization of methionine  $\alpha$ -lyase from *Brevibacterium linens* BL2 Appl. *Environ. Microbiol.* 64:3327.

Broadbent, J.R., M. Strickland, **B. Weimer**, M.E. Johnson, and J.L. Steele. 1998. Peptide accumulation and bitterness in Cheddar cheese made using single-strain *Lactococcus lactis* starters with distinct proteinase specificities. *J. Dairy Sci.* 81:327.

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.

Gao, S., D-H. Oh, J. Broadbent, M. Johnson, **B. Weimer**, and J. Steele. 1997. Aromatic amino acid catabolism by lactococci. *Le Lait* 77:371.

**Weimer, B. C.**, C. Brennand, J. Broadbent, J. Jaegi, M. Johnson, F. Milani, J. Steele, and D. Sisson. 1997. Influence of flavor adjunct bacteria on the flavor and texture of 60% reduced fat Cheddar cheese. *Le Lait* 77:383.

#### Theses:

Ben Dias - Ph.D. (completed spring '99)

Kim Seefeldt - Ph.D. terminated due to student quitting

S. Ghosh - M.S. - in progress

**Published Abstract:**

**Presentations:**

Weimer, B. C., 1999. Flavor compounds – bacteria and cheese. Land O'Lakes, Minneapolis, MN.

Weimer, B. C., 1999. Production of flavor compounds by bacteria in cheese. University of California – Davis, Dept. of Food Science and Nutrition and Dept. of Enology.

Weimer, B. C., 1999. Sulfur metabolism in dairy related bacteria. Institute of Food Technologists annual meeting, Biotechnology Section, Chicago, IL.

Weimer, B. C., 1999. Sulfur metabolism in dairy related bacteria. IFT Biotechnology Section Keynote.

Weimer, B. C., 1999. Sulfur metabolism in bacteria associated with cheese. 6<sup>th</sup> FEMS International Conference, Keynote speaker. Veldhoven, The Netherlands.

Weimer, B. C., B. Dias, M. Ummadi, M. Stickland, J. Broadbent, M. Johnson, J. Jeaggi, J. Steele, and J. Harper. 1997. Improving Cheddar cheese flavor with the addition of *brevibacteria*. American Dairy Science Association Annual meeting.

Weimer, B. C., 1997. Strategies for improving cheese flavor. Dairy Management, Inc. Conference on Cheese Flavor. Chicago, IL.

**Patent/Invention Disclosures:**

<b>Technology Transfer Activities</b>
Contact was made with 2 large cheese companies in the US about working together on sulfur metabolism in cheese flavor
For information on licensing contact: Bart Weimer <a href="mailto:Milkbugs@cc.usu.edu">Milkbugs@cc.usu.edu</a> 435 797 3356

**Visitors Hosted:**

Paul Chiak –IFF

Chakra Wijesundera – Food Science Australia

**Invention Disclosures: (Title, Date)**

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**Patents: (Title, Date, #)**

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**Licensing Activities:**

GB has the license for bug sales.

Rhodia is also interested in getting a brevi for their culture line. We have sent 2 cultures at this point. The cheese they made was analyzed by GC and taste panel. The specific details are not available to me, but indications were that they want to look for some other cultures.

**Discoveries:**

new enzyme has been purified. Could be useful as a 2nd licensed product, further work needs to be done on the stability in cheese, preliminary studies indicate the enzyme is stable in slurries. These data suggest the enzyme will be stable in cheese. Experiments to verify this will be done in the next few months.

MGL is stable in cheese slurries, suggesting that it can be added as a pure enzyme in cheese. Cost and addition issues need to be worked out before commercialization can begin.

# Western Dairy Center

## Project Report

Reporting Period November 1, 2000 — December 31, 2001

Principal Investigators: Dr. Jeff Broadbent  
Co-Investigators: Dr. Dennis L. Welker

Project Title: Understanding capsule production in *Streptococcus thermophilus* MR-1C

Institution's Project #: 00120

Project Completion Date: 12/31/01

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

Modifications to Project/Budget: none

Project Objectives: (Include any revisions to objectives)

1. Define the minimal genetic locus encoding production of the capsular extracellular polysaccharide in *Streptococcus thermophilus* MR-1C

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

*Streptococcus thermophilus* MR-1C enhances the functionality and yield of lowfat Mozzarella cheese. This effect is due to production of a capsular exopolysaccharide, and we have characterized several genes required for its synthesis. Since MR-1C does not have fast acid-producing ability, technology to make capsule-producing variants from fast acid-producing industrial *S. thermophilus* starters would benefit the US Mozzarella cheese industry. To reach this goal, we need to define the minimal genetic locus encoding capsule production in MR-1C. Thus, it is the objective of this project to identify regions of the MR-1C *cps* cluster that are not present in the industrial, fast acid-producing, EPS-negative, Mozzarella cheese starter bacterium *S. thermophilus* TAO61.

### 1. Significant Progress against Objectives:

We have characterized, by DNA sequence analysis, large regions of the MR-1C and TAO61 chromosomes, respectively, that contain genes associated with EPS production. Although 3 gaps remain in the TAO61 sequence, comparative sequence analysis has revealed TAO61 contain a number of *eps* genes found in MR-1C and other EPS-producing *S. thermophilus*. Once the sequence effort is complete, we will determine if introduction of MR-1C genes lacking in TAO61

can produce a capsule-positive phenotype in the latter strain. Finally, it is significant to note that the information generated in this project has allowed Drs. Broadbent and Welker to secure USDA-NRI support to investigate the biochemistry of capsule production in MR-1C.

**2. Significant Conclusions:**

See progress, above

**3. Anticipated Problems/Delays:**

None

**Publications:**

Broadbent, J. R., D. J. McMahon, C. J. Oberg, and D. L. Welker. 2001. Use of exopolysaccharide-producing cultures to improve the functionality of low fat cheese. *Int. Dairy J.* 11:433-439.

**Theses:**

None

**Published Abstract:**

None

**Presentations:**

Oberg, C.J., J.R. Broadbent, and D.J. McMahon. Applications of EPS production by lactic acid bacteria. Invited oral presentation for the symposium on exopolysaccharide production by lactic acid bacteria at the annual meeting of the American Dairy Science Association. July 21-25, Quebec City, Quebec, Canada.

Welker, D. L., C. J. Oberg, A. D. Cefalo, D. J. McMahon, and J. R. Broadbent. 2002. Insight on the mechanism(s) for loss of exopolysaccharide synthesis phenotype in *Streptococcus thermophilus*. Poster presentation at the 102<sup>nd</sup> general meeting of the American Society for Microbiology. May 19-23, Salt Lake City, UT.

Broadbent, J. R. 2001. Exopolysaccharide production in *Streptococcus thermophilus*: biochemistry, genetics, and applications. Invited oral presentation for Fifth Annual Symposium on Industrial and Fermentation Microbiology. April 27, University of Wisconsin, La Crosse.

**Patent/Invention Disclosures:**

None

**Technology Transfer Activities**

For information on licensing contact:  
Jeff Broadbent

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

None

**Patents: (Title, Date, #)**

None

**Licensing Activities:**

None

**Discoveries:**

See above

# Western Dairy Center

## Project Report

Reporting Period October 1, 1999 — December 31, 2001

**Principal Investigators:** Carl Brothersen, Utah State University

**Co-Investigators:** Bart Weimer, Utah State University

**Project Title:** Effect of size, hydrophobicity and temperature on the diffusion of molecules within the Cheddar cheese matrix.

**Institution's Project #:** 99110

**Project Completion Date:** 4/1/2000

**National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1**

### Modifications to Project/Budget:

**Project Objectives:** (Include any revisions to objectives)

1. Determine the extent of diffusion for macro molecules in Cheddar cheese.
2. If significant diffusion is detected in objective 1, determine the effect of storage temperature on the migration of molecules in Cheddar cheese.
3. If significant diffusion is detected in objective 1, determine the effect of age at injection on the migration of molecules in Cheddar cheese.

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

### 1. Significant Progress against Objectives:

This project was delayed in starting because of problems with the confocal microscope and problems with personnel. We now have a person trained on the microscope and have determined the sample preparation procedures. We are currently collecting and analyzing images from the microscope.

### 2. Significant Conclusions:

### 3. Anticipated Problems/Delays:

### Publications:

### Theses:

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Project Report

Reporting Period January 1, 2001 — December 31, 2003

**Principal Investigators:** Dr. Daren Cornforth, Utah State University

**Co-Investigators:**

**Project Title:** Dried milk mineral as an antioxidant in various processed meats.

**Institution's Project #:** 01130

**Project Completion Date:** December 31, 2002

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

### **Modifications to Project/Budget:**

**Project Objectives:** (Include any revisions to objectives)

1. Compare storage stability and sensory acceptability of fresh port sausage, turkey roll and summer sausage made with MM and BHT.

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

**1. Significant Progress against Objectives:**

**2. Significant Conclusions:**

**3. Anticipated Problems/Delays:**

**Publications:**

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

<b>Technology Transfer Activities</b> For information on licensing contact:
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**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**



# Western Dairy Center

## Final Project Report

Reporting Period July 1, 2000 – December 12, 2001

**Principal Investigators:** Mark McGuire, University of Idaho  
Michelle McGuire, Washington State University,  
Kathy Beerman, Washington State University

**Project Title:** Effect of butter and margarine intake on human milk CLA and fat concentrations in lactating women.

**Institution's Project #:** 99106

**Project Completion Date:** 6/30/2000

**National Research Plan:** Priority: 4; Goal: 4; Tactic: 1

**Modifications to Project/Budget:** None

**Project Objectives:** (Include any revisions to objectives)

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

Lactating women were randomly assigned to a treatment sequence in a 3 x 3 Latin square design. Treatments consisted of muffins and cookies made with either 1) butter (containing CLA and no industrially-produced trans fatty acids), 2) regular margarine (containing no CLA and high amounts of industrially-produced trans fatty acids) and no trans margarine (containing either CLA nor industrially-produced trans fatty acids). Women consumed approximately 30 g of each lipid source daily for the 7-d periods. Milk samples were collected at the end of each period by complete breast expression. Milk composition was determined and fatty acid composition was analyzed using gas chromatography. Statistical analysis of the over-all Latin square design suggested that there was no effect of lipid source on milk fat or protein concentration. However, subsequent analyses showed a clear reduction in milk fat (40%) in lean subjects during the regular margarine period, as compared to the butter and no trans margarine periods. This is important as a relatively large literature suggests that milk fat percent is somehow influenced by body composition. It is possible that this may be mediated by dietary fatty acids. Further, we found a small but significant decrease in lactose concentration during the butter treatment. The fatty acid profile of milk was altered by dietary supplementation with the different lipid sources. Mean CLA concentration was highest during the butter period and lowest during the no trans margarine period; interestingly, consumption of regular margarine resulted in significantly higher milk CLA than consumption of no trans margarine. This suggests that women can produce CLA

from fatty acids found in regular margarine. Further, butter consumption resulted in higher concentrations of palmitic acid in milk fat. Conversely, butter consumption decreased linoleic acid and linolenic acid concentrations compared to either margarine.

### 1. Significant Progress against Objectives:

This project was carried out by Nicole Anderson, a graduate student in the Department of Food Science and Human Nutrition at Washington State University, under the direction of Drs. Shelley McGuire, Kathy Beerman and Mark McGuire. Lactating women ( $n = 12$ ) were enrolled in this 32 d experiment consisting of 6 periods: baseline (3 d), intervention I (5 d), washout I (7 d), intervention II (5 d), washout II (7 d) and intervention III (5 d). Interventions consisted of the consumption of the diets enriched with butter (containing CLA and no industrially-produced trans fatty acids), regular margarine (containing no CLA and high amounts of industrially-produced trans fatty acids) and no trans margarine (containing either CLA nor industrially-produced trans fatty acids). Blood and milk samples were collected on the last day of each period, dietary information on the last 3 d of each intervention period, milk output data on the penultimate day of each intervention period, maternal and infant weight data before and after the study and maternal body fat estimations (via dual energy x-ray absorptiometry) during the baseline period.

Subject recruitment was completed in August, 2000 with the final subject finishing the study in September, 2000. At that time, biochemical analyses (total fat, fatty acids, lactose and protein) began on the milk samples; these analyses are completed. We have now also analyzed the plasma samples for their fatty acid concentrations. Further, all of the data regarding maternal anthropometrics and dietary intake have been analyzed. Data were submitted for presentation at the upcoming Experimental Biology meetings (April, 2002) in New Orleans. Manuscript preparation will be completed by May, 2002; we intend to submit this paper to the *American Journal of Clinical Nutrition*.

### 2. Significant Conclusions:

Effect of dietary intervention on milk fat, lactose and protein concentrations

Overall data analyses, not accounting for differences in maternal adiposity, suggested initially that dietary intervention did not influence milk fat content in this study (see **Figure 1**). However, when women were divided into lower body fat (LBF;  $n = 6$ ) and higher body fat (HBF;  $n = 6$ ), it was clear that leaner women experience significant milk fat depression when consuming a diet high in regular margarine (**Figure 2**). This finding was also illustrated by the fact that we found very strong and significant interactions between the magnitude of milk fat depression caused by regular margarine consumption and body fat ( $r = 0.7403$ ;  $P < 0.001$ ; **Figure 3**).

Data also show that consumption of a high butter diet resulted in the production of milk with significantly ( $P < 0.05$ ) lower milk lactose than diets high in no trans

margarine or regular margarine ( $71.1 \pm 1.8$ ,  $73.0 \pm 1.3$  and  $74.0 \pm 2.1$  mg/g, respectively; **Figure 4**). This was not an anticipated result of this study, and we do not yet have a hypothesis as to the effect of butter. However, the difference is small (<5%) and may not be important biologically.

Supporting our original hypothesis that dietary treatment would not influence milk protein, data analyses suggest no effect of dietary intervention period on milk protein content (see **Figure 5**).

### Effect of dietary intervention on milk fatty acids

The dietary intervention had significant effects on most fatty acids that were considered (see **Table 1**). As expected, CLA was significantly higher during the butter period than during either of the margarine periods. Note that consumption of regular margarine resulted in greater concentrations of CLA than consumption of no trans margarine suggesting that regular margarine either supplied CLA which is unlikely or more likely provided substrate, trans-11 18:1 vaccenic acid, for synthesis of CLA by the  $\Delta^9$  desaturase enzyme. Butter consumption resulted in higher concentrations of C16:0, palmitic acid. Conversely, butter consumption decreased C18:2 (linoleic acid) and C18:3 (linolenic acid) concentrations.

## Table 1

Effect of dietary intervention period on milk fat acid concentrations (%), mean  $\pm$  SEM) in lactating women (n = 12).

Fatty acid	Dietary Intervention Period		
	Butter	No Trans Margarine	Regular Margarine
16:0, palmitic	21.5 $\pm$ 0.7 <sup>b</sup>	14.2 $\pm$ 0.6 <sup>a</sup>	15.0 $\pm$ 0.6 <sup>a</sup>
18:0, stearic	9.0 $\pm$ 0.2	6.8 $\pm$ 0.4	7.8 $\pm$ 0.3
18:1, oleic	30.9 $\pm$ 0.7 <sup>a</sup>	40.3 $\pm$ 1.0 <sup>b</sup>	30.4 $\pm$ 0.6 <sup>a</sup>
18:2, linoleic	11.1 $\pm$ 0.5 <sup>a</sup>	15.4 $\pm$ 0.8 <sup>b</sup>	21.7 $\pm$ 1.0 <sup>c</sup>
c9,t11-18:2, CLA	0.56 $\pm$ 0.04 <sup>c</sup>	0.27 $\pm$ 0.04 <sup>a</sup>	0.36 $\pm$ 0.05 <sup>b</sup>
18:3, linolenic	0.65 $\pm$ 0.04 <sup>a</sup>	1.22 $\pm$ 0.17 <sup>b</sup>	1.57 $\pm$ 0.09 <sup>c</sup>

### 3. Anticipated Problems/Delays:

As outlined in our previous report, we initially encountered some important delays in being able to transfer funds from the University of Idaho to Washington State University. However, after that point the initiation and completion of the sample collection period ran very smoothly. Since that time, we were working to enhance our fatty acid analyses so that plasma CLA could be detected in relatively small sample sizes (i.e., 100 :L). Nicole Anderson, the graduate student working on this project for her Masters degree, is a very careful and studious individual and has taken additional time to be as accurate and precise as possible in all stages of this research. Although this is highly desirable, it has resulted in a bit of a delay in finishing up the project. She plans to defend

# Western Dairy Center

## Project Report

Reporting Period February 1, 2001 — December 31, 2001

**Principal Investigators:** Don McMahon, Utah State University

**Co-Investigators:**

**Project Title:** Rehydration and structure of reconstituted casein micelles.

**Institution's Project #:** 01129

**Project Completion Date:** February 1, 2004

**National Research Plan (1997): Priority:** milk/whey powder **Goal:** 2 **Tactic:** 2

### Modifications to Project/Budget:

This is a non-DMI funded project.

### Project Objectives: (Include any revisions to objectives)

1. Characterization of the structure of casein micelles reconstituted from dry powders in comparison to the structure of native casein micelles in milk.
2. Investigate any differences in coagulation properties of milk containing reconstituted casein micelles.

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

Microstructure of casein micelles reconstituted from sodium caseinate, calcium caseinate, calcium caseinate formed from acid casein dissolved using calcium hydroxide, and non-fat dried milk (NFDM) were studied using transmission electron microscopy. Solutions of all the dried products were made to a casein concentration of 2.4% and the pH of the solution adjusted to  $6.65 \pm 0.05$ . The powders were hydrated at 40 °C, and allowed to stabilize for 4 h. Sodium caseinate did not form any micelles but rather a network very similar to a gel-like structure. Calcium caseinate formed large micellar structures, which were stained heavily. Casein micelles in non-fat dried milk appeared to have lost their perfect spherical shape forming incomplete spherical shapes. When acid

casein was dissolved in water by the addition of calcium hydroxide, the structure of the calcium caseinate formed was different from the former. These formed smaller particles with irregular shapes. Similar structures were formed from adjunct starter culture media.

### Methods:

Microstructure of caseins in sodium caseinate (NaCN), calcium caseinate (CaCN), and non-fat dried milk (NFDM) were studied using transmission electron microscopy. Solutions of all the dried products were made to a casein concentration of 2.4% and the pH of the solution adjusted to  $6.65 \pm 0.05$ . The powders were hydrated at 40 °C, and allowed to stabilize for 18 h with moderate mechanical stirring. Samples were drawn at 4, 10, and 18 h of hydration. In another experiment the powders were hydrated at high shear for 5 min using a hand held high speed blender (Omni 5000) and subsequently stabilized for 1h with moderate mechanical stirring.

Casein solutions were diluted 100 times and the casein micelles were adsorbed on to parlodion coated copper grids. Parlodion coated copper grids were coated with poly-L-lysine to improve the adsorption of protein on to the parlodion film. These grids were stained using uranyl acetate and oxalic acid and washed to remove excess stain. The stained grids were quick frozen in liquid nitrogen cooled liquefied Freon 22, and freeze dried so that casein micelles or particles in a form as close to their native state was imaged. Images were photographed at 30,000 $\times$ , 85,000 $\times$  and 140,000 $\times$  at 80 kV using a Ziess 902 transmission electron microscope.

Coagulation properties such as rennet coagulation time and curd firmness of skim milk fortified with NaCN or CaCN or NFDM or ASM to a protein concentration of 2.99%, 3.17% and 3.35% were measured using a Formagraph. Rennet coagulation time (RCT) and curd firmness at 60 min ( $A_{60}$ ) after rennet addition were calculated. At each level of protein concentration, the sodium caseinate treatments were added with different levels of calcium chloride and calcium caseinate treatments with potassium-dihydrogen-phosphate so as to bring the coagulation properties within the range of the control skim milk. Protein solutions for fortification were prepared by high-shear mixing the powder in water so as to make a final concentration of 12% protein. These were allowed to stabilize for 8 h with moderate stirring. Skim milk was added with 1, 3 or 5 % of the above 12% protein solutions. This fortification increased the protein content in skim from 2.91 % to 2.99%, 3.17% and 3.35% respectively. In this report, when names of protein powders are preceded by 1, 3, and 5 it represents the level of fortification of milk with that 12% protein powder solution. The supplemented milks were allowed to stabilize for 1h before preparing it for Formagraph testing. As higher amounts of CaCN and NaCN reflected undesirable coagulation properties (such as longer RCT), phosphate in the form of  $\text{KH}_2\text{PO}_4$  was added to CaCN and calcium in the form of  $\text{CaCl}_2$  was added

to NaCN 30 min prior to rennet addition in the Formagraph. Calcium and phosphate was added up to a concentration of 2.4 and 72 mM of Ca and PO<sub>4</sub> respectively. Ten milliliters of these milks were brought to 35 °C and allowed to stand for 30 min at that temperature when 100 µl of double strength rennet diluted 1:100 was added and allowed to set in a Formagraph to get time-firmness curves.

### Significant Conclusions

After 4h of dispersion in water, NFDM produced agglomerates of small micelles ( $\leq 100$  nm). By increasing the hydration time or the shear rate of mixing, these agglomerates were not seen. The individual micelles showed a hairy microstructure. Calcium caseinate dissolved to form an opaque milky white solution. In case of calcium caseinate, higher hydration times or shear separated the micelles from each other whereas, for a 4-h hydration time, the micelles were connected with proteins. Large micelles ~300 nm were still present and were darkly stained. Sodium caseinate formed a mesh like structure at 4 h of moderate mechanical stirring. When hydrated at high shear or for 10 h, it appeared as strands or small agglomerates of proteins. The particles were still larger at 10 h of hydration than the high-shear treatment. Thus, a longer hydration time ~10 h or a higher shear rate of mixing can disperse the micelles effectively when milk protein powders are hydrated in water.

Rennet coagulation time of NFDM was quicker with higher amounts of fortification. RCT for milks supplemented with CaCN was longer with higher rates of supplementation. These differences diminished with phosphate addition and the RCT was comparable to that of milk. A similar trend of longer RCT with increased fortification and diminishing RCT with calcium addition was seen in NaCN supplemented milks. Curd firmness ( $A_{60}$ ) increased with higher fortification with NFDM. Curd firmness for CaCN supplemented milks decreased when compared to the control (skim milk with no fortification) while it increased with addition of phosphate. In case of milk supplemented with NaCN there was a reduction in curd firmness which was apparent for NaCN. Addition of Ca increased the firmness of all the treatments.

Even though milk supplementation with various milk proteins can destabilize the coagulation properties, these can be brought back to that of original milk by addition of various salts such as calcium hydroxide and potassium phosphate.

**Publications:** None

**Theses:** None

**Published Abstract:**

Oommen, B. S., D. J. McMahon and W. R. McManus. 2001. A comparative study of the microstructure of caseins in dried milk. 96th American Dairy Science Association Meeting, J. Dairy Sci. 84(Supp. 1):381

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Final Report

Reporting Period November 1, 2000 — December 31, 2001

**Principal Investigators:** Marie K. Walsh  
Charles Carpenter

**Co-Investigators:**

**Project Title:** Production of an extruded whey protein snack food

**Institution's Project #:** 00119

**Project Completion Date:** December 31, 2001

**National Research Plan (1997): Priority:** milk/whey powder **Goal:** 2 **Tactic:** 2

### Modifications to Project/Budget:

**Project Objectives:** (Include any revisions to objectives)

**Project Summary:** (Suitable for inclusion in Center documents released to the public)  
High protein snack foods are currently finding a large market as protein supplements, and we have described the use of thermoplastic extrusion to produce a high protein snack from whey protein. Our WPS is puffed during extrusion, although we have not yet achieved the expansion (puffing) desirable in this type of snack product. The purpose of this proposed research is to optimize the formulation and extruder configuration to allow extrusion into a puffed snack having acceptable texture. Variables being explored include concentration of whey protein and type of starch.

### 1. Significant Progress against Objectives:

- 0 Operational parameters to permit pilot scale production of an extruded whey protein



snack food.

- 1 Determine appropriate starch type (corn, modified corn, potato, modified potato, rice and bran), pH and use level which permits production of a puffed product.
- 2 Product evaluation including sensory and stability analysis.

Extruder configuration is being optimized to allow shear and pressure development vs conveyance which is typically used in meat extenders.

Corn starch, rice starch, tapioca starch, oat bran, wheat bran, corn bran and modified starches including Crisp Film, BAKA-SNAK and Purity NC (National Starch Company) have been obtained and are being used for the production of a whey protein snack product.

We have recently purchased new extruder shafts, screws and paddles and are optimizing the extrusion of a snack product.

We have also designed a new extruder die and had that manufactured for the production of a snack product.

**Table 1.** The following extruder runs have been conducted and samples collected.

WPC80 Source	% Protein	High Amylose		High Amylopectin	
Davisco	16%	T 140/140/100	O 147/153/125	T 140/140/125	O 123/144/125
		S 200 rpm	Q not available	S 160 rpm	Q 65%
		F 600	P 930	F 475	P 330
			M 178.0		M 168.2
	32%	T 140/135/125	O 141/142/125	T 100/120/115	O 136/121/116
		S 160 rpm	Q 65%	S 110 rpm	Q 60%
		F 500	P 700	F 500	P 310
			M 163.0		M 171.3
	40%	T 135/135/120	O 137/136/120	T 130/130/120	O 131/133/119
		S 125 rpm	Q 55%	S 100 rpm	Q 75%
		F 625	P 470	F 600	P 390
			M 157.7		M 154.1
Plainview	16%	T 130/130/120	O 124/152/120	T 135/135/115	O 110/133/112
		S 150 rpm	Q 75%	S 150 rpm	Q 85%
		F 550	P 940	F 450	P 270
			M 176.0		M 153.0
	32%	T 135/135/120	O 117/145/120	T 130/130/115	O 133/135/115
		S 200 rpm	Q 65%	S 200 rpm	Q 70%
		F 575	P 910	F 450	P 290
			M 168.2		M 156.5
	40%	T 135/130/125	O 136/136/124	T 130/125/120	O 132/134/120
		S 175 rpm	Q 70%	S 175 rpm	Q 70%
		F 475	P 810	F 500	P 660
			M 158.1		M 157.1

Controllable conditions: Temperature set (t), screw speed (S), dry feed rate (f), wet feed rate (held constant). Non-controllable (dependent) conditions: Temperature observed (O), torque (Q), die pressure (P), melt temperature (M).

Each sample shown in Table 1 will be characterized with respect to:

density (g/m<sup>3</sup>)

SME (energy input as indicated by torque)

soluble starch

soluble protein

Using methods developed by Hale et al (in press).

**2. Significant Conclusions:**

The type of starch significantly influences the expansion of an extruded snack product. In addition the concentration of whey protein influences the expansion of a snack product with higher whey levels leading to a more dense product.

Table 2. Observations on snack products produced as described in Table 1.

WPC80 Source	% Protein	High Amylose	High Amylopectin
Davisco	16%	Consistent, light, crunchy product. Mild flavor. System was slightly unstable.	High expansion, but system was unstable.
	32%	Consistent, light, crunchy product. Lightly toasted Cheerio-type flavor.	Consistent, light, crunchy product. "Raw" whey flavor very strong.
	40%	Limited, inconsistent expansion. Limited browning. Very little tooth pack, but product has strong raw whey flavor.	Inconsistent, incomplete expansion. Product very wet, excessive browning. Hard crunch and strong flavor.
Plainview	16%	Consistent, light, crunchy product. No tooth pack. Mild toasted flavor.	High, consistent expansion. Light color, high toothpack. Strong raw whey flavor.
	32%	Limited, incomplete expansion. Mild flavor.	High, inconsistent expansion. Hard crunch, high tooth pack. Strong flavor.
	40%	Complete but limited expansion. Golden brown color, but strong raw whey flavor.	Inconsistent expansion with large air cells. Very brittle. Strong raw whey flavor.

**3. Anticipated Problems/Delays:**

None

**Publications:**

Hale, A.B., C.E. Carpenter, and M.K. Walsh. Sensory and instrumental evaluation of beef patties containing extrusion-textured whey protein. Accepted by J. Food Sci.

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

patent pending

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Final Report

Reporting Period July 1, 1999 to December 31, 2001

**Principal Investigators:** Bart Weimer, Utah State University

**Co-Investigators:**

**Project Title:** Use of non-lactic acid bacterial proteolytic enzymes to reduce bitter peptides in dairy products.

**Institution's Project #:** 99109

**Project Completion Date:** December 31, 2001

**National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1**

**Modifications to Project/Budget:**

None

**Project Objectives:** (Include any revisions to objectives)

**Hypothesis:**

Proteolytic enzymes associated with non-lactic acid bacteria will increase proteolysis that subsequently improves flavor development and reduces bitter peptides during cheese aging.

**Objectives:**

- 0 Determine the ability of 3 previously isolated proteases to degrade  $a_{s1}$  - casein (f1-9) in Jenness-Koops buffer.
- 1 Determine the ability of 3 previously isolated proteases to degrade B-casein (f193-209) in Jenness-Koops buffer.
- 2 Determine the ability of these proteases to degrade  $a_{s1}$  - casein (f1-9) and B-casein (f193-209) in direct acid cheese slurries.

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

Proteolysis is an important process in Cheddar cheese manufacture and plays a role in flavor and texture development of the cheese. Initial hydrolysis of intact caseins is catalyzed by chymosin and endogenous milk enzymes. Subsequently, the concerted action of the proteolytic systems of the starter and non-starter bacteria hydrolyzes peptides during aging. The interaction between these enzymes is directly linked to bitter peptide production in Cheddar cheese. Bitter peptides are hydrophobic and usually originate from  $_{s1}$  - and  $_{s2}$  - casein. Two peptides commonly associated with bitterness are  $_{s1}$  - casein f(1-9) and  $_{s2}$  - casein f(193-209). Hydrolysis of these peptides is associated with decreased bitterness in

Cheddar cheese. The aim of this study was to investigate the ability of flavor adjunct bacteria and non-starter lactobacilli to degrade bitter peptides.

Cheddar cheese was made with *Lactococcus lactis* ssp. *cremoris* S1 with and without *Brevibacterium linens* BL2 and *Lactobacillus helveticus* CNRZ32 as adjuncts. In addition, Cheddar cheese was made with *Lactococcus lactis* ssp. *lactis* S2 with and without *B. linens* BL2 and *L. helveticus* CNRZ32 combined as adjuncts. Each bacterial population was followed during ripening. RP-HPLC analysis was used to determine  $\alpha_1$ -casein f(1-9) and  $\alpha_2$ -casein f(193-209) content and sensory attributes were evaluated by a trained taste panel.

The  $\alpha_1$ -casein f(1-9) and  $\alpha_2$ -casein f(193-209) content in each cheese, with and without adjuncts, increased during ripening. However, the concentration of these peptides was lower in cheeses made with adjuncts. When the adjuncts were added in combination they did not produce an appreciable reduction in  $\alpha_1$ -casein f(1-9) and  $\alpha_2$ -casein f(193-209) content in comparison to the individual adjunct addition. Addition of the adjuncts did not affect the overall flavor acceptability. But the Cheddar cheese did receive lower bitter scores. Cheddar cheese made with *B. linens* BL2 was rated the best by the panel, in addition to having lower  $\alpha_1$ -casein f(1-9) and  $\alpha_2$ -casein f(193-209) content. These studies suggest that adjunct bacteria reduce bitterness during ripening.

The NSLAB counts remained low for the initial aging period compared to a consistently high count for the starter population. Further analysis of the peptides is underway.

### 1. Significant progress against objectives:

Objectives 1 and 2 are done with the finding of each protease degraded these peptides. We added a few enzymes that will expand the amount of information compared to the original objectives.

A delay was encountered due to a personnel change and expansion of the number of strains and enzymes used in objective 1 and 2. However, the data from these additional strains is proving to be very interesting. At first inspection, it appears that there are very different peptide degradation products from each strain.

The profile analysis for each treatment is done and the data set is being evaluated for statistical differences.

### 2. Significant Conclusions:

Enzymes added to pure peptides degraded the peptides within 90 minutes. Each of these peptides rose in cheese made without added enzymes. With addition of the enzymes, these two peptides decreased and the overall peptide profile changed. These enzyme treatments also accelerated and enhanced the flavor profile. These studies indicate that addition of enzymes from non-lactic acid

bacteria is a viable method to decrease the hydrophobic peptide pool and accelerate flavor changes during ripening.

### 3. Anticipated Problems/Delays:

We changed slurries to cheese production. The cheese has been made and the peptide content has been determined. The final data analysis of the results for the peptide content is underway and should be done in the next few weeks.

#### Publications:

Koka, R., and **B. C. Weimer**. 2000. Isolation and characterization of a protease from *Pseudomonas fluorescens* RO98. J. Applied Microbiol. 89:280-288.

Koka, R., and **B. C. Weimer**. 2000. Investigation of the ability of a purified protease from *Pseudomonas fluorescens* RO98 to de-bitter cheese. Int. Dairy J. 10:75-79.

**Weimer, B. C.**, Xie Yi, and Rod Brown. 2000. Autocatalytic processing of the protease from *Brevibacterium linens* BL2: a kinetic analysis for the degradation of casein. International Dairy Federation Biennial Cheese Flavor Conference, Banff, Alberta.

#### Theses:

**P. Joseph** – Ph.D. in progress

#### Published Abstract:

**Weimer, B. C.**, P. Joseph, J. Petersen, and J. Steele 2000. The proteinases of lactobacilli. International Dairy Federation Biennial Cheese Flavor Conference, Banff, Alberta.

**B. Weimer**, P. Joseph, R. Koka, and Y. Xie. 2002. Use of non-lactic acid bacterial enzymes to degrade casein. Institute of Food Technology, Anaheim, CA.

#### Presentations:

Joseph, P., **B. C. Weimer**, J. Petersen, and J. Steele 2000. The proteinases of lactobacilli. Intermountain Graduate Forum, Logan, UT.

#### Patent/Invention Disclosures:

None

#### Technology Transfer Activities

None

#### Visitors Hosted:

#### Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:



# Western Dairy Center

## Final Report

Reporting Period July 1, 1999 — December 31, 2001

**Principal Investigators:** Dr. Bart Weimer

**Co-Investigators:**

**Project Title:** The proteome of lactic acid bacteria

**Institution's Project #:** 99111

**Project Completion Date:** December 31, 2001

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

**Modifications to Project/Budget:** None

**Project Objectives:** (Include any revisions to objectives)

1. Obtain access to the genome sequence database for *Lactobacillus acidophilus* that is being determined by CalPoly.
2. Determine which proteins are expressed during optimal growth.
3. Determine which proteins are expressed during stress (sugar starvation, heat shock, cold shock, pH, and bile).

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

**1. Significant Progress against Objectives:**

**2. Significant Conclusions:**

**3. Anticipated Problems/Delays:**

**Publications:**

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

<b>Technology Transfer Activities</b> For information on licensing contact:
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**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**

# Western Dairy Center

## Project Report

Reporting Period July 1, 1999 — December 31, 2001

**Principal Investigators:** Bart Weimer, Utah State University  
**Co-Investigators:** Carl Brothersen, Utah State University  
Paul Grossl, Utah State University

**Project Title:** Determination of the oxidation/reduction potential of cheese

**Institution's Project #:** 99207

**Project Completion Date:** 6/30/00

**National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1**

**Modifications to Project/Budget:**

None

**Project Objectives:** (Include any revisions to objectives)

### Hypothesis

The redox potential has an impact on cheese flavor development, but due to lack of adequate analysis methods the role of residual oxygen has not been delineated

### Objectives

1. Evaluate commercially available probes for use in milk and milk products (Brothersen).
2. Use the methods to determine the redox potential of cheese slurries made with direct acid and lactic acid bacteria.

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

**Significant progress against objectives:**

Objective 1 has been completed. We have located a redox probe which can be used in both liquids and cheese.

We have used the redox probe to measure the redox and pH in both slurries inoculated with lactic acid bacteria.

We have monitored the pH and redox of cheese as it ages.

**Significant Conclusions:**

We have been able to control pH and redox independently in liquids. The redox potential of cheese decreases slowly and uniformly as it ages.

**Anticipated Problems/Delays:**

**Publications:**

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**

**Visitors Hosted:**

**Invention Disclosures: (Title, Date)**

**Patents: (Title, Date, #)**

**Licensing Activities:**

**Discoveries:**