Western Center for Dairy Protein Research and Technology

Researching the Western U.S. Dairy Industry’s Future

Annual Report
Fiscal Year 1996
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Annual Project Reports:

- Rheology and microstructure of Mozzarella cheese.............................................
  PI: Donald McMahon

- Milk protein interactions and gelation during thermal processing........................
  PI: Rodney Brown

- Influence of increased milkfat interfacial area and surfactant proteins on the attributes and acceptability of fat-reduced Cheddar cheese............................
  PI: Lynn Ogden

- Casein modification in skim milk with improved color/body for spray drying and use in frozen/fermented dairy products..................................................
  PI: Lynn Ogden

- Protein and mineral distribution in the bovine casein micelle................................
  PI: Donald McMahon

- Novel process to produce low and reduced fat Cheddar cheese of consistent quality and at a lower cost
  PI: J. Antonio Torres

- Rheological properties and texture of low-fat cheese and effect of moisture
  PI: Joseph Irudayaraj

- Suitability of low-fat Cheddar cheese as an ingredient in other foods
  PI: Charlotte Brennand

- Tryptophan metabolism by starter and adjunct bacteria in low-fat Cheddar cheese (low-fat cheese project) .............................................................
  PI: Bart Weimer
Arginine metabolism by starter and adjunct bacteria in low-fat Cheddar cheese (low-fat cheese project) .......................................................... PI: Bart Weimer

Role of amino acids and carbohydrate in the production of volatile hydrophobic flavor compounds in low-fat Cheddar cheese by Brevibacterium linens (low-fat cheese project) ........................................................................................................................................ PI: Bart Weimer

Methionine metabolism by starter and adjunct bacteria in low-fat Cheddar cheese (low-fat cheese project) .......................................................................................................................... PI: Bart Weimer

Peptide characterization and analysis of flavor components from low-fat Cheddar cheese (low-fat cheese project) ................................................................................................. PI: Jeff Broadbent/Bart Weimer

Indole production by lactobacteria in low-fat Cheddar cheese (low-fat cheese project) .................................................................................................................................. PI: Jeff Broadbent

Tyrosine metabolism and the formation of para-cresol in low-fat Cheddar cheese (low-fat cheese project) .................................................................................. PI: Jeff Broadbent

Influence of alternative starter cocci on the physical properties of low-fat Mozzarella cheese (low-fat cheese project) ........................................................................................................ PI: Jeff Broadbent

Moisture movement in low-fat cheese ........................................................................................................ PI: Antonio Torres

Time and processing effects on water interaction with the protein-reduced fat matrix (low-fat cheese project) .......................................................................................... PI: Conly Hansen

Development of thermophilic cultures for manufacture of low-fat and non-fat Mozzarella cheese: 1. Increasing casein proteolysis (low-fat cheese project) ........................................................................ PI: Donald McMahon

Development of thermophilic cultures for manufacture of low-fat and non-fat Mozzarella cheese: 2.
Exopolysaccharide producing cultures (low-fat cheese project) .................................................................
PI: Donald McMahon

WCDPRT ACTIVITIES SUMMARY

The Western Center for Dairy Protein Research and Technology (WCDPRT) was very active during the 1995 fiscal year. The activities of the Center are listed below.

1. Seventeen research projects were active during the year including thirteen projects in the new low-fat cheese area.

2. The Center Annual Meeting was held on August 23, 1996, at Utah State University, Logan Utah. A large group representing both dairy producers, processors and researchers attended and provided significant input onto the future direction of the Center.

3. One new member was added to the Operational Advisory Committee, Swiss Valley Farms, Davenport, Iowa.

4. The Center cosponsored two conferences to facilitate technology transfer. They were: The Second Biennial Symposium on Ultra-high Temperature Processing of Milk, and The Twelvth Cheese Management Short Course, both of which were held at Utah State University;

5. A meeting to plan and coordinate the research activities for the Low-fat Cheese Project was held at Snowbird Utah on November. The meeting was attended by Western Center researchers as well industry leaders interested in production of low-fat cheese.
Pursuant to the WDFRC proposal and contract with the National Dairy Promotion and Research Board, the voting members of the Operational Advisory Committee are:

Linda Racicot  
Dairy Management Inc  
10255 W. Higgins Road, Suite 900  
Rosemont, IL 60018-5616  
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Madison WI 53701  
(608) 276-3600

Raj G. Narasimmon  
Schreiber Foods, Inc.  
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Green Bay WI 54305  
(414) 437-7601

Mr. Carl E. Zurborg  
Swiss Valley Farms  
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Davenport IA 52808  
(319) 391-3341
OPERATIONAL ADVISORY COMMITTEE
(Continued)

Gale Moser
United Dairymen of Idaho
1864 South Hulls Crossing
Preston ID 83263
(208) 852-0560

Ann Sorenson, Head
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(503) 737-6520

Thomas C. Jenkinson
Western Dairy Farmers Promotion Assoc.
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Barney Krueger
Avonmore West, Inc.
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Twin Falls, ID 83301

Ted Whitehead
Tillamook County Creamery Association
P.O. Box 313
Tillamook, OR 98414
# Western Center for Dairy Protein Research and Technology

## Principal Investigators

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
<th>Address</th>
<th>Phone</th>
</tr>
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<tbody>
<tr>
<td>Floyd W. Bodyfelt</td>
<td>Dept. of Food Science &amp; Technology</td>
<td>Oregon State University</td>
<td>Corvallis OR 97331</td>
<td></td>
</tr>
<tr>
<td>Rodney J. Brown</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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</tr>
<tr>
<td>Donald J. McMahon</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
<td></td>
</tr>
<tr>
<td>Mark A. Daeschel</td>
<td>Dept. of Food Science &amp; Technology</td>
<td>Oregon State University</td>
<td>Corvallis OR 97331</td>
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</tr>
<tr>
<td>Bruce L. Geller</td>
<td>Dept. of Microbiology</td>
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<td>Corvallis OR 97331</td>
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<tr>
<td>Conly L. Hansen</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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<tr>
<td>Jeff Broadbent</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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<tr>
<td>Paul A. Savello</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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<tr>
<td>William E. Sandine</td>
<td>Dept. of Microbiology</td>
<td>Oregon State University</td>
<td>Corvallis OR 97331</td>
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<tr>
<td>Gerald Schelling</td>
<td>Dept. of Animal &amp; Veterinary Science</td>
<td>University of Idaho</td>
<td>Moscow ID 83843</td>
<td></td>
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<tr>
<td>Lynn V. Ogden</td>
<td>Dept. of Food Science &amp; Nutrition</td>
<td>Brigham Young University</td>
<td>Provo UT 84602</td>
<td></td>
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<tr>
<td>J. Antonio Torres</td>
<td>Dept. of Food Science &amp; Technology</td>
<td>Oregon State University</td>
<td>Corvallis OR 97331</td>
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<tr>
<td>Bart Weimer</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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<tr>
<td>Joseph Irudayaraj</td>
<td>Dept. of Nutrition &amp; Food Sciences</td>
<td>Utah State University</td>
<td>Logan UT 84322-8700</td>
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WESTERN CENTER FOR DAIRY PROTEIN
RESEARCH AND TECHNOLOGY

BUDGET REPORT
FISCAL YEAR 1996

NATIONAL DAIRY PROMOTION AND RESEARCH BOARD $500,000

REGIONAL/INDUSTRY SUPPORT:
Utah Dairy Commission $50,000
United Dairymen of Idaho $50,000
Oregon Dairy Products Commission $20,000
Western Dairy Farmers' Promotion Association $10,000
Kraft General Foods, Inc. $5,000
Schreiber Foods, Inc. $5,000
Marschal-Rhone Poulenc, Inc. $5,000
Tillamook Co. Cream. Assoc $5,000
Avonmore West, Inc. $5,000

TOTAL REGIONAL/INDUSTRY SUPPORT $155,000

FY96 TOTAL DAIRY RESEARCH CONTRIBUTIONS $655,000
FY95 BALANCE FORWARD $83,173
TOTAL AVAILABLE FUNDS FOR FY96 RESEARCH $738,173

FY96 COMMITTED RESEARCH FUNDS
Western Dairy Foods Research Center ($604,321)
Technology Transfer (20,000)
Administrative ($60,000)

TOTAL FY96 COMMITTED RESEARCH FUNDS ($684,321)
FY96 BALANCE FORWARD $53,852
## Financial Summary of Approved Projects 1993 - 1996

<table>
<thead>
<tr>
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<th>FY93</th>
<th>FY94</th>
<th>FY95</th>
<th>FY96</th>
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<td>Production of Extracellular Proteases of Brevibacterium linens for Use in Low-fat Cheese - Weimer, USU</td>
<td>$35,625</td>
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<td>Bacteriophage-Resistance Gene Replacement in Lactococcus lactis-Geller, OSU</td>
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<td>Purification of Monospecific, Polyclonal Antibodies from Bovine Cheese Whey -Brown, USU</td>
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<td>Rheology and Microstructure of Mozzarella Cheese - McMahon, USU</td>
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<td>Function of Whey Proteins and Lactose in Age Gelation of UHT-Processed Milk Concentrate-Part 2-McMahon, USU</td>
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<td>Extrusion Processing of Whey Proteins -Hansen, USU</td>
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<td>Effects of Iron Fortification on Chemical Physical, Microbiological and Nutritional Properties of Yogurt - McMahon, USU</td>
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<td>Interactions Between Milk Proteins, Starter Cultures, and Hydrocolloidal Milkfat Replacers - Weimer, USU</td>
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<td>Milk Protein Interactions and Gelation During Thermal Processing - Brown, USU</td>
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<td>Using a Natural Nutrient Process to Improve Milk Quality and Extend Milk Shelf-Life Through the Reduction in Lipid Oxidation and Off-Flavors with Tocopherol (Vitamin E) Supplementation to Dairy Cows - Schelling, U. of Idaho</td>
<td>19,838</td>
<td>19,438</td>
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<td>Influence of Preadsorbed Protein on Adhesion of Listeria monocytogenes to Dairy Food Contact Surfaces -Daeschel, OSU</td>
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<td>Using Whey for Improvement of Exposed Subsoils and Sodic and Saline-Sodic Soils - Hansen, USU</td>
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<td>Growth of Bifidobacteria in Milk Association with Streptococcus thermophilus and Lactobacillus Species and Measured by Genetic and Enzymatic Probes - Sandine, OSU</td>
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<td>Development of High Protein Low-Fat Fermented Foods from Yogurt Cheese -Hansen, USU</td>
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Financial Summary of Approved Projects 1993 - 1996 (continued)

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<td>Protein and Mineral Distribution in the Bovine Casein Micelle - McMahon, USU</td>
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<td>Casein Modifications in skim milk with improved color/body for spray drying and use in frozen/fermented dairy products - Ogden, BYU</td>
<td>0</td>
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<td>Influence of Increased Milkfat Interfacial Area and Surfactant Proteins on the Attributes and Acceptability of Fat-reduced Cheddar Cheese - Ogden, BYU</td>
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<td>Novel process to produce low and reduced fat Cheddar cheese of consistent quality and at a lower cost - Torres, OSU</td>
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<td>Rheological properties and texture of low-fat cheese and effect of moisture - Irudayaraj, USU</td>
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<td>Suitability of low-fat Cheddar cheese as an ingredient in other foods - Brennand, USU</td>
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Low-fat Cheese Project

Tryptophan metabolism by starter and adjunct bacteria in low-fat Cheddar cheese - Weimer, USU

Arginine metabolism by starter and adjunct bacteria in low-fat Cheddar cheese - Weimer, USU

Role of amino acids and carbohydrate in the production of volatile hydrophobic flavor compounds in low-fat Cheddar cheese by *Brevibacterium linens* - Weimer, USU

Methionine metabolism by starter and adjunct bacteria in low-fat Cheddar cheese - Weimer, USU

Peptide characterization and analysis of flavor components from low-fat Cheddar cheese - Broadbent/Weimer, USU

Indole production by lactobacteria in low-fat Cheddar cheese - Broadbent, USU

Tyrosine metabolism and the formation of para-cresol in low-fat Cheddar cheese - Broadbent, USU
Influence of alternative starter cocci on the physical properties of low-fat Mozzarella cheese - Broadbent, USU

Moisture movement in low-fat cheese - Torres, OSU

Time and processing effects on water interaction with the protein-reduced fat matrix - Hansen, USU

Proteinase activities from new strains of Lactococcus lactis subsp. cremoris (low-fat cheese project, final report) - Geller, OSU

Preliminary examination of hazelnut enzymes extracts for flavor enhancement of reduced fat Cheddar cheese - Bodyfelt, OSU

Development of thermophilic cultures for manufacture of low-fat and non-fat Mozzarella cheese: 1. Increasing casein proteolysis - McMahon, USU

Development of thermophilic cultures for manufacture of low-fat and non-fat Mozzarella cheese: 2. Exopolysaccharide producing cultures - McMahon, USU

<table>
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<th>Low-fat Cheese Project Total</th>
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<td>TOTAL</td>
<td>$324,854</td>
<td>$733,964</td>
<td>$616,133</td>
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Introduction

Current trends in the American diet clearly indicate that lowfat dairy products will be one of the most important research areas of the 1990's. Unfortunately, traditional cheese flavor is presently not available in reduced-fat ripened cheese, and the inferior flavor and texture of these varieties limits their acceptability among consumers. In general, the lower the fat content, the more difficult it is to produce a cheese similar in quality to full-fat cheese. Starter cultures and media which perform well in the production of full-fat cheese often are not suited to low-fat varieties because culture-related flavor defects are frequently enhanced in low-fat cheese. These varieties, for example, are generally more susceptible to off-flavors such as bitterness. Bitterness is associated with the accumulation of short (2-27 amino acids) hydrophobic peptides whose evolution may be related to the level and specificity of bacterial proteolysis in the cheese. At present, there is a pressing need to develop specialized culture systems that overcome low-fat cheesemaking constraints. Development of new starter systems for low-fat products would be facilitated by more sophisticated knowledge of the role microbial enzymes and metabolites play in cheese flavor development. This project seeks to identify peptides which contribute to bitter flavor defect in lowfat Cheddar and to characterize the enzymes and conditions which lead to their accumulation. Elucidation of key biochemical pathways involved in undesirable cheese flavor production would have immediate application in the development of starter systems for manufacture of high-quality lowfat Cheddar cheese.

Objective:
1. To identify various peptides which influence flavor attributes in lowfat Cheddar cheese and to characterize conditions which lead to their production.

Results:
This study investigated peptide accumulation and bitterness in 50% reduced-fat Cheddar cheese manufactured with single-strain Lactococcus lactis starters which produced a P₁-, P₃⁻, or P₁/P₃ intermediate-type cell envelope proteinase. Micellar electrokinetic capillary chromatography of aqueous cheese extracts detected three
large peaks, designated O, P, and Q, that eluted with peptide standards and whose area increased with maturation time in a pattern that was distinct for each starter. Regression analysis between bitter flavor scores from trained sensory panels and individual O-Q peak areas suggested peaks P and Q had a negative and positive correlation, respectively, to this defect. Since bitterness is caused by a buildup of hydrophobic peptides, HPLC, capillary electrophoresis, peptide sequencing, and mass spectroscopy were used to identify peptides which accumulated in 6 mo old cheeses. Five peptides from αs1-casein, one from β-casein, and one from αs2-casein were found to accumulate in a manner that corresponded with starter proteinase specificity and oligoendopeptidase activity. αs1-CN (f1-23) products in cheese made with S1, for example, were consistent with those predicted by studies with native P1-type CEP under cheese conditions. That enzyme preferentially hydrolyzed αs1-CN (f1-23) at the 13-14 position, but it also generated αs1-CN (f1-9) and αs1-CN (f1-16). The presence of αs1-CN (f1-14) in S1 cheese can be explained through studies which demonstrated the CEP products αs1-CN (f1-16) and αs1-CN (f1-17) can be hydrolyzed to αs1-CN (f1-13) and αs1-CN (f1-14) by the lactococcal neutral oligoendopeptidase, PepO, which would be present in the cheese as a consequence of starter autolysis.

αs1-CN (f1-23) products in SK11 cheese may be explained in similar fashion. Cell-bound PIII-type CEP in cheese cleaves the 1-23 peptide into αs1-CN (f1-16) and αs1-CN (f1-17), and these peptides could be hydrolyzed to αs1-CN (f1-13) and αs1-CN (f1-14) by PepO. Finally, data collected from cheese made with the bitter L. lactis strain S3 suggests this bacterium’s CEP has a preference for the 9-10 position of αs1-CN (f1-23), but the enzyme is also able to hydrolyze the 13-14 bond. To further characterize the role of starter CEP specificity in the development of bitterness, we now propose to exchange the S1 and SK11 CEPs then evaluate the effect of each isogenic construct on bitterness in Cheddar cheese.

The αs2-CN (f1-21) fragment we have detected is probably produced by plasmin and the β-CN fragment (f193-203) is a known product of chymosin. The latter peptide is quite hydrophobic (Q = 1753) and has been associated with bitterness in cheese. That observation is consistent with our data, which found highest levels of this peptide in bitter S3 cheese. The only other peptide found at high concentration in S3 cheese was the 1-9 fragment of αs1-CN. αs1-CN (f1-9) has not previously been associated with bitterness, but some evidence is available to suggest it may contribute to this defect. First, peak Q provided good correlation to bitter flavor in 4 and 6 mo old cheeses (r² = 0.62 and 0.69), and αs1-CN (f1-9) was the only peptide shown to elute in this peak. Second, αs1-CN (f1-9) has an average hydrophobicity of 1422 and a MW < 6,000, which, according to the Q-rule, predict this peptide to be bitter. Sensory studies are now underway to investigate the role of αs1-CN (f1-9) and β-CN (f193-203) on bitterness.

Significance to the Dairy Industry

Future acceptance and demand for low-fat cheese will be heavily dependent on the availability of high-quality products. Solutions to the flavor and textural problems that have dogged low-fat cheese manufacture will require a more comprehensive understanding of the role other microbial enzymes and metabolites play in cheese flavor development. This project seeks to identify peptides which
contribute to bitter flavor defect in lowfat Cheddar and to characterize the enzymes and conditions which lead to their accumulation. Identification and characterization of these properties will facilitate the development of low-fat starter systems, through strain combinations or recombinant DNA technology, for the manufacture of high-quality low-fat cheese. Low-fat cheese with organoleptic qualities of full-fat varieties will increase consumer acceptance of low-fat dairy products and expand consumer demand for these goods to individuals that avoid cheese for reasons of diet and the absence of high quality low-fat alternatives.

Publications


Abstracts


