1996 Annual Report

Various Authors
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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 PI: Bart Weimer

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 PI: Bart Weimer

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

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 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.
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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.
WESTERN CENTER FOR DAIRY PROTEIN RESEARCH AND TECHNOLOGY

OPERATIONAL ADVISORY COMMITTEE

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  
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Thornton, CO 80233-0120

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Tillamook County Creamery Association
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Tillamook, OR 98414
WESTERN CENTER FOR DAIRY PROTEIN RESEARCH AND TECHNOLOGY

PRINCIPAL INVESTIGATORS

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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:**

<table>
<thead>
<tr>
<th>Organization</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Utah Dairy Commission</td>
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<td>United Dairymen of Idaho</td>
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<td>Oregon Dairy Products Commission</td>
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<td>Western Dairy Farmers' Promotion Association</td>
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<td>Kraft General Foods, Inc.</td>
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<td>Schreiber Foods, Inc.</td>
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<tr>
<td>Marschal-Rhone Poulenc, Inc.</td>
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<tr>
<td>Tillamook Co. Cream. Assoc</td>
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<tr>
<td>Avonmore West, Inc.</td>
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</table>

**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**

<table>
<thead>
<tr>
<th>Category</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Western Dairy Foods Research Center</td>
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<tr>
<td>Technology Transfer</td>
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<tr>
<td>Administrative</td>
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**Total FY96 Committed Research Funds**  
($684,321)

**FY96 Balance Forward**  
$53,852
## Financial Summary of Approved Projects 1993 - 1996

<table>
<thead>
<tr>
<th>Project Title</th>
<th>FY93</th>
<th>FY94</th>
<th>FY95</th>
<th>FY96</th>
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</thead>
<tbody>
<tr>
<td>Production of Extracellular Proteases of Brevibacterium linens for Use in Low-fat Cheese - Weimer, USU</td>
<td>$35,625</td>
<td>$39,293</td>
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<td>$0</td>
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<tr>
<td>Bacteriophage-Resistance Gene Replacement in Lactococcus lactis-Geller, OSU</td>
<td>40,860</td>
<td>41,571</td>
<td>44,228</td>
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<tr>
<td>Purification of Monospecific, Polyclonal Antibodies from Bovine Cheese Whey -Brown, USU</td>
<td>0</td>
<td>44,875</td>
<td>42,360</td>
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<tr>
<td>Rheology and Microstructure of Mozzarella Cheese - McMahon, USU</td>
<td>19,950</td>
<td>44,340</td>
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<td>56,980</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>
<td>22,075</td>
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<tr>
<td>Extrusion Processing of Whey Proteins - Hansen, USU</td>
<td>47,200</td>
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<tr>
<td>Effects of Iron Fortification on Chemical Physical, Microbiological and Nutritional Properties of Yogurt - McMahon, USU</td>
<td>26,700</td>
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<td>Interactions Between Milk Proteins, Starter Cultures, and Hydrocolloidal Milkfat Replacers - Weimer, USU</td>
<td>42,620</td>
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<tr>
<td>Milk Protein Interactions and Gelation During Thermal Processing - Brown, USU</td>
<td>0</td>
<td>60,735</td>
<td>67,925</td>
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<tr>
<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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<tr>
<td>Influence of Preadsorbed Protein on Adhesion of Listeria monocytogenes to Dairy Food Contact Surfaces - Daeschel, OSU</td>
<td>29,671</td>
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<tr>
<td>Using Whey for Improvement of Exposed Subsoils and Sodic and Saline-Sodic Soils - Hansen, USU</td>
<td>14,000</td>
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<tr>
<td>Growth of Bifidobacteria in Milk Association with Streptococcus thermophilus and Lactobacillus Species and Measured by Genetic and Enzymatic Probes - Sandine, OSU</td>
<td>26,315</td>
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<td>Development of High Protein Low-Fat Fermented Foods from Yogurt Cheese -Hansen, USU</td>
<td>0</td>
<td>39,242</td>
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Financial Summary of Approved Projects 1993 - 1996 (continued)

<table>
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<tr>
<th>Project Title</th>
<th>FY93</th>
<th>FY94</th>
<th>FY95</th>
<th>FY96</th>
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</thead>
<tbody>
<tr>
<td>Protein and Mineral Distribution in the Bovine Casein Micelle - McMahon, USU</td>
<td>0</td>
<td>0</td>
<td>19,730</td>
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<tr>
<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>
<td>0</td>
<td>18,500</td>
<td>21,600</td>
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<tr>
<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>47,500</td>
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<tr>
<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>0</td>
<td>42,105</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>33,880</td>
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<td>Suitability of low-fat Cheddar cheese as an ingredient in other foods - Brennand, USU</td>
<td>0</td>
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<td>27500</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>
<thead>
<tr>
<th>Low-fat Cheese Project Total</th>
<th>0</th>
<th>300,000</th>
<th>325,000</th>
<th>325,000</th>
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<tbody>
<tr>
<td>TOTAL</td>
<td>$324,854</td>
<td>$733,964</td>
<td>$616,133</td>
<td>$684,321</td>
</tr>
</tbody>
</table>
Project Title: Peptide characterization and analysis of flavor components in lowfat Cheddar cheese.

Bart C. Weimer, Dept. Nutrition and Food Sciences, Utah State University.
Marie Strickland, Dept. Nutrition and Food Sciences, Utah State University.
Mark E. Johnson, Center for Dairy Research, Univ. Wisconsin-Madison.
James L. Steele, Dept. Food Science, Univ. Wisconsin-Madison.

Funding: Western Center for Dairy Protein Research and Technology Utah Agricultural Experiment Station

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-, P₃₁-, 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 $\alpha_s$-casein, one from $\beta$-casein, and one from $\alpha_2$-casein were found to accumulate in a manner that corresponded with starter proteinase specificity and oligoendopeptidase activity. $\alpha_{31}$-CN (f1-23) products in cheese made with S1, for example, were consistent with those predicted by studies with native $P_1$-type CEP under cheese conditions. That enzyme preferentially hydrolyzed $\alpha_{31}$-CN (f1-23) at the 13-14 position, but it also generated $\alpha_{s1}$-CN (f1-9) and $\alpha_{s1}$-CN (f1-16). The presence of $\alpha_{s1}$-CN (f1-14) in S1 cheese can be explained through studies which demonstrated the CEP products $\alpha_{31}$-CN (f1-16) and $\alpha_{s1}$-CN (f1-17) can be hydrolyzed to $\alpha_{s1}$-CN (f1-13) and $\alpha_{s1}$-CN (f1-14) by the lactococcal neutral oligoendopeptidase, PepO, which would be present in cheese as a consequence of starter autolysis.

$\alpha_{s1}$-CN (f1-23) products in SK17 cheese may be explained in similar fashion. Cell-bound $P_{iii}$-type CEP in cheese cleaves the 1-23 peptide into $\alpha_{s1}$-CN (f1-16) and $\alpha_{s1}$-CN (f1-17), and these peptides could be hydrolyzed to $\alpha_{s1}$-CN (f1-13) and $\alpha_{s1}$-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 $\alpha_{s1}$-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 SK17 CEPs then evaluate the effect of each isogenic construct on bitterness in Cheddar cheese.

The $\alpha_{s2}$-CN (f1-21) fragment we have detected is probably produced by plasmin and the $\beta$-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 $\alpha_{s1}$-CN. $\alpha_{s1}$-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 ($r^2 = 0.62$ and 0.69), and $\alpha_{s1}$-CN (f1-9) was the only peptide shown to elute in this peak. Second, $\alpha_{s1}$-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 $\alpha_{s1}$-CN (f1-9) and $\beta$-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