1996

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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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 Manatgement Short Course, both of which where 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:

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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

Project Title	FY93	FY94	FY95	FY96
Production of Extracellular Proteases of Brevibacterium linens for Use in Low-fat Cheese - Weimer, USU	$35,625	$39,293	$0	$0
Bacteriophage-Resistance Gene Replacement in Lactococcus lactis-Geller, OSU	40,860	41,571	44,228	0
Purification of Monospecific, Polyclonal Antibodies from Bovine Cheese Whey -Brown, USU	0	44,875	42,360	0
Rheology and Microstructure of Mozzarella Cheese - McMahon, USU	19,950	44,340	50,890	56,980
Function of Whey Proteins and Lactose in Age Gelation of UHT-Processed Milk Concentrate-Part 2-McMahon, USU	22,075	26,000	0	0
Extrusion Processing of Whey Proteins -Hansen, USU	47,200	29,650	0	0
Effects of Iron Fortification on Chemical Physical, Microbiological and Nutritional Properties of Yogurt - McMahon, USU	26,700	0	0	0
Interactions Between Milk Proteins, Starter Cultures, and Hydrocolloidal Milkfat Replacers - Weimer, USU	42,620	49,276	0	0
Milk Protein Interactions and Gelation During Thermal Processing - Brown, USU	0	60,735	67,925	68,026
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	19,838	19,438	0	0
Influence of Preadsorbed Protein on Adhesion of Listeria monocytogenes to Dairy Food Contact Surfaces -Daeschel, OSU	29,671	29,544	0	0
Using Whey for Improvement of Exposed Subsoils and Sodic and Saline-Sodic Soils - Hansen, USU	14,000	0	0	0
Growth of Bifidobacteria in Milk Association with Streptococcus thermophilus and Lactobacillus Species and Measured by Genetic and Enzymatic Probes - Sandine, OSU	26,315	0	0	0
Development of High Protein Low-Fat Fermented Foods from Yogurt Cheese -Hansen, USU	0	39,242	0	0

Financial Summary of Approved Projects 1993 - 1996 (continued)

Project Title	FY93	FY94	FY95	FY96
Protein and Mineral Distribution in the Bovine Casein Micelle - McMahon, USU	0	0	19,730	29,230
Casein Modifications in skim milk with improved color/body for spray drying and use in frozen/fermented dairy products - Ogden, BYU	0	0	18,500	21,600
Influence of Increased Milkfat Interfacial Area and Surfactant Proteins on the Attributes and Acceptability of Fat-reduced Cheddar Cheese - Ogden, BYU	0	0	47,500	0
Novel process to produce low and reduced fat Cheddar cheese of consistent quality and at a lower cost - Torres, OSU	0	0	0	42,105
Rheological properties and texture of low-fat cheese and effect of moisture - Irudayaraj, USU	0	0	0	33,880
Suitability of low-fat Cheddar cheese as an ingredient in other foods - Brennand, USU	0	0	0	27500

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				
Low-fat Cheese Project Total	0	300,000	325,000	325,000
TOTAL	$324,854	$733,964	$616,133	$684,321

Project Title: Peptide characterization and analysis of flavor components in lowfat Cheddar cheese.

Personnel: Jeffery R. Broadbent, Dept. Nutrition and Food Sciences, Utah State University.
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 P_I-, P_{III}-, or P_I/P_{III} 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 α_{S1}-casein, one from β-casein, and one from α_{S2}-casein were found to accumulate in a manner that corresponded with starter proteinase specificity and oligoendopeptidase activity. α_{S1}-CN (f1-23) products in cheese made with S1, for example, were consistent with those predicted by studies with native P_I-type CEP under cheese conditions. That enzyme preferentially hydrolyzed α_{S1}-CN (f1-23) at the 13-14 postition, but it also generated α_{S1}-CN (f1-9) and α_{S1}-CN (f1-16). The presence of α_{S1}-CN (f1-14) in S1 cheese can be explained through studies which demonstrated the CEP products α_{S1}-CN (f1-16) and α_{S1}-CN (f1-17) can be hydrolyzed to α_{S1}-CN (f1-13) and α_{S1}-CN (f1-14) by the lactococcal neutral oligoendopeptidase, PepO, which would be present in cheese as a consequence of starter autolysis.

α_{S1}-CN (f1-23) products in SK11 cheese may be explained in similar fashion. Cell-bound P_{III}-type CEP in cheese cleaves the 1-23 peptide into α_{S1}-CN (f1-16) and α_{S1}-CN (f1-17), and these peptides could be hydrolyzed to α_{S1}-CN (f1-13) and α_{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 α_{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 SK11 CEPs then evaluate the effect of each isogenic construct on bitterness in Cheddar cheese.

The α_{S2}-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 α_{S1}-CN. α_{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), andα_{S1}-CN (f1-9) was the only peptide shown to elute in this peak. Second, α_{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 α_{S1}-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

Strickland, M., B.C. Weimer, and J.R. Broadbent. 1996. Capillary electrophoresis of Cheddar cheese. J. Chromatogr. A 731:305-314.

Abstracts

Broadbent, J.R., B.C. Weimer, and M. Strickland. 1996. Influence of single-strain *Lactococcus lactis* starters on the accumulation of casein-derived peptides in 60% reduced fat Cheddar cheese. J. Dairy Sci. 79(Suppl. 1):100.

Strickland, M, J. Broadbent, and B. Weimer. 1996. Methods for characterizing the water soluble peptides in Cheddar cheese. J. Dairy Sci. 79(Suppl. 1):91.

Strickland, M, J. Broadbent, and B. Weimer. 1996. A combined HPLC and capillary electrophoresis study of casein degradation during aging of Cheddar cheese. J. Dairy Sci. 79(Suppl. 1):99.

Johnson, M.E., J.L. Steele, B.C. Weimer, and J.R. Broadbent. 1996. Improvement of reduced-fat Cheddar cheese through identification, isolation and analysis of enzymes and metabolites produced by starters and adjunct cultures. J. Dairy Sci. 79(Suppl. 1):99.

Weimer, B., C. Brennand, M. Strickland, J. Broadbent, J. Jaegi, M. Johnson, and J. Steele. 1996. Flavor and chemical analysis of lower fat Cheddar cheese. J. Dairy Sci. 79(Suppl. 1):100.