1991

1990-1991 Annual Report

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Western Dairy Foods Research Center

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

Annual Report
1990-1991
WESTERN DAIRY FOODS RESEARCH CENTER

ANNUAL REPORT
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B. Annual Financial Reports by Project
WDFRC ACTIVITIES SUMMARY

The Western Dairy Foods Research Center (WDFRC) organized many activities during its operating year 1990-91. These activities included:

- continuation of important dairy products/processing research projects
- holding its annual meeting at Utah State University (together with the American Dairy Science Association's annual meeting)
- sponsoring seminars by outside speakers
- communicating through its semi-annual newsletter, Communique, to the NDPRB, WDFRC sponsors, and interested parties
- undergoing an external review of the WDFRC by the NDPRB, and
- reporting many project results of WDFRC projects at scientific meetings.

Twenty-three (23) research projects were on-going during this past year. The five (5) research areas of the WDFRC had active projects with some projects coming to completion. Many projects were actively continuing into the next operating year (1991-92). However, all projects have shown sufficient progress to report the projects' activities.

The WDFRC Annual Meeting was held on August 10, 1991 on the Utah State University campus. This date of the Annual Meeting was planned in order to coordinate with the American Dairy Science Association's annual meeting held on this same campus on August 12 to 15, 1991. The WDFRC Annual Meeting provided the opportunity for the Operating Advisory Committee (OAC) to give significant input into the future direction and focus of the WDFRC. Update reports of all WDFRC-sponsored projects were presented to the OAC at this Annual Meeting.

On August 22 and 23, 1990, an external review/evaluation of the WDFRC was conducted by the NDPRB. This two-day review included in-depth evaluation of the WDFRC's administration, research area objectives, projects, research proposal review process, accounting, contract/subcontract arrangements with Oregon State University and Brigham Young University (of the WDFRC consortium), communications to industry, and general progress of the Center. The overall recommendation from NDPRB was to support extension of the Center's activities for an additional three (3) years after the termination of the present five (5) year operating period.

Activities and operation management have instituted nearly all the evaluation team recommendations. The input of the OAC has been strongly solicited to determine the direction and focus of the WDFRC during its operating in the extension years. A semi-annual newsletter, Communique, is written in lay language and mailed to WDFRC sponsor organizations and other industry parties. The WDFRC Director is administering the Center without the aid of an administrative assistant as recommended. However, this recommendation is being discussed as to time allotment, required experience of assistant, and job description and roles.
The WDFRC total budget was $1,707,395 of which $949,778 was targeted for dairy research. Of this latter amount, $649,778 represented funding for direct WDFRC research projects. The remaining $300,000 represented dairy research programs underway at Utah State University that are funded by USDA-ARS and the newly created “Center for Dairy Foods Technology,” a State of Utah Center of Excellence. Major partners of the WDFRC are the National Dairy Promotion and Research Board, Utah Dairy Commission, United Dairymen of Idaho, Oregon Dairy Products Commission, Western Dairy Farmers' Promotion Association, Kraft-General Foods, Inc. Schreiber Foods, Inc. Marschall-Rhone Poulenc, Inc. and Borden, Inc.

The three (3) universities of the WDFRC contributed $757,617 in support resources. This institutional support provides for eighteen (18) principal investigators in the WDFRC and in facilities important to both the institutions and the WDFRC.

Full accounting of the WDFRC was prepared and submitted for a qualified internal auditing. This accounting was performed of all WDFRC projects and submitted October 18, 1991.

The Technical Advisory Committee (TAC) met in Salt Lake City, Utah on May 24, 1991 to review ten (10) research proposals. The TAC membership included three (3) dairy industry technical representatives, one (1) dairy researcher of the USDA-ARS, one (1) food science faculty professor of Oregon State University, one (1) NDPRB staff member, and the WDFRC Director. Seven (7) projects were approved by the TAC. The TAC recommended modifications (financial or technical) to some of these seven projects. One approved (with modifications) project was withdrawn by the principal investigator before the OAC Annual Meeting. The remaining six (6) projects were discussed by the OAC and approved for funding.
A proposal for a three-year extension of funding from the NDPRB was prepared and reviewed by WDFRC supporters. The proposal was forwarded to NDPRB staff on April 2, 1991. The proposal was reviewed by the appropriate committees of NDPRB. A recommendation of the NDPRB was made to the Director that the WDFRC consider its strengths and channel these strengths into a more focused and directed dairy research program. The direction and focusing of WDFRC research was discussed by the OAC at the Annual Meeting.

The OAC agreed with the NDPRB recommendation. The overall suggestion by the OAC was that the Director should look at all aspects of the Dairy Center and institute the direction that will best utilize the expertise of research personnel and the facilities available to do the research. As an outcome of this OAC discussion, the Director prepared a modified proposal (for a three-year extension of funding). This proposal was sent to the NDPRB in September 1991.

The proposal for funding extension include the following changes:

- The name of the Center is changed to the Western Center for Dairy Protein Research and Technology. This name change clearly defines and portrays the Center's focused research activities.
- The two (2) research program areas include: 1) Fundamental research in the physical/chemical properties and microstructure of dairy proteins, and 2) Practical research in the effects of various processing technologies on dairy proteins as these affect characteristics of milk and dairy foods.
- Research activities that will be used to investigate these research areas include: thermal processing, drying technologies, membrane processing, enzymes, lactic cultures, nutrition, and whey utilization.

The revised proposal includes research area objectives and research project objectives. Strategies to meet these objectives are under discussion.

The Dairy Center is excited about the direction and focus that the NDPRB and OAC have helped institute through its valuable participation. The Dairy Center will continue its highest quality research efforts in the area of dairy protein research and technology.
APPENDIX A

New WDFRC Projects 1991-92


Identifying batch of origin of finished cheese made in continuous processes. Lynn Ogden, Brigham Young University.

The influence of preadsorbed protein on adhesion of Listeria monocytogenes to dairy food contact surfaces. Mark Daeschel, Oregon State University.

Using whey for improvement of exposed subsoils and sodic and saline-sodic soils. Conly Hansen, Utah State University.

Comparative effects of whey protein concentrate (WPC), lactose, salt, phosphate, and pH on cooked yield, bind, and acceptability of turkey rolls and boneless hams. Daren Cornforth, Utah State University.

Prediction and determination of the efficacy of nisin in dairy foods. (Project renewal with increased funding.) Mark Daeschel and Floyd Bodyfelt, Oregon State University.
APPENDIX B

Annual Financial Reports by Project
To National Dairy Promotion
and Research Board
Logan, Utah

QUALIFIED INTERNAL AUDITORS' STATEMENT

We have performed an accounting of the Annual Financial Report by Project, of the Western Dairy Research Center, from July 1, 1987 to June 30, 1991.

Keith Sedgwick, Director
Internal Audits

Utah State University
October 18, 1991
## Western Dairy Center Listing of Funded Projects By Account Number

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***TOTAL***

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LEGEND:

☐ - Increase in project funding approved for FYs
• - Decrease in project funding approved for FYs
(____) - Extension (no cost) approved
(____) - Project revision approved
To National Dairy Promotion and Research Board
Logan, Utah

QUALIFIED INTERNAL AUDITORS' STATEMENT

We have performed an accounting of the Annual Financial Report by Project, of the Western Dairy Research Center, from July 1, 1987 to June 30, 1991.

Keith Sedgwick, Director
Internal Audits

Utah State University
October 18, 1991
QUALIFIED INTERNAL AUDITORS’ STATEMENT

We have performed an accounting of the Annual Financial Report by Project, of the Western Dairy Research Center, from July 1, 1987 to June 30, 1991.

Keith Sedgwick, Director
Internal Audits

Utah State University
October 18, 1991
# ANNUAL FINANCIAL REPORT BY PROJECT

**Western Dairy Research Center**

**Annual Report Ending:** June 30, 1991, Final  
**Project Term:** 07/01/87 - 06/30/89

**Dairy Center Director:** Dr. Paul A. Savello  
**Principal Investigator:** HANSEN CONLY L

**Project Title:** COGENERATION OF BIOGAS AND SINGLE CELL PROTEIN FROM ULTRAFILTRATION PERMEATE AND WHEY

**Project Status:** Closed  
**USU Project Number:** 189  
**USU Account Number:** 547766

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/87 - 06/30/92
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: KONDO JEFFERY K
Project Title: CLONING THE NISIN AND OTHER GENES OF LACTIC STREPTOCOCCI INTO LEUCONOSTOC SPECIES AND AMPLIFICATION OF NISIN PRODUCTION
Project Status: Active
USU Project Number: 188
USU Account Number: 547767

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## ANNUAL FINANCIAL REPORT BY PROJECT

Western Dairy Research Center

Project Term: 08/01/87 - 06/30/90

Dairy Center Director: Dr. Paul A. Savello  
Principal Investigator: MAHONEY ARTHUR W

Project Title: IRON FORTICICATION OF CHEESE CURD

Project Status: Closed  
USU Project Number: 182  
USU Account Number: 547768

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The budget summary shows the allocation and expenditure of funds for the project from 08/01/87 to 06/30/90. The total funds allocated include salaries and wages, fringe benefits, supplies, and equipment. The NDPRB funds and local funds are balanced as shown in the table.
ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Annual Report Ending: June 30, 1991, Final  Project Term: 07/01/87 - 06/30/90
Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: RICHARDSON GARY H

Project Title: ACQUISITION OF ZYMARK II ROBOT FOR LABORATORY AUTOMATION STUDIES

Project Status: Closed  USU Project Number: 190  USU Account Number: 547769

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/90 - 06/30/92
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: SAVELLO PAUL A

Project Title: DAIRY CENTER GENERAL EXPENSES FROM NATIONAL DAIRY BOARD FUNDS

Project Status: Active
USU Project Number: USU Project Account Number: 547778

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: SAVELLO PAUL A
Project Title: DAIRY CENTER GENERAL EXPENSES FROM NON-NATIONAL DAIRY BOARD FUNDS

Project Status: Active
USU Project Number: USU Account Number: 547779

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/87 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: RICHARDSON GARY H

Project Title: IMPROVING YIELD AND PHYSICAL PROPERTIES OF MOZZARELLA CHEESE

Project Status: Active
USU Project Number: 181
USU Account Number: 547780

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**ANNUAL FINANCIAL REPORT BY PROJECT**
Western Dairy Research Center

Project Term: 09/01/87 - 08/31/89

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: ERNSTROM C ANTHON

Project Title: CONTINUOUS PRODUCTION OF COTTAGE CHEESE FROM ULTRA-FILTRATED SKIM MILK RETENATE

Project Status: Closed  USU Project Number: 183  USU Account Number: 547781

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# ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

**Annual Report Ending:** June 30, 1991, Final  
**Project Term:** 07/01/87 - 06/30/90  
**Dairy Center Director:** Dr. Paul A. Savello  
**Principal Investigator:** CORNFORTH DAREN P

**Project Title:** EVALUATION OF MILK PROTEINS AS WHITENING AGENTS IN PROCESSED MEATS AND POULTRY PRODUCTS

**Project Status:** Closed  
**USU Project Number:** 184  
**USU Account Number:** 547782

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| **TOTALS**           | 31839       | 33072       | -1233       | 21227       | 21686       | -459        | 10612       | 11386       | -774       |
**ANNUAL FINANCIAL REPORT BY PROJECT**

**Western Dairy Research Center**

Project Term: 07/01/87 - 06/30/89

Dairy Center Director: Dr. Paul A. Savello  
Principal Investigator: OLSEN ROBERT L

Project Title: INTERACTION OF PROTEIN AND POLYSACCHARIDES IN CHYMOSIN AND ACID COAGULATION OF MILK

Project Status: Closed  
USU Project Number: 186  
USU Account Number: 547783

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/87 - 06/30/91

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: RICHARDSON GARY H

Project Title: IMPROVED CONTROL OF CHEESE MANUFACTURE THROUGH VAT MONITORING

Project Status: Active   USU Project Number: 187   USU Account Number: 547784

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Project Term: 07/01/87 - 06/30/92
Principal Investigator: SAVELLO PAUL A
Project Title: WESTERN DAIRY FOODS RESEARCH CENTER ADMINISTRATIVE ACCOUNT

Project Status: Active    USU Project Number: 191    USU Account Number: 547785

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- NDPRB Funds: 105105
- Local Funds: 41118

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ANNUAL FINANCIAL REPORT BY PROJECT  
Western Dairy Research Center  

Project Term: 07/01/87 - 06/30/88  
Dairy Center Director: Dr. Paul A. Savello  
Principal Investigator: ERNSTROM C ANTHON  
Project Title: EFFECT OF MILK CLOTTING ENZYMES ON THE CURING AND QUALITY OF CHEDDAR CHEESE  
Project Status: Closed  
USU Project Number: 192  
USU Account Number: 547802  

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Annual Report Ending: June 30, 1991, Final  Project Term: 07/01/87 - 06/30/92
Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: SANDINE W E

Project Title: CLONING THE NISIN AND OTHER GENES OF LACTIC STREPTOCOCCI INTO LEUCONOSTOC SPECIES AND AMPLIFICATION OF NISIN PRODUCTION

Project Status: Active  USU Project Number: 188  USU Account Number: 547804

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| Supplies       | Alloc: 54024 | Spent: 38385 | Balance: 15639 |
| Equipment      | Alloc: 3955  | Spent: 3955  | Balance: 0   |
| Travel         | Alloc: 0     | Spent: 2099  | Balance: -2099 |
| Publication    | Alloc: 0     | Spent: 0     | Balance: 0   |
| TOTALS         | Alloc: 165679| Spent: 14730 | Balance: 180409 |

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| Fringe         | NDPRB Alloc: 20959  | NDPRB Spent: 20959  | NDPRB Balance: 0   |
| Supplies       | NDPRB Alloc: 54024  | NDPRB Spent: 38385  | NDPRB Balance: 15639 |
| Equipment      | NDPRB Alloc: 3955   | NDPRB Spent: 3955   | NDPRB Balance: 0   |
| Travel         | NDPRB Alloc: 0      | NDPRB Spent: 2099   | NDPRB Balance: -2099 |
| Publication    | NDPRB Alloc: 0      | NDPRB Spent: 0      | NDPRB Balance: 0   |
| TOTALS         | NDPRB Alloc: 165679 | NDPRB Spent: 14730  | NDPRB Balance: 180409 |
ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/87 - 06/30/90
Dairy Center Director: Dr. Paul A. Savello
Project Title: CHARACTERIZATION OF BACTERIOPHAGE RECEPTOR SITES OF LACTIC STREPTOCOCCI
Principal Investigator: SANDINE W E

USU Project Number: 194
USU Account Number: 547810

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 08/01/87 - 06/30/91

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: BODYFELT FLOYD W

Project Title: RAPID ASSAY FOR HEAT RESISTANT MICROBIAL PROTEASES IN RAW MILK BY A SIMPLE CASEIN DENATURATION METHOD

Project Status: Active
USU Project Number: 195
USU Account Number: 547811

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 09/01/87 - 12/31/90
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: BODYFELT FLOYD W

Project Title: PRODUCTION OF OMEGA-3 FATTY ACIDS BY GENETICALLY ALTERED FUNGI AND LACTIC ACID BACTERIA

Project Status: Closed
USU Project Number: 196
USU Account Number: 547812

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 01/01/88 - 12/31/90
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: MCGUIRE JOSEPH
Project Title: CHARACTERIZATION OF THE POST-ABSORBTIVE BEHAVIOR OF B-LACTOGLOBULIN FOR CONTROL
OF SPORE AND MICROBIAL ADHESION
Project Status: Closed
USU Project Number: 197
USU Account Number: 547813

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: SANDINE W E

Project Title: STUDIES ON THE GROWTH AND SURVIVAL OF BIFIDOBACTERIUM SPECIES IN MILK

Project Status: Closed  USU Project Number: 198  USU Account Number: 547814

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Annual Report Ending: June 30, 1991, Final  Project Term: 09/01/87 - 08/31/89
Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: OGDEN LYNN V

Project Title: CONTINUOUS PRODUCTION OF COTTAGE CHEESE FROM ULTRAFILTRATED SKIM MILK RETENATE

Project Status: Closed  USU Project Number: 183  USU Account Number: 547822

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: OGDEN LYN V

Project Title: METHOD FOR IDENTIFYING BATCH OF ORIGIN OF SEMI-CONTINUOUS CHEESE PROCESSES

Project Status: Closed
USU Project Number: 199
USU Account Number: 547823

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**ANNUAL FINANCIAL REPORT BY PROJECT**

**Western Dairy Research Center**


Project Term: 07/01/88 - 06/30/91

Dairy Center Director: Dr. Paul A. Savello

Principal Investigator: BROWN RODNEY J

Project Title: APPLICATION OF FOURIER TRANSFORM INFRARED TECHNOLOGY TO MILK AND DAIRY PRODUCTS

Project Status: Active

USU Project Number: 201

USU Account Number: 547825

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- Salaries/Wages: 53593 (Total), 36958 (NDPRB), 16635 (Local)
- Fringe: 16078 (Total), 12119 (NDPRB), 3959 (Local)
- Supplies: 3000 (Total), 11720 (NDPRB), -8720 (Local)
- Equipment: 50250 (Total), 41015 (NDPRB), 9235 (Local)
- Travel: 2000 (Total), 3865 (NDPRB), -1865 (Local)
- Publication: 0 (Total), 60 (NDPRB), -60 (Local)

TOTALS: 124921 (Total), 105736 (NDPRB), 19185 (Local)
ANNUAL FINANCIAL REPORT BY PROJECT

Western Dairy Research Center

Project Term: 07/01/88 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: BROWN RODNEY J

Project Title: ESTIMATION OF INDIVIDUAL MILK PROTEINS AND GENETIC VARIANTS BY MULTICOMPONENT ANALYSIS OF AMINO ACID PROFILES

Project Status: Active
USU Project Number: 267
USU Account Number: 547827

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Annual Report Ending: June 30, 1991, Final  Project Term: 08/01/88 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: SAVELLO PAUL A

Project Title: USE OF ULTRAFILTRATION AND DIFFERENT HEAT TREATMENTS ON YOGURT FLAVOR AND PHYSICAL PROPERTIES

Project Status: Active  USU Project Number: 204  USU Account Number: 547828

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Annual Report Ending: June 30, 1991, Final  Project Term: 07/01/88 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello  Principal Investigator: DAESCHEL MARK A

Project Title: PURIFICATION OF A BACTERIOCIN FROM PEDIOCOCCUS PENTOSACEUS AND GENETIC TRANSFER OF THE PLASMID BORNE DETERMINANT

Project Status: Active  USU Project Number: 203  USU Account Number: 547833

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: TORRES J ANTONIO
Project Title: CHEDDAR CHEESE BLOCKS: EFFECT OF CHEESE COMPOSITION AND COOLING METHOD

Project Status: Closed
USU Project Number: 202
USU Account Number: 547834

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Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Project Term: 11/01/88 - 12/31/91
Principal Investigator: HANSEN CONLY

Project Title: COMPARISON BETWEEN 40 AND 640 LB BLOCKS OF UNIFORM COOLING OF 640 LB BLOCKS

Project Status: Active
USU Project Number: 206
USU Account Number: 547838

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Western Dairy Research Center

Project Term: 09/01/88 - 08/31/91

Dairy Center Director: Dr. Paul A. Savello  
Principal Investigator: MCMAHON DONALD J

Project Title: VARIATIONS IN CASING COMPOSITION OF MILK HIGH YIELD, LOW MOISTURE CHEESE FROM HOMOGENIZED UF MILK

Project Status: Active  
USU Project Number: 200  
USU Account Number: 547839

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Annual Financial Report by Project

Western Dairy Research Center

Project Term: 10/04/88 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: MAHONEY ARTHUR W

Project Title: EVALUATION OF IRON-PROTEIN COMPLEXES IN IRON-FORTIFIED DAIRY PRODUCTS

Project Status: Active    USU Project Number: 208    USU Account Number: 547841

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Annual Financial Report by Project
Western Dairy Research Center

Project Term: 03/01/89 - 03/01/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: HANSEN CONLY L

Project Title: A NEW METHOD FOR MEASURING SYNERESIS OF RENNETED GELS APPLIED TO DEVELOPMENT OF CHEESE

Project Status: Closed
USU Project Number: 210
USU Account Number: 547848

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ANNUAL FINANCIAL REPORT BY PROJECT

Western Dairy Research Center

Project Term: 11/01/88 - 12/31/91

Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: TORRES J ANTONIO

Project Title: COOLING RATE OF CHEDDAR CHEESE: COMPARISON BETWEEN 40 AND 640 LB BLOCKS OF UNIFORM COOLING OF 640 LB BLOCKS

Project Status: Active
USU Project Number: 206
USU Account Number: 547862

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Project Term: 07/01/88 - 06/30/90
Principal Investigator: MCDANIEL MINA R
Project Title: OPTIMIZATION OF THE SENSORY QUALITIES OF FLAVORED YOGURT

Project Status: Active  USU Project Number: 209  USU Account Number: 547863

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Annual Financial Report by Project
Western Dairy Research Center

Project Term: 07/01/88 - 06/30/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: DAESCHEL MARK A
Project Title: PREDICTION AND DETERMINATION OF THE EFFICACY OF NISIN IN DAIRY FOODS
Project Status: Active
USU Project Number: 207
USU Account Number: 547864

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Dairy Center Director: Dr. Paul A. Savello
Project Term: 07/01/89 - 06/30/91
Principal Investigator: MCMAHON DONALD J

Project Title: FUNCTION OF WHEY PROTEINS AND LACTOSE IN AGE GELATION OF ULTRA-HIGH TEMPERATURE STERILIZED MILK CONCENTRATE

Project Status: Active  USU Project Number: 211  USU Account Number: 547867

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# ANNUAL FINANCIAL REPORT BY PROJECT

**Western Dairy Research Center**

**Annual Report Ending:** June 30, 1991, Final  
**Project Term:** 07/01/89 - 06/30/91

**Dairy Center Director:** Dr. Paul A. Savello  
**Principal Investigator:** TORRES J ANTONIO

**Project Title:** ACID WHEY UTILIZATION: FUNCTIONAL PROPERTIES OF A FOOD GRADE STABILIZER PRODUCED BY LACTOBACILLUS PLANTARUM FROM ACID WHEY

**Project Status:** Active  
**USU Project Number:** 212  
**USU Account Number:** 547877

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 09/01/90 - 08/31/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: SAVELLO PAUL A

Project Title: MEMBRANE FRACTIONATION OF IMMUNOGLOBULINS FROM MILK AND WHEY

Project Status: Active
USU Project Number: 204
USU Account Number: 547898

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/90 - 06/30/92
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: HANSEN CONLY L
Project Title: DEVELOPMENT OF A PROCESS FOR PRODUCTION OF UF MILK RETENTATE POWDER
Project Status: Active
USU Project Number: 210
USU Account Number: 547899

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center

Project Term: 07/01/90 - 06/30/92
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: MCMAHON DONALD J
Project Title: CONTROLLING AGG GelATION OF UHT STERILIZED MILK CONCENTRATES

Project Status: Active
USU Project Number: 211
USU Account Number: 547900

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ANNUAL FINANCIAL REPORT BY PROJECT

Western Dairy Research Center

Project Term: 07/01/90 - 08/30/91
Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: RICHARDSON GARY H
Project Title: CAUSES AND PREVENTION OF STICKY TEXTURE IN MOZZARELLA CHEESE

Project Status: Active
USU Project Number: 181
USU Account Number: 547901

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Fringe: Total 2380, Allocated 2380, Spent 2794, Balance 0
Supplies: Total 3000, Allocated 3000, Spent 2381, Balance 619
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Travel: Total 0, Allocated 0, Spent 0, Balance 0
Publication: Total 0, Allocated 0, Spent 102, Balance -102
TOTALS: Total 19380, Allocated 19380, Spent 19615, Balance -235
**ANNUAL FINANCIAL REPORT BY PROJECT**

**Western Dairy Research Center**

**Annual Report Ending:** June 30, 1991, Final

**Project Term:** 08/07/90 - 06/30/92

**Dairy Center Director:** Dr. Paul A. Savello

**Principal Investigator:** SANDINE WILLIAM E

**Project Title:** GROWTH OF BIFIDOBACTERIA IN MILK: ASSOCIATION WITH STREPTOCOCCUS THERMOPHILUS AND LACTOBACILLUS SPECIES AS MEASURED BY GENETIC AND ENZYMATIC PROBES

**Project Status:** Active

**USU Project Number:** 198

**USU Account Number:** 547902

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ANNUAL FINANCIAL REPORT BY PROJECT
Western Dairy Research Center


Dairy Center Director: Dr. Paul A. Savello
Principal Investigator: PENNER MICHAEL

Project Title: UTILIZATION OF ACID WHEY AS A SUBSTRATE FOR THE PRODUCTION OF FOOD GRADE CELULASES

Project Status: Active
USU Project Number: 212
USU Account Number: 547903

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**ANNUAL FINANCIAL REPORT BY PROJECT**

**Western Dairy Research Center**

**Annual Report Ending:** June 30, 1991, Final  
**Project Term:** 09/01/90 - 06/30/92

**Dairy Center Director:** Dr. Paul A. Savello  
**Principal Investigator:** BROWN RODNEY J

**Project Title:** CHARACTERIZATION OF MILK PROTEOLYSIS BY LACTOCOCCAL STARTER CULTURE STRAINS USING AMINO ACID ANALYSIS

**Project Status:** Active  
**USU Project Number:** 243  
**USU Account Number:** 547910

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### ANNUAL FINANCIAL REPORT BY PROJECT

**Western Dairy Research Center**

**Summary Totals for All Projects**

Project Term: 07/01/87 - 06/30/90

Dairy Center Director: Dr. Paul A. Savello

**Project Title:** SUMMARY TOTALS FOR ALL WESTERN DAIRY CENTER PROJECTS

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MEMORANDUM

2 January, 1991

To: WDFRC Operational Advisory Committee Members

From: Janet C. Williams, Ph.D.
      Director of Research, NDB

Re: WDFRC Evaluation Document

As per the request of Dr. Paul Savello, Director of the Western Dairy Foods Research Center, I have attached a copy of selected pages from the Program evaluation that was conducted on behalf of the National Dairy Promotion and Research Board this past summer. The sections included are those from the scientific review team, as well as the NDB staff recommendations that were accepted by the Board of Directors of the National Dairy Board this past November.

Since the Board meeting, I have met with Drs. Savello and Brown to discuss the report, and to review all of the recommendations. Overall, the Board was positive about the outcome of the review and supportive of the Western Dairy Foods Research Center. We were pleased to be able to request a proposal for a continuation of program support for the Center, and look forward to reviewing the proposal when it is submitted this spring.

If you have questions regarding the report that is attached, please contact Dr. Savello to establish his reaction to your questions or comments. I would be happy to discuss any of the report with you once you have had the opportunity to discuss it with Dr. Savello.

We sincerely appreciate your interest in and support of the Western Dairy Foods Research Center, and look forward to many opportunities in the future to work with you as we direct product and process development research for the benefit of the entire dairy industry.

cc: Dr. Paul Savello
    Dr. Rodney Brown
    Dr. Robert G. Bursey, NDB
The Western Dairy Foods Research Center was evaluated on August 22 and 23, 1990. The team members included: Dr. David M. Barbano (Center Director, Northeast Dairy Foods Research Center), Dr. James Moran (Kraft, Inc.), Dr. Charles Morr (The Ohio State University), Mr. Harry Papageorge (National Dairy Promotion and Research Board), Dr. Bibek Ray (University of Wyoming), Mr. Harold Rice (National Dairy Promotion and Research Board), Dr. Ronald Richter (Texas A&M University), Dr. Charles White (Mississippi State University), and Dr. Carolyn Berdanier, Team Chair, (University of Georgia). Dr. Lowell Satterlee of The Pennsylvania State University was a guest participant/observer. The Center evaluation was organized and managed by Dr. Janet C. Williams (National Dairy Promotion and Research Board), assisted by Ms. Marykate Ginter (Dairy Research Foundation). Dr. Robert G. Bursey and Dr. Thomas Bongiorno (National Dairy Promotion and Research Board staff) were in attendance.

The Western Center was established in 1987 and has been in operation for three years. Faculty at Utah State University, Oregon State University and Brigham Young University comprise the research faculty involved in the conduct of the work. The Center research areas are as follows: Microbiology of Starter Cultures, Coagulation Properties of Milk, Ultrafiltration and Reverse Osmosis, and Product Quality. Research projects in these areas are conducted at all three institutions.

INSTITUTIONS OF THE CENTER

Utah State University

The "Lead" Institution of the Center is the Utah State University, located in Logan, Utah. As such, Utah State University holds the contract with the National Dairy Promotion and Research Board (NDPRB) for the conduct of the Western Dairy Foods Research Center, and assumes the overall administrative function for the Center. The services provided the Center by Utah State University include contracts and grants administration, financial accounting, provision for the Center Director, and overall coordination of the Center activities. Research grant administration including contracts, subcontracts, and financial accountabilities is handled through the Contracts and Grants Office of the University. Subcontracts between the Center and Oregon State University, and the Center and Brigham
Young University are coordinated between the Research Grants office of the two institutions negotiating the subcontracts.

Program strengths of the Utah State University faculty include coordination of basic and applied science, inclusion of nutrition science in dairy science research, and coordination of faculty within the Institution.

Oregon State University, ("OSU")

The second institution of the Western Dairy Foods Research Center, Oregon State University, is located in Corvallis, Oregon. The administration of OSU has not expressed concerns over the administrative load placed on the institution as they participate in the Center. The financial as well as program administration are handled effectively, and there are not apparent problems with subcontracting or funding research projects approved for administration at OSU.

The faculty participating in Center activities are largely in the Department of Food Science and Technology, although an important research component for the Center comes from the Department of Microbiology. Faculty have support from the University administration for the conduct of research through the Center, and have facilities for basic as well as applied research. There appears to be a blend of research interests and significant coordination among faculty at OSU. Some attention is being given to coordination with faculty at USU as well; however, given the geographical proximity of the institutions, this coordination is somewhat problematic. Support should be given to increased faculty coordination and cooperation with Center funds.

The OSU faculty appear to be very responsive to industry needs and build their program effectively with that input. The Center Co-Director has made the effort to include industry considerations in program direction and the effort appears to provide programs that meet some specific industry needs. Coordination with faculty in the microbiology department has significantly strengthened not only the Oregon State University Food Science Program, but also the Center Program overall.

Brigham Young University, ("BYU")

The third institution of the Western Dairy Foods Research Center is Brigham Young University, in Provo, Utah. Support from the BYU administration appears to be substantial, although the size of the program is extremely limited. Only one researcher from BYU has been involved in Center activities; and there does not appear to be the impetus to move other researchers into dairy-related research activities. The effort should be made by both Center personnel, and personnel at BYU to expand the level of
interest in Center activity among BYU faculty, and to commit to increasing program coordination with this institution.

**Administration**

Center administration exists on two levels - one through the university and the other through the Department of Nutrition and Food Sciences (where the Director is housed). The university administrative personnel appear to be supportive of the Center, and the Center activities. The university has committed to responsibility for the accounting activities of the Center, including contracting and subcontracting for research projects, and tracking finances for each of the projects funded. The subcontracting process could be streamlined for multi-year projects, and the administrators stated that they will look into the possibility and that an effort will be made to move in that direction. The financial tracking is somewhat arduous owing to multi-source funding within most of the projects funded through the Center. Accounting seems to be adequate and manageable, and there appears to be significant coordination between the Center personnel and the university accounting personnel. The contracting process for multi-year projects may be over­cumbersome, and needs careful study by the administration. If the process could be streamlined, it would be helpful to the investigators, and to the Director.

The Center has had several Directors. The first was Dr. C. Anthon Ernstrom. He was followed by Dr. Gary H. Richardson who in turn was followed by Dr. Jeffrey K. Kondo. A new Director is to be named in the next few months. The co-Director located at Oregon State University is Mr. Floyd Bodyfelt. This large turnover in Directors at the Western Center is unfortunate because it confounds the need for program stability and consistency. The job is time consuming and historically has taken time away from the primary teaching and research duties of the Director. Therefore, consideration needs to be given to the best means by which the Center can be administered and the Director can continue to progress in his research teaching program.

The position of Dairy Center Director should utilize no more than 0.2 to 0.3 FTE of the effort of the researcher. In order to protect the research/teaching/extension time of the Director, it is recommended that an administrative assistant be hired by the Dairy Center. This individual should aid in the preparation of reports and in the management of all aspects of Dairy Center activities. Once the day-to-day management of the Center is delegated to a qualified administrative assistant, the Director can work to streamline the process of project approval and funding where multi-year projects are proposed.
It is very important that an excellent working relationship be maintained between the Dairy Center program and the departments within which the Dairy Center projects are carried out. The Director should work with the chairman/head of each department to establish an appropriate amount of funds to be allocated within each project to cover departmental expenses (clerical, accounting, payroll) that are incurred as a result of Dairy Center projects. This will help foster a good working relationship between the departments and the Dairy Center program and will permit adequate attention to be paid to the administrative details incumbent in managing Center funded research activities.

The Dairy Center Director needs to ensure that producers and processors on the Operational Advisory Committee (OAC) have adequate input into program planning and project evaluation for the Center. The Director should consider methods to obtain more active participation of the OAC members in evaluation and recommendation of individual research projects. The Center may want to consider including non-university members of the OAC on the Technical Advisory Committee (TAC). The TAC should make recommendations on each project to the OAC and they should make recommendations to the Center Director on each project. If modifications of the NDPRB contract are necessary to do this, the NDPRB staff should work with the Center Director to make appropriate revisions.

**Technology Transfer**

The Center's efforts at Technology Transfer are accomplished via the following:

a. Publication in scientific journals.

b. Presentation at national scientific meetings (IFT, ADSA).

c. Hosting regional technical conferences.

1. Utah State Biennial Cheese Conference.

2. Utah State Annual Cheesemaking Shortcourse.


4. Extension efforts at Oregon State University.
These efforts are effective at reaching the academic community, large industry processors and investors in the program with R&D staff (Kraft, Land O' Lakes), and some small processors, primarily through Oregon State University efforts. The effectiveness of these efforts is limited in reaching the small processors in Utah/Idaho through cheese conferences. The efforts have not been effective in communicating with the producer organizations. Producer representatives were very clear that they did not know "what was going on" nor did they have anything that they could pass on to their members.

Industry Relevance

The Milk Coagulation/Cheese Technology area and Product Quality areas are very relevant to the industry because of the applied nature of the projects. The benefits to the industry are primarily in product, process, and manufacturing improvements. Quality improvements should lead to increased consumer acceptance of products. The process and manufacturing improvements can lead to product quality improvements and/or profit improvement for dairy processors. Projects on new products (Iron Fortified Cheese) and consumer acceptance of yogurt are being conducted and clearly demonstrate industry relevance.

The ultrafiltration/reverse osmosis section projects are more basic but are still application-type research. Projects are directed at a near-term potential, but with equipment and processes that are not currently widely used. The benefits of the ultrafiltration/reverse osmosis applications are clearly explained; but the new focus on UHT could be more clearly explained. The benefit of ultrafiltration technologies for yogurt to the U.S. dairy processor was not readily apparent. A possible benefit for the dairy producer for exporting products can be influenced by political factors and other market conditions. The use of ultrafiltration/reverse osmosis does however have tremendous potential and commercial impact.

The Microbiology of Starter Cultures section is the area of most basic research. The goals however are clearly stated and simple, with significant commercial benefit of eliminating or reducing losses due to phage infection. The studies on inhibitors and the production of usable products from dairy waste streams are very relevant. The benefits would be increased confidence of consumers in the safety and quality of dairy products, leading to increased sales.

A wide range of microbiological projects are being conducted, and proposed at the Western Center, largely at Oregon State University. While the specific objective area is microbiology of starter cultures, other projects which appear to be product or process specific (i.e., they address a specific concern in the
state or region) are being initiated. The OAC may wish to prioritize the various areas of food safety/microbiology research they wish to have conducted at the Western Center.

There is demonstrated capability to conduct good quality and industry relevant microbiological research at the Western Center. This is a clear strength of the Center, as there exist nationally known scientists with capabilities to transfer important information to the industry on the faculty of the Western Center. It would strengthen the program significantly if cooperative projects could be initiated which involve a food safety/microbiology component with other objective areas. Also cooperation in this research area among scientists at the three institutions could strengthen the Center program. Questions arose as to the facilities for the conduct of some of the microbiological work proposed, and the TAC, as well as the OAC need to be sensitive to this issue as they approve projects for Center funding. Further, the time commitment to long term microbiological studies needs to be established within the objective areas of the Center so that appropriate funds can be made available to meet the long term needs for this type of research.

Several innovative projects, with clear industry relevance, were discussed during the review. It appears that considerable industry input on a variety of levels has been gained- now the direction for the research needs to be established. The program is somewhat fragmented, and needs focus in order to make consistent progress for the benefit of the industry.

Communications

Communication of research results, as well as progress, is pivotal to the continued support of the Dairy Centers Program and of the individual Centers. The necessity to transfer this information, in a meaningful way, to appropriate audiences requires a well designed system/mechanism, support from the Institutions and the Center, and the inherent desire of the researchers to see their research results communicated for the benefit of the dairy industry. Incumbent in this system would be the means by which researchers would be provided adequate information to establish their obligation for information transfer and communication. There needs to be a more dedicated extension effort, targeted for dairy research, since the Center is now in a more productive plan.

Furthermore, communication about the program needs to be expanded to enhance the probability of including scientists from other disciplines in the research effort. During the progress of research studies, activity needs to be better communicated to investors in the program. This
communication can take place on a variety of levels - in formal presentations at local dairy industry meetings, on an informal basis with interested parties, and in publications that could be widely disseminated in the region.

Along the lines of improving communications to producers, processors, sponsors of the Dairy Center, other Dairy Foods Research Centers, and dairy scientists worldwide, it is felt that a newsletter which describes the research and other Center activities being pursued by the Center in lay terms would be a beneficial communications vehicle for the Center. The NDPRB staff should aid all Dairy Centers by identifying a mailing list of key producer groups nationwide that should receive the newsletter.

**Research Areas**

One of the strengths of the Western Dairy Foods Research Center is the collegiality of the faculty from the three universities that participate in the Center. This collegiality has had a very positive impact on research cooperation and productivity within the Center.

Historically, milk coagulation properties was well supported as a research area at the Western Center. A significant number of good quality studies were supported in the area of cheese making. Evaluation of cheese, development of manufacturing guidelines for cheese, and quality parameters in cheese have been funded. Further, some yogurt studies have been funded. There appears to have been a shift in research emphasis from curd formation as it relates to cheese. Many of the studies currently funded could be termed "curd formation" but they are grouped with microbiology of starter cultures, UF/UHT milk, and product quality.

**Curd Formation**

Previous research on milk coagulation at the Dairy Center has provided the basis for the development of devices for measuring milk coagulation during the manufacture of cheese. Anticipated research with USDA through ARS should establish the Western Dairy Center as the leading institute for research in the United States on milk coagulation.

**Ultrafiltration and Reverse Osmosis**

Present research is directed to the use of ultrafiltration and reverse osmosis systems for the development of new products and for improvement of existing products. Use of new membrane systems, including ceramics, offers promise for the fractionation of milk components, and the development of products not possible with current membrane technology. The use of ultrafiltration/RO
to concentrate milk prior to ultrapasteurization might provide a method to prevent age gelation in concentrated UHT. This should be accomplished by studying the role of the minerals, lactose, and proteolytic enzymes in age gelation.

The proposed Center for Excellence on UHT processing would enhance the image of the Western Dairy Center if successful. However, a Center for Aseptic Processing exists in North Carolina. Precautions should be taken to avoid duplication of efforts with this Center and to establish any possible avenues for program coordination.

The UHT processing project mainly considered the technological aspect of the process. Proper consideration should be given on the microbiological aspects, and could well become a coordinated program area within the Center if effort were put forth to manage it as such.

Control runs with known levels of high heat resistant spores should be included and the effect of temperature/time on the level of destruction of spores should be evaluated.

Product Quality

Very diversified projects exist within the objective area; all are responsive to needs expressed by the local dairy industry. One example is the project which developed a very useable assay for detecting heat-stable microbial proteinase in raw milk. The project was prompted by shelf-life problems at several dairy plants in the Northwest. The iron fortified cheese project appears to have excellent potential but the concern was expressed to evaluate the possibility of growth by Clostridium botulinum. Again, this type of microbiological study could provide the impetus for cooperative research within the Center institutions.

The project on multicomponent analysis has exciting potential and will give the dairy industry good support. Also, very practical problems are being addressed through projects such as that on batch cheese identification, and the projects on cheese cooling rates in manufacturing situations.

The OAC may wish to establish the priority rank for "curd formation," or "milk clotting," and focus the research in specific product categories, or establish the need to reclassify such projects under other objective areas.

In closing, the recommendations of the review committee will take an already strong Dairy Center and make it even stronger. The Western Dairy Foods Research Center and the NDPRB personnel and administration have learned, and will continue to learn how to efficiently operate the Dairy Center.
Recommendations

1. We commend individual faculty on their research efforts both in terms of its breadth and application to problems of importance to producers, processors and consumers. However, the direction of the Center in the long-term should reflect the needs and concerns of the producers, processors and consumers. Thus, the Center needs to use the OAC so that their input is solicited and used more effectively in meeting the established objectives of the Center.

2. We recommend that the procedure for the best scientific review of proposed projects be used. TAC should include industry representatives who are research oriented. Also producers should be represented. Likewise, we recommend that the OAC should review these projects prior to their meeting.

3. We recommend that a highly qualified administrative assistant, dedicated to the Center, be employed to assist the Director. This person would be responsible for the administration of the Center - to include the reporting process, oversee the budget process, schedule meetings, project reviews, etc.

4. We recommend that the results of the research be shared with producers, processors and other Dairy Centers via a newsletter written in lay language. Other activities and relevant items should be included. Include names of people to contact (i.e., administrative assistant).

5. We recommend that research results and Dairy Center activities be communicated to appropriate audiences through the cooperative extension service.

6. We recommend that a mechanism be put in place by the Director so that the project budget includes the costs associated with the conduct of the research (i.e., payroll, secretarial, etc).
WESTERN DAIRY FOODS RESEARCH CENTER
PROGRAM EVALUATION

Staff Report to Directors,
National Dairy Promotion and Research Board
November, 1990

The Western Dairy Foods Research Center, was established by contract with the National Dairy Promotion and Research Board in 1987, and is administered through personnel housed at the Utah State University. The Western Center is comprised of three institutions with faculty contributing to the research efforts of the Center. The institutions include Utah State University, Oregon State University, and Brigham Young University.

Administration: The institutional support for the Center appears to be considerable, at all three institutions. The largest burden for the administration is encumbered by the Utah State University, although there has been communication between Utah State University administrators and those from Oregon State University concerning the administrative load that the Center has placed on the institutions. Comments were generally positive about the progress that had been made in administering the Center, and the program management scheme that is in place. There are concerns within all institutions of the Center about the cumbersome process for contracting each of the projects approved for funding. That process does need some modification, and it is recommended that NDB staff work with the administrative personnel at all three institutions, as necessary to streamline the system.

The Center Director position has not been consistently filled at this Center, and is problematic. Some lack of direction is apparent probably in part because of the situation with the Director's position. However, it should be noted that significant, industry relevant research projects are being encouraged and funded through this Center. A new director has been named, although technically has yet to be approved by the Dean of the College of Agriculture. It is recommended that the NDB staff work closely with the new director to assure that direction for the program is more clearly defined within the next six months so that the last two years of the contract term have program clearly established.

Financial: A financial audit was conducted at the Utah State University, for the Western Center, on behalf of NDB in late 1988, as the Center Director position was being filled. A number of recommendations were made for more appropriate performance of contractual issues - those were reviewed with the university personnel, and corrective measures taken to assure that appropriate procedures would be in place in the future. A copy of the formal audit report is included in the full Center evaluation document. Additional, updated financial information is provided each year as part of the Center Annual Report.
Program: The research program objectives for the Western Center have shifted slightly over the first three years of the Program, and now include:

- Microbiology of Starter Cultures
- Curd Formation/Cheese Technology
- Ultrafiltration and Reverse Osmosis
- Product Quality
- Ultra-High Temperature Processing

Each of these areas has significant potential to deliver innovative research information to the dairy industry, if the research that is approved for funding within each is appropriately directed. The processes in place for research project approval need to be refined. The Technical Advisory Committee (TAC) is not adequately represented by dairy industry scientists who can provide insight into the scientific efforts in place within the industry. There are two committees established (TAC and OAC), by contract, to put into place an approved Program, and approved research projects for funding. The processes are not entirely effective for establishing programs that have clearly defined direction, clearly coordinated plans for the larger Program, or clearly defined and coordinated plans for disseminating the research information to the industry. Specifically, industry investors, and scientists from appropriate dairy industry organizations need to provide more input, and direction for the Program so as to enhance the effectiveness of the overall program. Recommended that NDB staff work with the new director to establish improved means for coordinating the committees, and to put into place the mechanisms whereby the effectiveness of those committees can be increased.

Communication: There is a significant need to transfer research technologies and information to the industry investors, and to the NDB in a manner in which the information can be made more useful. The investors in this Center have been particularly frustrated with the lack of information coming to them from the Center, and have felt that perhaps their dollars were not best utilized in this fashion. Over the past six months, significant strides have been made in improving this communication effort, to the benefit of both the Center, and the industry. Further efforts need to be made to communicate information from the Center, and to the Center from industry in order to enhance the research usefulness for the dairy industry in the Western region, and the United States. Recommended that NDB staff work with the Center personnel to establish clearly defined means by which the Center could provide more information, and more useful information to the industry about the research program; and to help put into place a mechanism to formalize the information transfer process for the benefit of the dairy industry.
WESTERN DAIRY FOODS RESEARCH CENTER

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

ANNUAL MEETING
Utah State University, Logan
August 10, 1991
WESTERN DAIRY FOODS RESEARCH CENTER

ANNUAL MEETING
Utah State University, Logan
August 10, 1991
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WESTERN DAIRY FOODS RESEARCH CENTER
1991 ANNUAL MEETING AGENDA
ECCLES CONFERENCE CENTER
UTAH STATE UNIVERSITY

Opening
8:30
Opening Remarks and Introductions
Paul A. Savello, Director
Floyd Bodyfelt, Liaison, Oregon State University

9:00–10:30
Oral Progress Reports

Curd Formation/Cheese Technology
Donald J. McMahon, Chair

Improved control of cheese manufacture through vat monitoring.
Gary Richardson and Michael LeFevre, Utah State University.

Cooling rate of Cheddar cheese: comparison between 40 and
640 lb blocks of uniform cooling of 640 lb blocks. Conly Hansen,
Utah State University; J. Antonio Torres, Floyd W. Bodyfelt, Jorge
Bouzas, and Connie Grazier, Oregon State University

with

Cheddar cheese blocks: effect of cheese composition and
cooling method. J. Antonio Torres, Oregon State University.

UHT Processing
Donald J. McMahon, Chair

Function of whey proteins and lactose in age gelation of UHT
sterilized milk concentrate. Donald McMahon, Mrudula
Kalpalathika, Venkatachalam Narayanaswamy, and Bashir
Yousif, Utah State University.

Controlling age gelation of UHT sterilized milk concentrates.
Donald McMahon, Utah State University.

Product Quality and Nutrition
Floyd W. Bodyfelt, Chair

Acid whey utilization: functional properties of a food grade
stabilizer produced by Lactobacillus plantarum from acid whey.
J. Antonio Torres, Mark Daeschel, Miriam Martino, Nilo Youssef-
Hakimi, and Carlos Kantt, Oregon State University.

Application of Fourier transform infrared technology to milk and dairy products. Rodney Brown and Ivan Mendenhall, Utah State University.

Estimation of individual milk proteins and genetic variants by multicomponent analysis of amino acid profiles. Rodney Brown and Carol Hollar, Utah State University.

10:30–11:00 BREAK. POSTERS ON DISPLAY DURING BREAK PERIOD.

11:00–12:00 Oral Progress Reports (continued)

**Product Quality and Nutrition (continued)**


Utilization of acid whey as a substrate for the production of food grade cellulases. Michael Penner and Soren Nordmark, Oregon State University.

Causes and prevention of sticky texture in Mozzarella cheese. Gary Richardson and Richard Merrill, Utah State University.


**Ultrafiltration/Reverse Osmosis**

Paul A. Savello, Chair


High yield, low moisture cheese from homogenized milk. Donald McMahon, Utah State University.

Development of a process for production of UF milk retentate powder. Conly Hansen, Donald McMahon and Yehia El-Samragy, Utah State University.

Membrane fractionation of immunoglobulins from milk and whey. Paul Savello and Reyad Mahmoud, Utah State University.
12:00–1:00  **CHICKEN BARBECUE LUNCH**  
ON GROUNDS IN FRONT OF CONFERENCE CENTER

1:00–2:00  Oral Progress Reports (continued)

**Microbiology of Starter Cultures**  
Jeffery Broadbent, Chair

Cloning the nisin and other genes of lactic streptococci into *Leuconostoc* species and amplification of nisin production. Jeffery Kondo, Jeffery Broadbent, Hua Wang, and Yat-Chen Chou, Utah State University; William Sandine, Herb Wyckoff, and Mary Barnes, Oregon State University.

Characterization of bacteriophage receptor sites of lactic streptococci. Bruce Geller and William Sandine, Oregon State University.

Production of omega-3 fatty acids by genetically altered fungi and lactic acid bacteria. Floyd Bodyfelt, Samuel Beattie, Daniel Selivonchick, and William Sandine, Oregon State University.

(Poster)  
Purification of a bacteriocin from *Pediococcus pentosaceus* and genetic transfer of the plasmid borne determinant. Mark Daeschel and Xintian Ming, Oregon State University.

(Poster)  
Prediction and determination of the efficacy of nisin in dairy foods. Mark Daeschel, Floyd Bodyfelt, and Dong-Sun Jung, Oregon State University.

Growth of Bifidobacteria in milk: Association with *Streptococcus thermophilus* and *Lactobacillus* species as measured by genetic and enzymatic probes. Joseph Booth, Janine Trempy, and William Sandine, Oregon State University.


2:00–2:30  **BREAK**

2:30–4:30  Operational Advisory Committee Business Meeting  
1.  New WDFRC projects for 1991–92  
2.  WDFRC Extension Proposal  
   • NDPRB Recommendations  
   • WDFRC OAC Responses and Actions
WESTERN DAIRY FOODS RESEARCH CENTER

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:

Janet C. Williams
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2111 Wilson Blvd., Suite 600
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WESTERN DAIRY FOODS RESEARCH CENTER

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(continued)

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WDFRC BUDGET ACTIVITY
1990-1991 FISCAL YEAR

INSTITUTIONAL SUPPORT (Utah State University,
Oregon State University, Brigham Young
University) $757,617

RESEARCH CONTRIBUTIONS:

NATIONAL DAIRY PROMOTION AND RESEARCH BOARD 400,000

REGIONAL/INDUSTRY SUPPORT:
- Utah Dairy Commission 50,000
- United Dairymen of Idaho 50,000
- Oregon Dairy Products Commission 40,000
- Western Dairy Farmers' Promotion Association 10,000
- Kraft General Foods, Inc. 5,000
- Schreiber Foods, Inc. 5,000
- Marschal-Rhone Poulenc, Inc. 5,000
- Borden, Inc. 5,000
- Western Dairymen Cooperative, Inc. 2,500
- USDA-ARS 280,000
- State of Utah Center of Excellence “Center for Dairy Foods Technology” 20,000

TOTAL REGIONAL/INDUSTRY SUPPORT 472,500

FY91 TOTAL DAIRY RESEARCH CONTRIBUTIONS $872,500

FY90 BALANCE FORWARD $77,278

TOTAL AVAILABLE FUNDS FOR FY91 RESEARCH $949,778

FY91 COMMITTED RESEARCH FUNDS
- Western Dairy Foods Research Center (517,740)
- USDA-ARS (280,000)
- State of Utah Center of Excellence (20,000)
- Administrative (50,000)

TOTAL FY91 COMMITTED RESEARCH FUNDS $867,740

FY92 BALANCE FORWARD $82,038
## SUMMARY OF TOTAL BUDGET FOR ALL YEARS
(AVAILABLE FUNDS FOR ALLOCATION AND COMMITMENTS)

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## Western Dairy Center Listing of Funded Projects By Account Number

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### Project Funding Allocated Thru FY 1992

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**LEGEND:**

- Increase in project funding approved for FYs
- Decrease in project funding approved for FYs
- Extension (no cost) approved
- Project revision approved
Project Title: Improved Control of Cheese Manufacture Through Vat Monitoring

Personnel: G. H. Richardson, Professor, Dept. of Nutrition and Food Sciences, Utah State University

Don McMahan, Professor, Dept. of Nutrition and Food Sciences, Utah State University

Michael J. LeFevre, Research Assistant, Dept. of Nutrition and Food Sciences, Utah State University.

Funding: Western Dairy Foods Research Center and Utah Agricultural Experimental Station.

Objectives:

1. Determine the ability of the hot-wire system to detect differences in coagulation time and curd strength. Compare these measurements with other coagulation instruments. These data would aid in preventing high moisture cheese due to late cutting or product losses due to early cutting of the curd.

2. Determine abilities of chymosin, calcium salts, and lactic cultures in milk for Cheddar cheese to overcome the inability of milk from cows in late lactation to coagulate.

3. Use the same system to monitor the coagulation of milk for cottage cheese manufacture. Establish software that would be most helpful for the cheese industry.

Results:

ADSA abstract 1991

Use of hot-wire viscometric measurements to predict physical curd firmness of renneted milk as a function of milk composition. M.J. LeFevre*, G.H. Richardson and D.J. McMahon, Utah State University, Logan

Curd firmness was predicted by viscometric measurements using the hot-wire method for renneted milk of various composition. A 2x5 factorial design was set up using 2 levels each of protein, fat, Ca, pH and chymosin. The protein content of skim milk was adjusted by ultra-filtration to 3.25% and 2.75% and the pH was adjusted to 6.54 and 6.28 using lactic acid. Fat and Ca were adjusted to a zero level (no addition) and to 3.25% and .01% respectively by the addition
of cream and CaCl2. Rennet coagulation tests (.03 RU/ml and .015 RU/ml) were run simultaneously using the hot probe instrument, with pH and temperature sensors, and a Formagraph. The analysis of variance indicated that all 5 factors caused a significant (p<.05) effect on the time required to reach the K20 value (approximate cut-time for renneted milks) on the Formagraph. Significant interactions included chymosin x pH, chymosin x protein, and fat x protein (p<.05). Coagulation time, maximum first derivative value and selected area values of the first derivative curve of the hot probe data were used along with milk composition variables to predict the Formagraph K20 value using step-wise regressions. Linear correlation coefficients (R^2) ranging from .90 to .96 were obtained in predicting the time from chymosin addition to the K20 point.

Other tests have also been completed that demonstrate the effect of changing milk temperature on the hot-wire curve. Poor coagulating milk has been evaluated by the system and tests are presently underway using late lactation milk.

Impact of Research:

The data generated from such an instrument can be useful to provide improved control to every cheese vat. A curd cut time, based on coagulation, pH and temperature could decrease losses and improve cheese yield and quality. The ability to measure the length of heat time and the rates of change of pH and temperature would also benefit the cheese manufacturer. Software programs could be included that would provide more management guidance. Significant savings to the industry could result when enzyme coagulant and other additive costs can be reduced by fine tuning the process through continuous monitoring of the milk in the cheese vat.

Publications

Project Title: Cooling rate of Cheddar cheese: comparison between 40 and 640 lb blocks of uniform cooling of 640 lb blocks

Personnel: J. Antonio Torres, Principal Investigator, Dept. Food Sci. & Technology, Oregon State University

Floyd W. Bodyfelt, Principal Investigator, Dept. Food Sci. & Technology, Oregon State University

Conly L. Hansen, Principal Investigator, Dept. Nutrition & Food Sciences, Utah State University

Jorge Bouzas, Ph.D. Graduate Student, Dept. Food Sci. & Technol., OSU

Connie Grazier, M.S. Graduate Student, Dept. Food Sci. & Technol., OSU

Funding: Western Dairy Foods Research Center, Tillamook County Creamery Assn. (Tillamook, OR)

Objectives:

In spite of major research and process improvement efforts, there is still a wide variation in the sensory properties of the most popular cheese variety consumed, Cheddar cheese. Cooling of the freshly formed cheese is believed to be a processing step requiring closer control to achieve uniform and consistent flavor quality. The effect of time and temperature on Cheddar flavor quality was investigated on the basis of the following objectives:

1. To develop a computer program that calculates cooling rate of 40 lb and 640 lb blocks as a function of cooling conditions and Cheddar cheese composition.

2. To demonstrate that a more consistent quality can be achieved by controlling the cooling rate of 40 and 640 lb Cheddar cheese blocks.

3. To evaluate sensory properties and chemical composition of a commercial mild Cheddar cheese.

4. To identify temperature conditions leading to sensory characteristics similar to commercial Cheddar cheese and confirmed by chemical and microbial analysis.

5. To develop a mathematical model combining heat transfer calculations and kinetic expressions for the effect of time and temperature on selected sensory and chemical indexes.
6. To use the model developed in (5) to determine the cooling rate and aging room temperature conditions that result in the quality identified in (4) in a uniform manner for the entire cheese block.

7. To quantify the effect of heterogenous temperature distributions and microbial activity on the chemical and sensory characteristics of 640 lb cheese blocks.

Results:

The experimental procedures used in this research were developed primarily to accommodate the wide range of temperature effects to be covered (5-35°C) and the large experimental error associated with sensory analysis. Samples were obtained from the production of a commercial cheese manufacturer (Tillamook County Creamery Assn., Tillamook, OR) directly after the pressing operation. Forty pound blocks from the same vat lot were cut into 2.5 cm x 5 cm x 5 cm pieces. Each piece was vacuum shrink-wrapped in commercial O₂-barrier cheese film. Samples were small enough to reach storage temperature within 1-2 hours. Samples were randomly assigned to the five storage temperatures: 12, 15, 20, 25, and 35°C and tested at different stages during a three month period. Four batches with one replicate were used for sensory, microbial and chemical analysis tests. The following were the most significant findings:

1. Microbial, chemical and sensory analysis of samples stored at constant temperature for a three month period confirmed the importance of early temperature control to avoid flavor quality problems.

2. A kinetic analysis of microbial growth data suggests that microbial activities are controlled by diffusion phenomena involving the movement of nutrients and/or the diffusion of metabolic waste from the bacterial cell. This finding needs to be analyzed within the context of the temperature values observed during cooling and aging. Cheese blocks cooled at a slow rate have a high temperature during the first days of the ripening process and cause a rapid die-off of the starter culture. Faster cooling slows down the activity of the starter culture and this allows the starter culture to remain active for a longer period of time without reaching inhibitory conditions in the microenvironment surrounding the cell. A more extensive lactose utilization by the starter culture would lead to a reduction of the flavor quality problems associated with the growth of adventitious microorganisms.

3. Simulations using a computer model combining heat transfer calculations with kinetic equations for sensory and chemical changes during early ripening identified a large number of cooling (time and air temperature) and aging (air temperature) conditions leading to similar values for the chemical and sensory indexes used as flavor quality indicators. This observation implies that the optimum operating conditions for the cooling and aging process covers a range of temperatures and is not constrained to a single operating point.
Impact of research:

This project examined the sensory, chemical and microbial changes involved in the process to convert the rubbery, relatively flavorless matrix of fresh pressed curd into Cheddar cheese with distinct aroma, taste, body, and texture. For Cheddar cheese a period of at least 5 to 10 months refrigerated storage is required, during which operating costs and interests on capital involved in cheese aging significantly add to the cost of production. Our results have been used to develop models of the aging process to select cooling and aging conditions leading to improved and consistent flavor quality. Conversations with personnel at Tillamook County Creamery Assn. who have installed new equipment for the rapid cooling of cheese have confirmed predictions made on the basis of our experimental results.

Publications:


Bouzas, J., Kant, C.A., Bodyfelt, F.W., and Torres, J.A. Characterization and interpretation of time-temperature effects on chemical changes occurring during Cheddar cheese aging. Int. Dairy J. (IN REVIEW)


Grazier, C.L., Simpson, R., Roncagliolo, S., Bodyfelt, F.W., and Torres, J.A. Temperature effects on non-starter bacteria populations during cooling and aging of Cheddar cheese blocks. Int. Dairy J.
Function of whey proteins and lactose in age gelation of ultra-high temperature sterilized milk concentrate

Annual Report Date: 30 June 91  Project Term: 1 Jul 89 — 30 Jun 92

Personnel
Principal Investigator: Dr. D.J. McMahon
Research Associate: Dr. Mrudula Kalpalathika
Graduate Student: Mr. Venkatachalam Narayanaswamy
Graduate Student: Mr. Bashir Yousif

Funding Sources: Western Dairy Foods Research Center
USDA Agricultural Research Service

Objectives:
The mechanism by which age gelation in UHT sterilized milk concentrates occurs is still unknown. There have been many factors implicated and at best an empirical approach is taken to extend shelf life of sterilized milk products. UHT sterilization promotes association between κ-casein and β-lactoglobulin. In milk concentrates the concentration of whey proteins and lactose are increased. Their role in the age gelation process will be studied in this project. The specific objectives of this project are to:

1. Determine the fate of β-lactoglobulin during storage of UHT sterilized milk concentrates.

2. Determine the influence of lactose concentration of milk concentrates on age gelation.

3. Monitor changes in casein micelle structure during storage of UHT sterilized milk concentrates.

Results:
Objective 1: β-lactoglobulin.
The use of 14C-labelled β-lactoglobulin in UHT milk experiments has required that a laboratory scale UHT system be developed so that contaminated equipment can be properly handled. A laboratory UHT processor (built using 9 mm SS tubing) designed to duplicate the Alfa-Laval system with pre-heat treatment to 72°C over 60 s followed by indirect heating to 140°C over 90 s. Our commercial pilot-scale Alfa-Laval UHT system cannot be used for this work as its contamination would render it unusable for other work. Preliminary trials designed to provide equivalent heating profiles produced UHT milk that sedimented in the first month of storage. The system will need to be redesigned before 14C-labelled β-lactoglobulin can be used.

Objective 2: Lactose
Skim was concentrated 3X by ultrafiltration and extensively diafiltered with simulated milk ultrafiltrate to remove lactose. Lactose and sucrose were then added at levels of 3 and 6% and each of the concentrates UHT processed to 140°C for 4 s and stored at 4, 20 and 35°C. Shelf stability was monitored by measuring viscosity, extent of browning was measured using a color meter and proteolysis by electrophoresis. All samples at 4 and 20°C gelled but those at 35°C did not. No browning was observed in the samples containing <.1% sugars or the samples containing 3 and 6% sucrose. We concluded that sugar-
protein interactions through Maillard browning reactions of lactose with proteins is not a significant factor in causing age gelation. The streaking observed in electrophoresis of samples stored at 35°C were also not related to sugar content and therefore must relate to protein-protein interactions that occur at temperatures above 20°C.

Objective 3: Casein Micelle Structure
The microstructure of UHT milk concentrates was investigated using electron microscopy in conjunction with Dr. Miloslav Kalab, Food Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. It was observed that when 3X skim milk concentrate is heated to 140°C for 4 s, about 60% of the whey proteins are denatured and the casein micelles undergo a large increase in size. This size increase is due to complexing of ß-casein with denatured ß-lactoglobulin followed by further aggregation of denatured ß-lactoglobulin onto the micelle surface.

When such UHT samples are stored and eventually age gel, it was observed that many of the micelles were connected by thin threads of material. The microstructure of such gels was completely different to rennet milk gels. When rennet is added to non-UHT milk the casein micelles collide and form a gel structure in which the micelles are in close contact. However, if UHT milk concentrates (such as 3X UF concentrates) are renneted the gels have less integrity and there is not the same level of intimate contact between micelles. It appears that the layers of ß-lactoglobulin on the micelle surface interfere with aggregation.

Impact of Research:
The overwhelming success of dairy production in the United States has created a situation that calls for more attention to milk utilization and marketing. Development of new dairy products has now taken precedence over production of more milk.

International markets for U.S. dairy products could be developed if attention was directed to manufacture of stable products from our surplus dairy production. The competitive position of the U.S. would be enhanced by new and better quality products and our surpluses of dairy commodities would be reduced.

The major limitation to production of such a product is irreversible gelation after storage at higher-than-refrigerator temperatures over a long period of time. The gelling phenomena must be understood and means devised to prevent gel formation before rehydratable milk concentrates can be sold abroad.

Publications and Completed Theses:


Controlling age gelation of UHT sterilized milk concentrates

Annual Report Date: 30 June 91  Project Term: 1 Jul 90 — 30 Jun 92

Personnel
Principal Investigator: Dr. D.J. McMahon
Graduate Student:

Funding Sources: Western Dairy Foods Research Center
USDA Agricultural Research Service

Objectives:
Age gelation of ultra-high temperature (UHT) milk concentrates has hindered the commercial use of milk concentration as a means of lowering transport costs. There has been some work conducted on developing stable UHT milk concentrates but at best, an empirical approach is taken to extend shelf life. Composition of milk, severity of heat treatment, sequence of operation, homogenization, use of additives such as polyphosphates and sucrose, total solids, and enzyme treatment have all been shown to affect age gelation (and hence shelf-life) of UHT milk concentrates.

When milk is concentrated, its rheological and heat transfer characteristics are altered. Heat sterilization also affects these properties. To optimize the UHT processing of milk concentrates it is necessary to know how milk behaves throughout the UHT temperature profile. The effect of compositional changes and process parameters on age gelation will be studied in this project. By knowing how these parameters affect milk, it will then be possible to systematically develop a process for UHT sterilization of milk concentrates that can be successfully stored at ambient temperatures.

The specific objective of this project is to solve the age gelation problem by:

1. Determining effects of the process parameters of UHT heating on age gelation of milk concentrates.

2. Determining influence of compositional changes of milk concentrates on age gelation after UHT processing.

3. Using the data collected above to determine optimum conditions for UHT processing of milk concentrates.

Results:
It was anticipated that this work would commence in 1990 but because of research commitments on our UHT equipment it was not possible to start another graduate student on this project. Now that some of the other UHT projects in our department are coming to conclusion there is now time available.
Impact of Research:
The overwhelming success of dairy production in the United States has created a situation that calls for more attention to milk utilization and marketing. Development of new dairy products has taken precedence over production of more milk. At the same time, we are becoming increasingly aware that a global approach must be taken in the marketing of our dairy products. Two marketing strategies that are of importance are EXPORT and MILITARY. In both cases, transportation costs are of very high. By reducing the bulk of a product, such as by concentrating milk, transportation costs can be reduced and furthermore, for many military applications it would also alleviate space storage limitations of supplying "fresh" milk to military personnel.

International markets for U.S. dairy products could be developed if attention could be directed to manufacture of stable products from our surplus dairy production. The competitive position of the U.S. would be enhanced by new and better quality products and our surpluses of dairy commodities would be reduced. Production of dairy products other than powdered milk, butter and cheese with long shelf lives should be a major priority.

Specifically, for this project, a way of producing rehydratable milk concentrates that can be stored at ambient temperatures is to be developed. This is needed to make U.S. dairy products more widely available on the world market. The major limitation to production of such a product is the irreversible gelation of UHT sterile milk concentrates that occurs after exposure to higher-than-refrigerator temperatures over a long period of time. The gelling phenomena must be understood and means devised to prevent gel formation before rehydratable milk concentrates made from surplus U.S. milk can be sold abroad.

An additional benefit of this project is that it would provide information on extended shelf life of dairy products. This would be useful to dairy processors who are looking at entering the expanding food service business, and in which extended shelf life is of importance.

Publications and Completed Theses:
None
Project Title: Acid-whey utilization: Functional properties of a food grade stabilizer produced by *Lactobacillus plantarum* from acid whey

Personnel:

J. Antonio Torres, Principal Investigator, Dept. Food Science & Technology, Oregon State University

Mark A. Daeschel, Principal Investigator, Dept. Food Science & Technology, Oregon State University

Miriam Martino, Post-doctoral Research Associate, Dept. of Food Science and Technology, Oregon State University

Nilo Youssef-Hakimi, M.S. Graduate Student, Dept. Food Science & Technology, Oregon State University

Carlos A. Kantt, M.S. Graduate Student, Dept. Food Science & Technology, Oregon State University

Funding Sources: Western Dairy Foods Research Center
Centro de Investigaciones en Criotecología de Alimentos (CIDCA), La Plata, Argentina

Objectives:

A major problem faced by today's dairy industry is to find profitable uses for whey. Annually, approximately 1.2 millions tons of lactose and 200,000 tons of milk protein are converted into whey worldwide. However, less than 60% of whey is utilized for human food and animal feed, the remainder is discharged as waste with a continuously increasing disposal cost. In spite of progress made in utilizing whey, whey solids, and whey protein concentrates in the manufacture of dairy, bakery, and specialized products, whey disposal remains a serious industrial problem. The goal of this research were to explore additional commercially viable approaches for the utilization of whey, particularly to characterize the rheological and surface properties of a polysaccharide obtained by direct fermentation of acid whey. The specific objectives were:

1. To establish the fermentation parameters for the production of the polysaccharide produced by *Lactobacillus plantarum* 304 utilizing acid or sweet whey as the substrate.
2. To establish the procedures to recover and purify the polysaccharide.
3. To analyze the fermentation broth by HPLC analysis and establish lactose utilization and yield parameters.
4. To determine the surface tension properties of the unknown polysaccharide as affected by polysaccharide concentration, pH, temperature and ionic strength.
Results:

a. Fermentation Studies

*Lactobacillus plantarum* 304, originally isolated from a cucumber fermentation, was routinely maintained and transferred in MRS broth medium. In all experiments, cultures of *L. plantarum* 304 were incubated without aeration. Acid whey was obtained from a local dairy plant and stored frozen until used.

Spontaneously occurring non-polysaccharide producing variants of *L. plantarum* can be isolated at a frequency of 6.6x10^-3. A method was developed to optimize differentiation of the colonies of the polysaccharide producing (poly+) and non-producing (poly-) strain using MRS agar followed by incubation at 25°C for 5-7 days. Polysaccharide producing strain showed larger, opaque and viscous colonies. Reversion to the poly+ phenotype was not observed.

Growth rate of both strains was measured using optical density (650 max wavelength) at 20, 25, 30, 35 and 40°C. The results showed that growth rates were not significantly different between the two strains. Optimum temperature for the growth was 35-36°C and the observed minimum growth temperature was 15°C.

A modified MRS medium was prepared by using all the components of the commercial medium with the exception of the glucose. Different sugars were added to the medium as indicated by specific experiments. MRS media were heat sterilized and acid whey was filter-sterilized. Different sugars were examined as a sole carbon source for growth of the bacteria including glucose, fructose, galactose, lactose and sucrose at 2% concentration. Both strains showed growth utilizing these sugars; however, no growth was observed when ribose was the sole carbon source. Addition of calcium or magnesium to the media (at 2%) retarded the growth to some degree.

b. Functional Properties Studies

Poly- cultures were used as negative controls along with uninoculated broths in viscosity measurements using Cannon-Fenske viscometers. Maximum viscosity was observed at about 24 hours of growth with a subsequent decrease with extended incubation. Polysaccharide was produced in modified MRS broth supplemented with either glucose, fructose, mannose, galactose, lactose, sucrose, cellobiose, maltose, trehalose, or raffinose as the sole sugar source. Ribose and arabinose produced no polysaccharide. Acid whey supported growth as well as polysaccharide production. The best yield of polysaccharide from acid whey occurred when the pH (4.3) was adjusted to pH 6 or 7.

A polysaccharide isolation technique with a yield of about 3 g/l from MRS medium was also developed. The yield of polysaccharide produced at different incubation temperatures (18, 25, and 37°C) did not differ significantly. Samples were collected at the same broth pH value to obtain polysaccharide after equivalent fermentation levels.

The viscosity of the recovered polysaccharide solution was determined using a Brookfield Viscometer (spindle series LV). Aqueous solutions of the isolated polysaccharide, 1 - 5% w/v, exhibited low viscosity (less than 10 cp). Viscosity increased slightly with polymer concentration. Attempts to stabilize the viscous behavior observed in the fermentation broth, e.g. by addition of divalent salts (Ca++), were only partially successful. All tested solutions exhibited a viscosity decrease with higher shear rates, typical of solutions with pseudoplastic behavior.
The dynamic initial surface tension \( \gamma_0 \) of polysaccharide solutions (0.1-2.5%) were determined with the drop weight method at 25 and 6°C. \( \gamma_0 \) was calculated as follows:

\[
\gamma_0 = \frac{M_o \cdot g}{2 \cdot r \cdot f}
\]

where \( M_o \) is the drop weight extrapolated at infinite drop formation time \( t \), \( r \) is the radius of the tip, \( g \) is the gravitational acceleration constant and \( f \) is a correction factor. The extrapolation to infinite formation time to determine \( M_o \) was accomplished using an empiric correlation:

\[
M(t) = M_o + St^{-0.75}
\]

where \( M(t) \) is the drop weight at drop formation time \( t \) and \( S \) is a constant. The tip radius was calculated using an iteration procedure based on drop weight data for distilled water and correction factor \( f \) which were found in the literature tabulated as a function of tip radius.

The initial surface tension of 0.1 to 2.5% polysaccharide solutions were 2-20% lower than the value for pure water. The largest reduction was observed for a 1% solution adjusted to pH 4.6. Oil/water mixtures samples prepared with 1% w/v purified polysaccharide in water remained emulsified longer than samples with no polysaccharide. Microscopic examination showed that the polysaccharide facilitates the formation of emulsions with smaller droplets. This would be consistent with the initial surface tension measurements.

**Impact of Research:**

A major problem faced by today’s dairy industry is to find profitable uses for whey. In spite of new uses, whey utilization remains around 50%. The rest is discharged with a continuously increasing disposal cost. The production of polysaccharides with functional properties of commercial interest uses a waste to generate a by-product with commercial value.

**Publications:**


Project Title: Rapid Assay for Heat-Resistant Microbial Proteases in Raw Milk by a Simple Casein Denaturation Method

Personnel:
- Floyd W. Bodyfelt, Dept. of Food Science & Technology, Oregon State University
- Sergio C. Feijoo, Dept. of Food Science & Technology, Oregon State University
- Carlos Gonzalez, Dept. of Food Science & Technology, Oregon State University

Funding: Western Dairy Foods Research Center and Oregon Dairy Products Commission

Objectives:

Heat-resistant sporeforming psychrotrophic bacteria produce proteinases in raw or pasteurized milk that are responsible for causing marked deterioration of milk quality, e.g., bitter taste, unclean off-flavor and/or protein coagulation (sweet curdle). The typical microbiological procedures that are used for isolating, enumerating and confirming the presence of thermoinstable psychrotrophs (or their associated proteinases) are laborious, time consuming and do not provide directly information about potential proteolytic activity. Hence, the development and possible adoption of a simple, rapid and sensitive assay for relatively low concentrations of proteinases in raw milk supplies or pasteurized milk products could provide processors with an early awareness or warnings about possible quality problems.

1. Develop a diffusion casein-agar test capable of quantitating the proteolytic activity exhibited by heat-resistant sporeforming bacteria (Bacillus spp.) in either raw or pasteurized milk samples that have been subjected to a standardized heat treatment.

2. Determine the optimal conditions for: (1) the initial heat treatment (standardized) of milk samples, (2) preliminary incubation conditions, and (3) other necessary assay parameters.

3. Determine the most appropriate casein fraction and the optimal buffering and suspension systems for the substrate matrix for conduct of the proteinase assay.

4. Develop an alternative method for determining populations of Bacillus spp. in selected raw milk samples by a combination of preliminary incubation and a dye reduction test.
Results:

A sensitive diffusion casein-agar method was developed to detect the proteolytic activity exhibited by heat resistant sporeforming bacteria (Bacillus spp.) in raw milk. Initial experiments demonstrated β-casein to be the preferred substrate for assessing proteinase activity of Bacillus spp. Optimum test parameters for proteinase assays were determined; initial heat-treatment of milk samples at 75°C for 20 min.; pH optima (7.25) of the casein-agar matrix; the sample load (15μl); an indication of proteinase activity within 33-36 hrs.; and use of a plate-well method for assessing enzyme dispersion through the agar suspended substrate. To optimize sporulation of potential Bacillus spp. that may have survived the initial heat treatment of raw samples, 1 ml aliquots of each heat-treated milk sample were inoculated into a defined media (nutrient broth with 5 added minerals and 0.05% NDM), followed by 18 hr. incubation at 21°C. Next, 15 μl aliquots were added to formed wells (4.0 mm dia.) in Petri dishes (90.0 mm) that contained 15 ml of β-casein-agar matrix and were incubated for 18 hrs at 30°C. The extent of proteinase activity was proportional to the relative zone size of casein precipitation, which closely corresponded with the magnitude of milk sample spore and psychrotrophic spore counts.

Within the course of evaluating the best possible method(s) for assay for bacilli proteinases, experiments and trials were undertaken of a vertical, micro-hematocrit-tube and several dye reduction tests. However, lack of sensitivity, clear-cut endpoints and cumbersome laboratory handling characteristics appeared to preclude either of these methodologies. The plate diffusion assay with appropriate preliminary incubation for proteinase detection appeared to be the most promising technique for assessment of enzyme activity.

Impact of Research:

Five major milk processors in the Pacific Northwest incurred and reported serious and widespread consumer complaints related to "sweet curdle" defects in fluid milk and cream products in 1989-1991. Also, numerous school milk quality problems stem from "early" curdling and objectionable off-flavors due to proteinase activity of Bacillus spp. derived from raw milk supplies. Cottage cheese, and conceivably cheddar cheese, yields and product quality can be adversely affected when Bacillus spp. and their associated proteinases develop in cheese milk. The current test method for heat-resistant sporeforming psychrotrophs in raw milk sources requires at least 10 or 12 days to complete. A more rapid, method for determining objectional levels of Bacillus spp. in raw milk supplies would be a most advantageous analytical tool for inclusion in processor quality incentive (bonus) payment programs. Producer associations and processors across the U.S. have requested information about criteria for thermoduric microflora in raw milk for possible incorporation in milk quality incentive programs. Conservatively, assuming that approximately one fourth of all milk producers incur higher than desirable Bacillus spp. counts, there may be no better way to focus on this ubiquitous milk quality problem than development and adoption of a rapid, routine test
method for screening milk samples for this troublesome microflora. Such a reliable and economically feasible analytical tool could be an invaluable step forward for enhancing milk quality at the farm level. An effective tool for rapid and accurate detection of *Bacillus* spp. could serve as a keystone test within milk quality incentive programs for the U.S. dairy industry.

**Publications:**


Project Title: Application of Fourier Transform Infrared Technology to Infrared Technology to Milk and Dairy Products

Personnel: Rodney J. Brown, Dept. of Nutrition and Food Sciences, Utah State University

Ivan V. Mendenhall, Dept. of Nutrition and Food Sciences, Utah State University

Funding: Western Dairy Foods Research Center

Objectives:

1. Find a set of wavelengths in the infrared spectrum that respond to changes in fat, protein, and lactose concentrations.
2. Find a set of wavelengths in the infrared spectrum that do not respond to changes in saturation level, chain length, and level of free fatty acids.
3. Combine (1) and (2) to make a robust set of wavelengths common to all constraints.
4. Determine the individual spectra and common wavelengths to milk fat, protein and lactose.
5. Find a set of wavelengths common to the milk components (4) and to the robust set (3).
6. Statistically calibrate for testing samples of unknown composition using only this set of wavelengths (9) and milk samples chemically tested for fat, protein, lactose, and moisture. (Less than 1600 cm⁻¹ wavenumber should be used if possible.)
7. Calibrate, using wavenumbers greater than 2700 cm⁻¹, to determine saturation level of fat in dairy products (especially cheese).

Results:

To identify wavelengths which respond to changes in fat concentration, we prepared a series of milk samples where the fat level varied and all other components were held constant. The correlation coefficient for absorbance and fat concentration was calculated at each wavelength. A similar experiment was performed to determine wavelengths that linearly respond to changes in protein concentration. To determine wavelengths that linearly respond to changes in lactose concentration, a series of lactose solutions were prepared where the lactose concentration varied from 1 to 5.5%. We then selected wavelengths which were responsive to fat, protein, and lactose concentrations, and relatively unaffected by saturation, chain-length, and lipolysis. Nine calibration standards (each composed of milk from a separate herd) were purchased. The FTIR was calibrated using these standards and Partial Least Squares (PLS) statistics. The concentration of fat, protein, and lactose in these samples was then predicted using the
generated calibration equations. The standard deviations of difference between
chemical and predicted values were below the AOAC recommended SD of .06%. This
data was obtained with no homogenization or temperature control. A liquid ATR cell
was used and 64 scans at 4 cm⁻¹ resolution were averaged to obtain each sample
spectrum. Addition of whey powder to non-fat dry milk and non-dairy fat to cheese were
quickly and easily detected using FTIR.

Impact of Research:

This project was requested by one of the parties (Utah Dairy Commission) contributing
to the funding of the Western Dairy Foods Research Center. They wanted a fast
method for detecting non-dairy products, particularly fat, in products labeled or sold as
dairy products. Their justication is that adulterated products now being sold as dairy
products will be replaced by real dairy products if it is widely known that such a test
exists. In accomplishing this end. we also provided an improved method for measuring
fat, protein, lactose and water content in all dairy products. This should assist the dairy
industry as use of milk components in all foods becomes a larger share of total milk
utilization.

Publication:

measurement of fat, protein, and lactose in milk. 85th American Dairy Science

Richardson, G. H., R. J. Brown, T.C J. Yuan and I.V. Mendenhall, 1990, Combining
FTIR, conductance and reflective colorimetry for simultaneous estimation of milk
components, abnormal milk, microbial and antibiotic contents in raw milk. XXIII
International Dairy Congress. Montreal, Canada.

Mendenhall, I. V. and R. J. Brown, 1991, Spectroscopic detection of whey protein
powder in non-fat dry milk. Amer. Chem. Soc. Annual Meeting, Atlanta, GA.

Mendenhall, I. V. and R. J. Brown, 1991, Fourier transform infrared determination of

Mendenhall, I. V., and R. J. Brown, 1991, Spectroscopic determination of whey powder
74:Supp. 1, 93.

Mendenhall, I. V. and R. J. Brown, 1991, Spectroscopic determination of a non-dairy fat
in process cheese. In Preparation.

Mendenhall, I. V. and R. J. Brown, 1991, Robust infrared wavelengths for the
determination of fat, protein, and lactose in milk. In Preparation.
Project Title: Estimation of Individual Milk Proteins and Genetic Variants by Multicomponent Analysis of Amino Acid Profiles

Personnel: Rodney J. Brown, Dept. of Nutrition and Food Sciences, Utah State University

Carol H Hollar, Dept. of Nutrition and Food Sciences, Utah State University

Funding: Western Dairy Foods Research Center

Objectives:

1. Determine concentrations of groups of proteins in milk such as caseins or whey protein using amino acid analysis.

2. Determine concentrations of specific milk proteins; \( \alpha_s1, \alpha_s2, \beta, \) and \( \kappa \)-caseins, \( \alpha \)-lactalbumin, \( \beta \)-lactoglobulin, bovine serum albumin using amino acid analysis.

3. Separate genetic variants of specific milk proteins, and use amino acid analysis to quantify individual variants in a protein mixture.

4. Use techniques developed in 1-3 analyze milk and other dairy products.

5. Determine mathematical procedures to obtain the most accurate and reproducible methods for estimating milk protein concentrations.

Results:

In the past year, whole casein was separated into \( \beta \)-casein, \( \kappa \)-casein, \( \alpha_s1 \)-casein and \( \alpha_s2 \)-casein fractions using cation-exchange fast protein liquid chromatography. The \( \gamma \)-caseins and several unidentified peaks were also separated. A urea-acetate buffer at pH 5 and a NaCl gradient from 0 to .26 M was used to separate the casein fractions. Several \( \gamma \)-caseins and unidentified fractions eluted first, followed by three \( \beta \)-casein peaks, several \( \gamma \)-casein and unidentified peaks, \( \kappa \)-casein, \( \alpha_s1 \)-casein and \( \alpha_s2 \)-casein. Some \( \gamma \)-caseins eluted with \( \beta \)-casein. The four major caseins, which accounted for over 90% of the whole casein fractions, were accounted for with this method and the calculated compositions correlated well with values obtained using anion-exchange fast protein liquid chromatography at pH 7. \( \beta \)-casein genetic variants A\(^1\), A\(^2\) and B were separated using cation-exchange fast protein liquid chromatography. \( \beta \)-casein from a herd bulk casein sample eluted as a series of three peaks. Casein samples from individual cows containing known combinations of \( \beta \)-casein A\(^1\), A\(^2\) and B were used to confirm that the three peaks were \( \beta \)-casein genetic variants. An acid-PAGE gel confirmed the identity of the peaks that eluted from the column.

Impact of Research:

The goal of this project is to determine proportions of specific milk proteins (down to the level of specific genetic variants) or groups of proteins in milk and other dairy products from the information contained in a single amino acid analysis of a sample. Relative proportions of milk proteins found in traditional dairy products are subject to change as ultrafiltration and other new processes are used in their manufacture. To use these
emerging manufacturing processes to produce entirely new products without the information that will be made available when this project is completed is nearly impossible. Many areas of research will also be facilitated by the results of this research. We will be able to follow milk protein composition through lactation periods of individual cows (or other species), correlate content of each of the milk proteins with coagulation properties during cheese making, make artificial infant formula that more closely matches mothers' milk, etc.

Publications:


EVALUATION OF IRON-PROTEIN COMPLEXES
IN IRON-FORTIFIED DAIRY PRODUCTS

Personnel: Arthur W. Mahoney, Ph.D., Professor
Mohan I. Reddy, Ph.D.

Objectives:

The objective of this study is to determine the chemistry of iron-protein complexes in milk as related to cheese making. We determined the chemical conditions under which maximal iron binding occurs (the conditions in which the iron is bound most tightly). This information is needed 1) for developing the best processing conditions which will give the highest quality iron-fortified dairy products and 2) for determining the best iron-protein complexes which will give the highest iron absorption from the fortified dairy products.

Results:

**Diafiltration Binding Studies:** Binding of Fe(III) to individual caseins and whey protein fractions was studied. Molar binding ratio of Fe(III) to proteins increased as the free Fe(III) concentration increased. We found that $\alpha_s$-, $\beta$-, and $\kappa$-caseins and BSA have two groups of non-identical binding sites with differing affinities for binding Fe(III). It appears that the first set of binding sites ($n_1$) are preferentially filled, compared to the second set of binding sites ($n_2$). No precipitation of proteins as a result of Fe(III) binding occurred even at saturating concentrations of Fe(III). The relative order of binding capacities for Fe(III) to casein and whey fractions was: $\alpha_s$- casein > $\beta$- casein > BSA > $\kappa$-casein > $\kappa$-casein.

Further studies on the binding of Fe(III) to $\alpha_s$-casein as a function of pH and ionic strength indicated that these parameters had no influence on the total number of binding sites, although binding affinity of Fe(III) to protein decreased with increase in pH from 5.60 to 7.80. Thus, from the practical point of view, the binding affinity of Fe(III) increases as the pH of milk is lowered by microbial action during cheese making. Some displacement of protein-bound Ca(II) by Fe(III) was observed for $\alpha_s$-casein. Dephosphorylation of $\alpha_s$-casein decreased the binding of Fe(III) to protein indicating that phosphoserine groups are involved in the binding.

**Visible-Difference Spectra of Fe(III)-Protein Complexes:** Difference absorption spectra of Fe(III)-protein complexes in the visible region (350 to 650 nm) was carried out to determine the possible groups involved in the binding of Fe(III) to different casein fractions and whey proteins. Negative absorption bands in the 420 nm region were observed for caseins indicating that phosphoserine groups and carboxyl groups of aspartic and glutamic acids are possible Fe(III) binding sites. The positive absorption band at 470 nm observed in caseins and whey proteins is typical of the absorption maximum of transferrin-iron complex and possibly due to a chelate site involving histidine and tyrosine residues. Thus, phosphoserines and carboxyl groups of aspartic and glutamic acids seem to play a major role in the binding of Fe(III) by caseins.

**Ultraviolet Difference and Fluorescence Spectra of Caseins and Whey Proteins:** Conformational changes in proteins, especially changes in the environment of aromatic side chains within proteins due to binding Fe(III) were monitored by following UV-difference spectra and fluorescence emission. The UV-difference spectrum of $\alpha_s$-casein induced by Fe(III) had absorption bands at 310 nm indicating the possible involvement of tryptophan residues in charge-transfer type complex formation with Fe(III). Addition of Fe(III) to caseins and BSA caused a decrease in fluorescence intensity together with a red shift of the emission maximum. Iron addition to $\beta$-lactoglobulin caused a decrease in emission intensity without affecting emission the maximum. Studies on the
fluorescence emission spectra of $\alpha_{s1}$-casein as a function of Fe(III) concentration indicated progressive quenching together with a red shift of the emission maximum. This indicates that the binding of Fe(III) to proteins induced conformational changes resulting in the exposure of tyrosine and tryptophan residues to polar environment. However, not all the fluorophores (tryptophans) are uniformly quenched. All of the fluorophores are exposed to polar environment as a result of binding of Fe(III) to $\alpha_{s1}$-casein. Since conformational changes in general affect functional properties of proteins, it was of interest to see if binding of Fe(III) affects Ca(II) sensitivity of $\alpha_{s1}$-casein; addition of Fe(III) to $\alpha_{s1}$-casein increased the Ca(II) sensitivity of the protein.

Effect of Fe(III) on the Renneting of Milk: Since binding of Fe(III) induced conformational changes in milk proteins, it was of practical interest to see if addition of Fe(III) to milk during cheese making would affect renneting properties. Therefore, FeCl$_3$ was added to cold milk before pasteurization and to pasteurized milk (as in case of regular cheese making process) and chymosin hydrolysis and rennet clotting time were evaluated. We use approximately 0.20 mM FeCl$_3$ in making iron-fortified Cheddar cheese. Iron at this concentration had no effect on the chymosin hydrolysis of either milks. However, iron decreased the rennet clotting time of both whole and skim milk when added before pasteurization and increased the rennet clotting time when it was added to pasteurized skim milk. Iron barely affected the rennet clotting time of whole pasteurized milk. Thus, iron at the concentrations normally employed in making iron-fortified cheddar cheese, i.e., 0.2 mM, did not affect renneting properties of milk, and hence can be added to cheese milk. It was also observed that iron binding to casein micelles slightly decreased the calcium content of micelles, but it would not affect the calcium nutrition of cheese.

Significance to the Dairy Industry

Iron fortification would increase the iron intakes of people who consume large amounts of dairy products, and it would allow people concerned with their nutriture to consume larger amounts of dairy products to achieve greater calcium intakes. Thus, dairy products would be even more healthful in the diet if iron-fortified. This research provides basic information on the mechanisms of iron binding in dairy products, information essential to industrializing the technology of fortifying dairy products with iron.

Additional Findings: UV-difference spectral and fluorescence spectral studies indicate that binding of Fe(III) to proteins induces conformational changes leading to the exposure of tryptophan and possibly tyrosine residues to polar environment. This is an interesting phenomenon because binding of Ca$^{2+}$ to $\alpha_{s1}$- and $\beta$-caseins induces self aggregation leading to precipitation at high Ca$^{2+}$ concentrations whereas binding of Fe(III) to caseins does not result in precipitation. Further, binding of iron increased the Ca(II) sensitivity of $\alpha_{s1}$-casein. Change in conformation of caseins may have some implications in the functional properties of some cheeses such as mozzarella. Hence this issue needs to be addressed.

Publications:


4. Reddy, M.I. and Mahoney, A.W. Binding of Fe(III) to bovine $\alpha_{s1}$-casein. In Preparation.
5. Reddy, M.I. and Mahoney, A.W. A study of the interaction of Fe(III) with bovine $\alpha_{S1}$-casein as studied using ultraviolet and fluorescence spectroscopy. In Preparation.

Theses/Dissertations: Nil.

Abstracts:

1. Reddy, M.I. and Mahoney, A.W. Effect of iron on the renneting of milk. Accepted for presentation at '86th ADSA Annual Meeting' to be held at Utah State University, Logan, August 12-15, 1991.

2. Reddy, M.I. and Mahoney, A.W. Binding of Fe(III) to bovine $\alpha_{S1}$-casein. Accepted for presentation at '86th ADSA Annual Meeting' to be held at Utah State University, Logan, August 12-15, 1991.

3. Reddy, M.I. and Mahoney, A.W. A study of the interaction of Fe(III) with bovine $\alpha_{S1}$-casein using ultraviolet and fluorescence spectroscopy. Accepted for presentation at the 86th ADSA Annual Meeting to be held at Utah State University, Logan, August 12-15, 1991.


Patents: Nil.
Project Title: Utilization of Acid Whey as a Substrate for the Production of Food Grade Cellulases

Personnel: Michael H. Penner, Dept. Food Science and Technology Oregon State University
Soren Nordmark, Dept. Food Science and Technology Oregon State University

Funding: Western Dairy Food Research Center

Objectives:

The use of enzymes in the food and related industries is growing rapidly due to decreases in the costs of producing relatively pure enzymes of high activity. The cellulase enzymes typify this trend as they are increasingly used in fruit juice processing and in the utilization of food processing wastes. One of the most expensive parts of industrial enzyme production is the cost of the substrate used to sustain the enzyme producing microbes. Alternative low cost substrates are, therefore, of interest to industrial producers. Whey products have the potential to be an attractive substrate for enzyme production. This is because whey products are rich in utilisable energy and several industrial microbes are capable of growing at relatively low pH.

The study is designed to test the feasibility of utilizing acid whey and whey permeate as primary substrates for the production of food grade cellulases by the mold Trichoderma reesei. A more general aspect of the study is to demonstrate that acid whey has beneficial properties that make it an attractive substrate for the production of secondary metabolites and enzymes by the general class of acid tolerant fungi.

Results:

Our initial studies have found that cellulase enzymes are produced by Trichoderma when grown on acid whey, pH 4.4. Enzyme yields were estimated to be approximately 50% of that observed for the same fungus grown under optimum conditions. The quantity of enzyme produced in different cultures is based on the traditional cellulase filter paper unit of activity. Adjustment of the whey substrate to pH to 5.0, 5.5, 6.0 and 6.5 prior to Trichoderma inoculation resulted in more reproducible enzyme production but only minimal increases in the median levels of enzyme produced. Soluble protein production at days 4 through 8 post inoculation has followed that of the control culture. The pH of all inoculated whey cultures increases by approximately 1 pH unit over the course of the 8 day incubation. This 1 pH unit rise has been consistent for whey preparations starting at pH 4.4, 5.5 and 6.5. Current studies are evaluating the use of an initiator to further stimulate cellulase production in the acid whey cultures. Studies evaluating the use of whey permeate as a substrate for Trichoderma cellulase production and simultaneous production of mycelial protein are also in progress.
Impact of Research:

The optimum scenario for this research is that the results will clearly demonstrate that acid whey or whey permeate is a cost effective substrate that may/should be exploited by major enzyme suppliers. The fungus, Trichoderma, and the enzyme system, cellulase, which are the focus of this research should be considered exemplary, since acid whey may potentially be utilized by a range of fungi which are currently being used for the production of food grade enzymes. Other secondary metabolites, such as pharmaceuticals and pigments, are also potential products which could conceivably be produced from microbes growing on whey substrates. Consequently, this research will be of significance to the food, pharmaceutical and chemical industry in terms of demonstrating substrate properties of whey products. The impact to the dairy industry will be realized if industrial producers accept acid whey as a microbial substrate, thereby providing a mechanism for the utilization of currently underutilized acid whey.
Project Title: Causes and Prevention of Sticky Texture in Mozzarella Cheese

Personnel: Gary H. Richardson, Dept. of Nutrition and Food Sciences, Utah State University.
Richard K. Merrill, Dept. of NFS, USU

Funding: Western Dairy Foods Research Center

Objectives:

1. Survey local manufacturers of Mozzarella cheese to obtain data concerning prevalence and present measures used to deal with the problem.

2. Obtain commercial samples of Mozzarella cheese that exhibit stickiness properties and analyze their physical and microbiological properties. Properties to be examined include moisture, fat content, protein content, pH, manufacturing profile, salt concentration, microbial count, culture type and strain used, and calcium content.

3. Assess the affects of freezing temperature, storage time and temperature, shredding, and thaw time and temperature on stretch, melt and cook color of low moisture part skim Mozzarella cheese.

Results:

Data gathered to date supports the findings of Masi and Addeo (1986). They observed that cheese becomes softer and more difficult to shred with increasing FDB and moisture content. We have shown that freezing, storing, and shredding significantly affect the stretch and melt of Mozzarella cheese, but do not affect cook color. Also, storing, thawing, and shredding parameters have been established so as to obtain optimal stretch and melt in frozen Mozzarella cheese. Further data is being collected to see if manufacturing parameters, culture type, or the strain used may play a role in the development of sticky texture in Mozzarella cheese.

Impact of Research:

Knowledge of manufacturing and storage practices that lead to poor product quality will allow Mozzarella cheese producers to manufacture better quality product. However, manufacturers must balance stretch and melt, because freezing and storing frozen affect stretch and melt oppositely.

Publications:

**Project Title**: Characterization of the post-adsorptive behavior of β-lactoglobulin for control of spore and microbial adhesion to dairy product processing and packaging surfaces

**Personnel**: Joseph McGuire, Bioresource Engineering and Food Science & Technology, Oregon State University
Viwat Krisdhasima, Chemical Engineering and Food Science & Technology, Oregon State University

**Funding**: Western Dairy Foods Research Center, AIChE Research Institute for Food Engineering, National Science Foundation, Public Health Service Institutional Grant (OSU), Oregon Agricultural Experiment Station

**Objectives**: The project objectives for fiscal year 1991 were to use dynamic ellipsometry to continuously measure the changing thickness and refractive index of β-lactoglobulin films adsorbed from aqueous solution, and to relate changes in surface conformational state to time and solid surface properties.

**Results**: We have used ellipsometry to monitor the influence of contact surface hydrophobicity on the post-adsorptive behavior of β-lactoglobulin (β-lg). Cuvettes that allow the continuous in situ observation of surface-induced protein conformational changes were designed and constructed for that purpose. Silicon surfaces have been modified to be hydrophilic by a series of oxidizing washings, and hydrophobic by surface methylation with dichlorodimethylsilane. We have successfully varied the degree of methylation on these surfaces to create a series of surfaces exhibiting varying hydrophobicities. β-lg adsorption isotherms have been constructed for each of these surfaces as well.

In parallel with the Center-sponsored work, funding received from other sources has been used to construct β-lg adsorption isotherms on acrylic, glass, polycarbonate, polyester and #304 stainless steel surfaces (Al-Malah et al., 1991). The influence of pH and ionic strength on β-lg adsorption equilibrium has also been investigated (Luay et al., 1991).

As originally hypothesized, it appears that a surface-induced conformational change takes place upon β-lg adsorption. We formulated a simple model for protein adsorption, accounting for an initial reversible step followed by a conformational change generating an irreversibly adsorbed species. We derived an expression for adsorbed mass as a function of time, protein concentration, and reaction rate constants, then used nonlinear regression to fit in situ adsorption kinetic data to our derived expression. The model described adsorption well in all cases with statistical analysis indicating that all parameters are significant. As expected, plateau values of adsorbed mass were observed to increase with increasing surface hydrophobicity. This is consistent with the observation that protein adsorption is an entropically driven process, and in agreement with our own equilibrium studies.

Expressions describing the kinetic rate constants that define protein arrival and unfolding support the hypothesis that rates of arrival and unfolding increase with increasing solid surface hydrophobicity, while the tendency of adsorbed protein to desorb decreases.
Impact of Research:

It was the purpose of this research to quantify the post-adsorptive behavior of β-lg on several materials as a function of time and contact surface properties. This purpose was fulfilled. Interpretation of adsorption kinetics with reference to a molecular model was shown to yield useful relationships among the rate constants that describe interfacial behavior. We are now prepared to seriously address the issue of what protein surface activity really has to do with bacterial adhesion. Based on the type of experimentation described in a new project proposal to the Center (M.A. Daeschel and J. McGuire, co-PIs), knowing the protein content of a fluid product, we should be able to describe surface and protein chemical influences on bacterial adhesion in a truly quantitative manner.

Publications:

Project Title: Properties of Low-Fat Yogurt Manufactured from Ultrafiltered and Ultra-High Temperature Treated Milk

Personnel: Paul A. Savello, Dept. of Nutrition and Food Sciences, Utah State University.

Funding: Western Dairy Foods Research Center

Objectives:

1. To measure the effects on the yogurt properties of viscosity, gel strength, syneresis, and water holding capacity by ultrafiltering yogurt milk to different total milk solids or standardized 5% protein level and applying different heat treatments.

2. To measure the whey protein denaturation in the yogurt milk as a result of different heat treatments.

Results:

Intermediate-(IHT) to ultra-high temperature (UHT) heat treatments were compared to vat heat treatment of skim milk, ultrafiltered (UF) skim milk, and skim milk with added nonfat dry milk (NDM) for yogurt. Yogurt made from skim milk ultrafiltered to 5% protein had greater gel strength and viscosity than yogurts enriched to 5% protein by NDM addition. This effect was observed for all heat treatments, including indirect plate exchange heating at 100, 110, 120, 130, or 140°C for 4 or 16 seconds, or vat heating at 82°C for 20 minutes. Vat heated UF yogurts had lower syneresis than vat heated NDM yogurts. IHT treatment (100°C for 16 sec, 110°C for 4 or 16 sec, 120°C for 4 sec) showed highest gel strength, viscosity, WHC, and lowest syneresis compared to high-end temperatures in all yogurts. UHT treatment (140°C for 4 or 16 sec) was detrimental to textural properties of skim milk, NDM, and UF yogurts.

In trials to produce an aseptic yogurt using UHT technology, pectin addition from 0.2 to 1.5% and temperatures as low as 100°C did not successfully stabilize the low pH (4.2-4.6) yogurts. Most promising results were received from yogurt milks that were H2O2-treated rather than prior pasteurization. As aseptic yogurt could provide new non-refrigerated sales opportunities for dairy products.

Impact of Research:

Previous reports on this project indicated that yogurt made from skim milk ultrafiltered (UF) to increase total solids resulted in greater viscosity, gel strength, water holding capacity (WHC), and lower syneresis in IHT yogurts (104, 116, 127°C for 4 sec)
compared to vat heated yogurts with added NFDM to comparable solids levels. Results presented here suggest that ultrafiltration also provides enhanced yogurt physical properties when used to enrich yogurt milks to a standardized 5% protein level as compared to traditional enrichment to 5% protein by addition of NDM. The use of UF to fortify yogurt may offer improved textural properties at lower total solids levels than traditional fortification, and potentially lower cost.

Previously reported data on skim milk, skim milk adjusted to 1% milkfat, and 12.5 and 15% solids UF skim milk indicated that IHT treatment of milk for yogurt optimizes the yogurt physical properties that can be achieved from indirect heat treatments between 104 to 138°C. If indirect plate heat exchange is to be used to process yogurt milks, results from 5% protein level data suggest IHT temperatures (100°C for 16 sec, 110°C for 4 or 16 sec, or 120°C for 4 sec) provide comparable or better yogurt properties in this study were achieved without the use of stabilizers. The use of UF and IHT technology both represent non-additive approaches to enhance yogurt physical properties.

Publications/Abstracts:


High Yield, Low Moisture Cheese from Homogenized UF Milk

Annual Report Date: 30 June 91  Project Term: 1 Sep 88 — 31 Aug 91

Personnel:
Principal Investigator:  Dr. D.J. McMahon
Graduate Student:  Mr. Brian Orme

Funding Sources:  Western Dairy Foods Research Center
Utah Agricultural Experiment Station

Objectives:
Ultrafiltration (UF) of milk can be used for the manufacture of high moisture cheeses. Its economics lay mainly in increased retention of fat and protein. There are, however, some difficulties and complexities of making low moisture cheese using UF concentrated milk in that high fat losses occur and it is difficult to remove moisture. The specific objectives of this project are:

1. Determine effects of homogenization treatment on fat losses from UF retentate curd.

2. Design a cheese making process so as to obtain cheese in the range of pH 5.0—5.4 and moisture content < 40%.

3. Determine effects of milk heat treatment on moisture, texture and body of cheese made from UF retentate.

4. Provide a manufacturing procedure for making acceptable low moisture cheese from pre-fermented UF retentate that could be adopted for a continuous cheese making process.

Results:
Objective 1: Homogenization and Fat Loss.
Experiments have been conducted to determine effects of homogenizing whole milk on the extent of fat/casein complexing that occurs and the subsequent retention of fat in cheese curds made from 5X UF retentate.

In one experiment, pasteurized whole milk was divided into two lots and one lot homogenized at 3500 psi, while the other was not homogenized. Both lots were ultrafiltered without diafiltration to 38% solids. Cheeses were made simultaneously from 3.5 kilogram batches of retentate from each lot using standard cheddar make parameters. A one half kilogram, pH 5.0 water overlay, was used to float curds.

Over the course of agitating and cooking curds to 39°C, a 1 cm deep free-fat layer developed in whey from the un-homogenized sample, while whey from the homogenized sample contained only a discontinuous film of free-fat. The extent of fat/protein complexing has been determined over a range of homogenization temperatures and pressures.

Objective 2: Cheese making Process
5X UF Retentate. Our experiments have shown that less rennet is required to coagulate 5X retentates if pH is below 6.4. UF retentates are also much less viscous and more easily handled if they have been adjusted to pH 6.4—6.0.
We have considered that if retentates were pre-fermented to pH 6.4 at approximately 30°C, temperature could then be reduced to 20°C to slow acid production and provide a larger make-window. If retentates were renneted and cut at 20°C, curds could be cooked over a 19°C range instead of a 9°C range to increase syneresis.

4X UF Retentate. To overcome the processing difficulties in using 5X retentate we have looked at the use of 4X UF retentate as the starting material for cheesemaking. Diafiltration levels required to reduce lactose to a level so that fermentation stops at pH 5.1 during cheesemaking have been determined.

Objective 3: Milk Heat Treatment
Work on this objective has been delayed until an appropriate cheesemaking procedure has been determined.

Objective 4: Cheese Quality
Cheese quality is affected by the microorganisms present in the cheese curd. Unpredictable fermentation rates have been observed when UF retentate is used to make cheese. We studied the functioning of a variety of strains of Lactococcus lactis ssp lactis and ssp cremoris in 4X retentate and found that there are significant strain differences in how they perform. The growth of some strains is inhibited in UF retentate while the generation time of other strains was not affected.

Impact of Research:
The technology of concentrating milk by ultrafiltration (UF) has progressed over the past ten years. Much of this technology has been utilized in the development of new methodology for manufacture of cheese. The most successful applications have been in the production of high moisture cheeses by European companies. However, UF has been of only limited use in the manufacture of low moisture cheeses because concentration of milk in most UF systems reaches a limit at approximately 40% total solids, or in other words it reaches a minimum of 60% moisture content.

It is our intention that through the development of a new cheesemaking procedure, as described in this research project, it will be possible to make low moisture cheeses that will retain the high yield advantages of UF. Cheeses in the moisture range of 35-45% are the most widely consumed cheeses in the U.S.A. The introduction of new varieties of cheese would have the greatest opportunity for success if they were in this category.

Cheesemaking technology to be developed in this project has the potential to provide opportunities to produce new low moisture cheese products on a cost effective basis.

Publications and Abstracts:
Project Title: Development of a process for production of UF milk retentate

Personnel:
Conly L. Hansen, Dept. of Nutrition & Food Sciences, Utah State University
Donald J. McMahon, Dept. of Nutrition & Food Sciences, Utah State University
Yehia A. El-Samragy, Dept. of Nutrition & Food Sciences, Utah State University

Funding: Western Dairy Foods Research Center

Background:
Several methods are available for processing surplus milk to extend its shelf life. Skim milk powder has been the standard means for storing surplus milk solids. Recently a frozen concentrate has also been developed (U.S. Patent 4,844,923). The major problem associated with frozen milk concentrate is the gradual destabilization of casein micelles that occurs during storage. Successful ultra-high temperature (UHT) concentrated milk has not yet been achieved because of the gelation that occurs in such concentrates upon storage of room temperature.

Production of milk powder by evaporative concentration and drying is an established segment of the dairy industry. However, product quality considerations favor the use of membrane separation to concentrate milk rather than thermal processes. Extensive heating during evaporative concentration often causes product degradation, primarily through change of color and flavors, and high denaturation of protein.

Milk powder with better functional and nutritional quality can be produced employing membrane separation such as ultrafiltration for concentrating milk.

Objectives:
The overall objective of this project is to develop a process for production of high protein UF milk retentate powder.

Specific Objectives:
1. Determining effects of heat treatment, pH and limited enzyme treatment of UF retentate on the functional properties of its resultant spray dried, high protein, milk powder.
2. Determining effects of drying parameters, such as particle size, temperature, solid concentration and foam spray, on properties of the retentate powder.

3. Evaluating product applications of the high protein milk powder.

GANTT CHART

| Months | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Objective 1 | (-----------------------------------------------) |
| Objective 2 | (-----------------------------------------------) |
| Objective 3 | (-----------------------------------------------) |

Effect of different pH and heat treatments on the properties of UF milk retentate powder:

Fresh raw skim milk was ultrafiltrated to 20% total solids. The UF milk was divided into three portions. pH of the first portion was adjusted to 6.4 using 1N HCL. The pH of the second portion was the normal pH of the UF retentate (6.7). The third portion was adjusted to pH 7.0 using 1N NaOH.

Each portion of adjusted pH retentate was divided into four parts. The first part was spray dried directly. The second part was heat treated at 65°C for 30 min, the third part was heat treated at 75°C for 28 sec while the forth part was heat treated at 85°C for 28 sec prior to spray drying. All powder samples were stored at 4°C until analysis for chemical composition, physical and functional properties.

Results:

Table 1 indicates the effect of pH values and heat treatments prior to spray drying on the chemical composition of skim milk retentate powder. The analysis of the powdered samples of the UF retentate for physical properties are on going, while the other functional properties will be completed at the end of next month.

We will be able to define the best combination of adjusted pH and heat treatment when finished with the statistical analysis of the obtained results, which forms about 80% of the first objective of the project. The remaining 20% of the first objective will be covered through the enzyme treatment in content with the best combination of adjusted pH and heat treatment. It will take about three months or until the end of December to complete this first objective.
Publications:


Two others are in preparation.
Table 1. Effect of pH values and heat treatments on the chemical composition of skimmilk retentate powder.

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HT: Heat Treatment.
NH: No Heat Treatment.
I: Heat Treatment at 65°C.
II: Heat Treatment at 75°C.
III: Heat Treatment at 85°C.
Project Title: Membrane Fractionation of Immunoglobulins from Milk and Whey

Personnel: Paul A. Savello, Dept. of Nutrition and Food Sciences, Utah State University
Reyad Mahmoud, Dept of Nutrition and Food Sciences, Utah State University

Funding: Western Dairy Foods Research Center

Objectives:
1. Fractionate immunoglobulin protein components from skim milk and whey using sequential micro-, ultra-, and nano-filtration technologies;
2. Optimize the fractionation procedures and conditions to obtain the highest purity of desired protein component per separation stream;
3. Concentrate and freeze dry the protein fraction streams for laboratory analysis of fractions' purity by HPLC.

Results:
Whey and skim milk were subjected to separation by a wide range of separation modules. Spiral wound polysulfone membranes (2,000, 5,000, 15,000 and 40,000), mineral membranes (10,000 and 80,000) molecular weight cut-off, and ceramic membranes (0.05, and 0.2 micron) pore size were tested. Permeates of sweet whey and skim milk were analyzed using HPLC gel permeation column.

The analysis of permeate was used to indicate the molecular range cut-off of the membranes. Polysulfone membranes below 4,000 molecular weight cut-off prevented almost all the whey proteins permeation. The 40,000 molecular weight cut-off membrane permeate indicated a complete retention of immunoglobulins in the retantate and partial permeation of α-LA and β-LG in the permeates of whey and skim milk. Permeates of skim milk and whey from ceramic membrane 0.2 micron allowed all the whey proteins to permeate including the immunoglobulins. However, the analysis of a ceramic membrane 0.05 micron permeates exhibited a slight permeation of α-LA and β-LG. Permeate of mineral membranes indicated partial permeation of α-LA and β-LG and retention of immunoglobulins.
Diafiltration and concentration of retentates of skim milk and whey of the three membranes (40,000 cut-off polysulfone, 100,000 mineral, and 0.05 ceramic) will be conducted to achieve the third objective.

Impact of Research:

The extraction of immunoglobulins from whey would have a positive impact in adding a value product.
Project Title: Cloning the Nisin and other Genes from Lactococcus into Leuconostoc Species and Amplification of Nisin Production

Personnel: Jeffery K. Kondo, Associate Professor, Dept. of Nutrition & Food Sciences, Utah State University, Logan.

William E. Sandine, Professor, Department of Microbiology, Oregon State University, Corvallis.

Jeffery R. Broadbent, Graduate Research Fellow, Dept. NFS, USU.

Herb Wycoff, Graduate Research Assistant, Dept. Microbiology, OSU.

Hua Wang, Graduate Research Assistant, Dept. NFS, USU.

Yat-Chen Chou, Graduate Research Assistant, Dept. NFS, USU.

Funding: Western Dairy Foods Research Center
National Science Foundation
Utah Agricultural Experiment Station
USDA-ARS National Needs Graduate Fellowship Program

Objectives:

1. To produce and characterize lactose positive Leuconostoc transconjugants obtained by conjugal matings between Lactococcus and Leuconostoc spp.

2. To develop transformation and gene cloning systems in Leuconostoc.

3. To introduce into Leuconostoc, plasmid-coded protease genes from lactococci (e.g. L. lactis ssp. cremoris Wg2).

4. To use the genetically constructed fast acid-producing Leuconostoc (lactose positive and protease positive) to produce different fermented dairy products such as cultured buttermilk, cottage and Gouda cheese.

5. To conjugally transfer plasmid-coded nisin genes from L. lactis ssp. lactis 7962 to Leuconostoc and to study this plasmid at the molecular level.

6. To amplify nisin production by gene cloning techniques.

7. To use nisin producing Leuconostoc in Swiss cheese manufacture to inhibit gas producing anaerobic sporeformers such as Clostridium tyrobutyricum.
8. To study the inhibition of *L. monocytogenes* by nisin and to use genetic engineering techniques to maximize its useful application.

Results, Utah State University:

Our research has been focused upon objectives 3 and 6 as outlined above. We have continued our investigation of conjugal transfer of nisin production and immunity in a lactococcal model system. These studies have examined the effects of various physiological and environmental factors upon transfer efficiency, in order to develop methodology which will permit transfer into *Leuconostoc* sp. Results from these experiments have allowed us to accomplish several important steps. First, as reported last year, was the development of the direct plate conjugation technique (DPC) which improved the efficiency of transfer for nisin-producing capability. This led to the successful transfer of nisin-producing ability into several strains of *L. lactis* ssp. *cremoris*. During the past year we were able to conjugally transfer the nisin genes into 3 strains of *Streptococcus salivarius* ssp. *thermophilus*. Transconjugants of these strains were immune to nisin and also acquired sucrose fermenting ability, but nisin production was not detected by an agar overlay assay. Hybridization data, however, indicated that the nisin structural gene, *nisA*, was present in transconjugants. Our investigations have also suggested that the conjugative enterococcal plasmid pAM-beta-1 facilitated the intergeneric transfer. Experiments are presently underway to determine if the use of pAM-beta-1 will also facilitate transfer of these genes to other dairy microorganisms such as *Leuconostoc* sp.

In related research, we used *Escherichia coli* to clone a 10 kb *Kpn I* fragment which contained the *nisA* gene from the nisin-producing strain *Lactococcus lactis* ssp. *lactis* 11454. Sequence data published by other investigators (1,2) indicated that the *nisA* gene was located approximately 1.5 kb downstream of the 5' end of our fragment and this orientation was supported by restriction analysis of the fragment. Published data also showed that our fragment lacked an RNA polymerase promoter sequence for transcription of *nisA* and one subsequent gene which are part of a polycistronic operon (4). Because genetics studies have indicated that the genes for nisin production and immunity, sucrose utilization, and reduced bacteriophage sensitivity are linked (3), we wished to determine whether our fragment would express one or more of these phenotypes in a lactococcal host. To accomplish this we constructed plasmid pJB100, which placed the fragment downstream of the promoter for the erythromycin resistance gene of the vector pGK13. After *Lactococcus lactis* ssp. *lactis* LM0230 was electrotransformed with pJB100, however, we were not able to detect any changes in the phenotypes of transformants. Additional studies are underway to determine whether the presence of pJB100 in nisin-producing lactococcal transconjugants may alter levels of nisin production through a gene dosage effect.

Impact of Research - Nisin:

Nisin, a peptide antibiotic produced by some strains of *Lactococcus lactis* ssp. *lactis*, is an effective inhibitor of Gram-positive bacteria. The antibiotic has been approved for use as a food preservative in over forty-five countries, including the United States. For
many years, investigators have been interested in transferring the nisin genes into other organisms used to manufacture fermented foods to enhance the shelf life of these products. Conjugation, a natural gene transfer process, may be useful to achieve this goal. Bacterial strains that are developed via conjugation contain genes that only come from other safe, food-grade lactic acid bacteria. Consequently, conjugally improved strains may face fewer FDA restrictions, with respect to industrial application, than strains which are improved through recombinant DNA technology.

Our studies of conjugation have yielded new methodology which has allowed us to investigate many of the parameters which affect transfer of the nisin genes. This work has led to improved transfer efficiency and the subsequent ability to transfer these genes into strains of *Lactococcus lactis* ssp. *cremonis* and *Streptococcus salivarius* ssp. *thermophilus*. These results indicate that we may be able to use conjugation to introduce the nisin genes into leuconostocs and other genera of lactic acid bacteria. We can foresee many applications for nisin-producing lactic organisms among dairy, food, and agricultural fermentations, to enhance the safety and shelf-life of foods.

**Construction of rapid acid-producing leuconostocs:**

*Leuconostoc* ssp. are slow acid-producers in milk and thus are unable to produce appreciable amounts of the important flavor compound, diacetyl, in pure milk cultures. This shortcoming has been attributed to the lack of milk proteolysis and limited lactose utilization. If the growth of leuconostocs is slow or inhibited, the resulting product lacks desirable flavor. Increasing the levels of proteolysis and lactose utilization, by gene transfer from lactococci to leuconostocs, may reduce or eliminate flavor defects in fermented milks. Improved *Leuconostock* ssp. may also allow the manufacture of specialty cheese similar to varieties now imported, or allow development of novel dairy products.

**Publications:**


**Abstracts:**


References:


Project Title: Cloning the Nisin and Other Genes of Lactic Streptococci into Leuconostoc Species and Amplification of Nisin Production

Personnel:

W. E. Sandine, Principal Investigator, Dept. of Microbiology, Oregon State University

M. B. Barnes, Technician, Dept. of Microbiology, Oregon State University

H. A. Wyckoff, Graduate Research Assistant, Dept. of Microbiology, Oregon State University

Funding Sources: Western Dairy Foods Research Center

Objectives:

This has been a joint project between USU and OSU. Research on nisin has been done at USU while development of Leuconostoc - Lactococcus genetic exchange procedures has been done at OSU. Leuconostoc bacteria are mixed with Lactococcus strains to produce and market different types of milk products. The Leuconostoc produce flavor (diacetyl) while the lactococci produce acid. Both are required and neither can produce what the other produces. Therefore in this research we are developing methods to introduce lactococcal genes into Leuconostoc in order that the latter can be used alone to produce traditional and innovative fermented dairy products.

1. To produce and characterize lactose positive Leuconostoc transconjugants obtained by conjugal matings between Lactococcus lactis and Leuconostoc spp.

2. To develop transformation and gene cloning systems in Leuconostoc.

3. To introduce into Leuconostoc, plasmid-coded-protease genes from lactic streptococci.

4. To use the genetically constructed fast acid-producing Leuconostoc to produce different fermented dairy products such as cultured buttermilk, cottage and Gouda cheese.

5. To conjugally transfer plasmid-coded nisin genes from Lactococcus lactis to Leuconostoc.

6. To amplify nisin production by gene cloning techniques.

7. To use nisin producing Leuconostoc in Swiss cheese manufacture to inhibit gas producing anaerobic spore formers such as Clostridium tyrobutyricum.

8. To study the inhibition of L. monocytogenes by nisin and to use genetic engineering to maximize its useful application

Results:

In our previous yearly reports, we have discussed the progress that we have made towards fulfilling the objectives of our studies on Leuconostoc. In summary, we have developed a genetic transformation system which has been applicable to all species of dairy Leuconostoc. By using this procedure, we have identified a number of cloning vectors which can be used in genetic studies pertaining to Leuconostoc. Although unsuccessful, preliminary attempts to express selected lactococcal genes in Leuconostoc have been made. In addition, a study dealing with the
efficacy of nisin inhibition of *Listeria monocytogenes* has been completed and published. This past year, we have focused primarily on the characterization of a small cryptic plasmid in *Leuconostoc dextranicum* 226 designated pMBB1. Our goal in this regards is two fold. One, we hope to gain some basic information about plasmid replication and maintenance in *Leuconostoc*. Unlike some of the industrial important plasmids in lactic streptococci, (lactose utilization and proteolysis) plasmids in *Leuconostoc* are very stable. Secondly, we plan to use this plasmid as the backbone for construction of cloning vectors which will be based on a *Leuconostoc* replicon and presumably have the same stability as native *Leuconostoc* plasmids.

Specifically, we have mapped pMBB1 with restriction endonucleases and sized it at approximately 2.85 kilobases in length. By using molecular cloning techniques, we have located the origin of replication on a 1.9 Kb Mbo1 fragment. We are currently sequencing pMBB1 and to date have the complete nucleotide sequence of the 1.9Kb fragment containing the origin of replication as well as most of the remaining sequence. Efforts are under way to determine the relatedness of pMBB1 to other plasmids, possible open reading frames, and other characteristics by comparing the sequence to known sequences in computer databanks. An in vitro procaryotic directed transcription-translation assay has allowed us to identify two proteins that are encoded on pMBB1. In addition, we have cloned a chloramphenicol resistance gene onto the 1.9 Kb fragment, completing the first step towards constructing a cloning vector. This construction has also allowed us to determine that this plasmid has the ability to replicate in *Lactococcus lactis* as well as *Leuconostoc* species other than *Leuconostoc dextranicum*.

**Impact of Research:**

The results that we have produced this last year augment those that we have achieved in the previous years. By increasing our understanding of the basic molecular mechanisms of the *Leuconostoc* spp. we increase our chances of being able to successfully engineer these organisms with the traits that the dairy industry deems benificial. By improving or altering the characteristics of *Leuconostoc*, current dairy fermentations can be improved or stabilized as well as allowing the development of new specialty dairy products or cheeses.

**Publications:**


Project Title: Characterization of Bacteriophage Receptor Sites of Lactococcus Bacteria

Personnel: B.L. Geller and W.F. Sandine, Oregon State University.

Funding: Western Dairy Foods Research Center

Objectives:
The overall objective is to understand the molecular mechanism of phage adsorption to the surfaces of lactococcus bacteria. The specific objectives are:

1. To identify the cell surface location of the bacteriophage receptor.
2. To define the bacteriophage receptor at the molecular level.

Results:
Our results can be summarized in four groups:

1. We have found that the receptor for the bacteriophage kh on *Lactococcus lactis* subsp. *cremoris* KH is the rhamnose of the extracellular wall polysaccharide. In addition, galactose is required, but appears not to directly interact with the phage. We also found that phage infection was prevented by adding rhamnose to either the phage or growing cultures.

2. The phage skl receptor on *L. lactis* subsp. *lactis* C2 was found to be the rhamnose of the exopolysaccharide. In addition, glucose appears to be required, although it is probably not the primary binding site.

3. We have tentatively identified the phage c2 receptor on *L. lactis* subsp. *lactis* C2 as the rhamnose of the exopolysaccharide. Neither galactose, glucose of N-acetylglucosamine are involved. The binding of phage c2 apparently is reversible. This is in contrast to the binding of phage skl, which is irreversible.

4. We have identified and partially purified a membrane protein required for bacteriophage c2 and skl infection of *L. lactis* subsp. *lactis* C2. The protein has an apparent *M*$_r$ = 350,000 and a subunit size of 32 KDa.

Impact of Research:
Over 80% of failed fermentation by mesophilic starter cultures are the result of bacteriophage attack. In order to design rational ways of combating this very
significant economical problem of the cheese-producing industry, more must be known about the mechanisms of phage infection in lactococci. One very sensible way of eliminating this problem is to prevent the initial attachment of the phage to the starter culture cells. Without attachment, infection is impossible. Our research has begun to define the molecular determinants of the phage receptors on the surface of different strains of *lactococcus lactis*. Two potential approaches for preventing phage attachment have been elucidated form our results:

1. Soluble rhamnose added to the growth medium can inactivate at least three phages, or prevent infection of a growing culture. While it would be impractical to add rhamnose to the milk used in making cheese, polymers of rhamnose might be added to milk to act as a phage "adsorbent". These polymers might even be secreted by lactococci genetically programmed to synthesize such polysaccharides.

2. A membrane protein which we have found can be mutated to an extent which prevents phage infection, but has no apparent effect on the growth of the bacteria. This would suggest that an improved strain could be constructed by genetically reprogramming the starter culture with an appropriately mutated and stable copy of the required protein.

**Abstracts of presentations at meetings:**


Publications:


Manuscripts:


Project Title: Production of Omega-3 Fatty Acids by Genetically Altered Fungi and Lactic Acid Bacteria

Personnel:

Floyd W. Bodyfelt, Dept. of Food Science & Technology, Oregon State University
Samuel E. Beattie, Dept. Food Science & Technology, Oregon State University
Daniel P. Selivonchick, Dept. of Food Science & Technology, Oregon State University
William E. Sandine, Dept. of Microbiology, Oregon State University

Funding: Western Dairy Foods Research Center

Objectives:

1. Examine *S. parasitica* for extra chromosomal DNA/plasmids with the goal of using an indigenous plasmid for cloning genes facilitating metabolism of lactose.

Since *S. parasitica* does not contain the enzymes necessary for lactose metabolism, it was necessary to genetically transform the organism. The first step to this was formation of stable protoplasts which were generated by use of a combination of enzymes and osmotic stabilization with 0.5M KCl (or 1M sorbitol). With this method, approximately 20% of the protoplasts regenerated. Subsequently, 0.6M sorbitol was found to stabilize protoplasts and allow higher regeneration percentages.

Finally, we successfully transformed *S. parasitica* to utilize lactose. Plasmid pKR1B-Lac4-1 (supplied by R. Dickson, U. of Kentucky) was used to transform *S. parasitica* by a polyethylene glycol procedure. This plasmid contains the β-galactosidase and lactose permease genes as well as an ARS section of the yeast *Kluveromyces lactis*. The transformed protoplasts were identified by their ability to grow and produce blue colonies on a lactose-based medium that contained the chromogenic indicator X-gal. Relatively high concentration of plasmid DNA were found to be required to achieve transformation and only 10-15 transformants for every 1X10⁶ protoplasts were obtained. Colony growth was quite poor on the regeneration medium.

The majority of the research was involved in the genetic alternation of *S. parasitica*. A transformation procedure was developed that allowed selection of transformants of *S. parasitica* based on the production of β-galactosidase. Because the plasmid DNA did not incorporate into chromosomal DNA, transformants were unstable. While transformants were shown to be unstable, the
transformation procedure should work for plasmids that incorporate into chromosomal DNA. A plasmid based on the *tripC* promotor of *Aspergillus nidulans* may lead to transformants.

2. Once genetically altered, determine growth and lipid accumulation (fatty acid profile of *S. parasitica* in a chemostat using lactose as a carbon source.)

Initial studies examined lipid profiles of *S. parasitica* grown in media of limited carbon and nitrogen sources. The defined medium that showed optimum omega-3 fatty acid (eicosapentenoic acid \{20:5\(\Delta\)5,8,11,14,17\}, EPA) production contained adequate carbon and nitrogen as glutamic acid and no glucose. When grown on this medium at 20°C, the fatty acid profile of *S. parasitica* was 24.6% EPA. When a casein hydrolysate was used as the sole nitrogen source, EPA production averaged 18.4% of the total fatty acid profile. Limiting the nitrogen source adversely affected the total growth and EPA production of *S. parasitica*, but increased total lipid production to 4.0% (wet weight basis). This data provided a baseline for further studies using genetically altered *S. parasitica*.

3. Develop a whey permeate based medium that will provide optimum growth and lipid accumulation by *S. parasitica*.

A most troublesome byproduct of cheese manufacture is whey permeate. Because of residual lactose, this byproduct is high in biological oxygen demand (BOD) and therefore expensive to dispose of through common means. A process that could reduce the BOD and simultaneously produce a marketable value-added product would be beneficial to cheese manufacturers. An intent of this research was to fulfill that need by possibly producing microbial oil that would be rich in omega-3 fatty acids; thus making it comparable to salmon or other fish oils.

One of the major objectives of this work was to examine the potential use of cheese whey permeate as a growth substrate for *Saprolegnia parasitica*. *S. parasitica*, when grown on a monosaccharide substrate, produces omega-3 fatty acids. Since the organism is unable to utilize lactose, much effort was expended on trying to clone lactose metabolizing genes into *S. parasitica*.

Since the transformants of *S. parasitica* exhibited instability (because the plasmid DNA did not incorporate into chromosomal DNA), the achievement of this objective was not consummated.

4. Determine the scale-up economics with an emphasis on optimum lipid extraction from large scale chemostat production into lactic acid bacteria.

The constraints and limits of achieving the first two objectives of this study made this objective superfluous. Also, recent Food and Drug Administration restrictions on making any health claims for omega-3 fatty acid profiles in food products served to lessen the nutritional impact of this approach.
Results:

Lipid production by microorganisms cannot be considered an economical means of disposing of cheese whey permeate when the lipid composition is similar to that of oilseeds. However, the current interest of nutritionists in the health promotion role of long chain polyunsaturated fatty acids (PUFAs), in particular the omega-3 fatty acids (e.g. 5,8,11,14,17-eicosapentaenoic acid {EPA}), suggests that commercial production of omega-3 fatty acids may be economically feasible. Relatively few microorganisms are able to produce omega-3 fatty acids; exceptions are phytoplankton and several filamentous fungi. The lower aquatic fungus, Saprolegnia parasitica, has been found to have a fatty acid profile that contains 20% EPA. Unfortunately, S. parasitica does not utilize lactose.

This research examined the ability of S. parasitica to produce omega-3 fatty acids with an emphasis on optimizing the yields of this PUFA by medium manipulation. We successfully transformed the organisms with the LAC5 (β-galactosidase) gene of Kluyveromyces lactis by use of the plasmid pKR1B-LAC4-1. Unfortunately, the resultant transformants were unstable and lost the capability to produce β-galactosidase after several transfers over a period of several months.

Impact of Research:

The primary objectives of this research were to develop an organism that would produce omega-3 fatty acids when grown on a medium that contained lactose (e.g. whey permeate). Saprolegnia parasitica was shown to produce an omega-3 fatty acid when grown on a medium that contained glucose. The influence of medium composition with respect to carbon and nitrogen source showed that optimum lipid and omega-3 fatty acid production occurred in a medium that contained glutamate as carbon and nitrogen sources. Omega-3 fatty acid production was also favored at 20°C regardless of the medium composition. Overall unsaturation also increased with decreased temperature. The major fatty acids affected by temperature were oleic, palmitic, and eicosapentaenoic acids. The data indicated that deactivation because of temperature may occur at the Δ12-desaturase leading to omega-3 fatty acids.

The economic considerations of this project are substantial. Lactose and cheese whey permeate are an economic burden to cheese producers and a potential environmental burden for the community. Production of microbial lipids from whey permeate has been shown to be feasible, provided the lipid can compete with traditional sources. In 1986, capsules of omega-3 fatty acid containing oil were selling for $0.33 to $0.66/gram ($327-$665/kg) fatty acid (D.H.S. Greene, personal communication). At that time, these figures made production of oil that contained omega-3 fatty acid economically desirable and feasible. More recently, the effectiveness of omega-3 fatty acids in control of various heart diseases has come under close scrutiny. In the past year the Food and Drug Administration has stopped manufacturers of fish oils from making health claims. Regardless of this action, scientific and epidemiological evidence shows that intake of omega-3 fatty acids can benefit cardiovascular health.
Publications:


Beattie, S. E. 1990. DNA transformation of Saprolegnia parasitica, an omega-3 fatty acid producing fungus, with the β-galactosidase gene of Kluyveromyces lactis. PhD Dissertation, Oregon University, Corvallis, 89 p.

Project Title: Purification of a bacteriocin from *Pediococcus pentosaceus* and genetic transfer of the plasmid borne determinants

Personnel: Mark A. Daeschel, Principal Investigator, Dept. of Food Science and Technology, Oregon State University

Xintian Ming, Dept. of Food Science and Technology, Oregon State University

Funding Sources: Western Dairy Foods Research Center

Objectives:

The primary objectives of this study are twofold:

1. To purify the bacteriocin, Pediocin A, using protein purification methodology to a purity suitable for the production of polyclonal antibodies.

2. To genetically transfer the Pediocin A plasmid (pMD136) into dairy fermentation strains via the current state of the genetic transfer systems that have been demonstrated with lactic acid bacteria.

Results:

Initial studies were focused on concentration and purification of Pediocin A to the extent where it could be incorporated into a selective medium suitable for the recovery of transformants. We have not been able to demonstrate activity in culture supernatants in which Pediocin A producing *Pediococcus pentosaceus* has been cultivated. Ultrafiltration or dialysis concentration of supernatants did not result in detection of Pediocin A activity when assayed by the well diffusion method. However, activity is clearly seen with the deferred antagonism method using the same medium (Trypticase Soy Broth).

During the 2nd year we concentrated on optimizing electroporation transformation procedures for Pediococci and Lactococci with the view of introducing the Pediocin A plasmid since we are unable at this time to use Pediocin A as a selective agent. Two alternative approaches are being explored. The first approach is to optimize electroporation conditions so that co-transformation of pMD136 and a directly selectable marker (antibiotic resistant plasmid) could occur. Co-transformants could then be individually screened for Pediocin A production. The second approach is to clone into a directly selectable plasmid vector the Pediocin A genes for production and immunity to Pediocin A. Both approaches are dependent on high transformation frequencies. We have been able to transform 3 of 5 strains of pediococci using the plasmid vector pNZ12. Transformation frequencies were in the range of 2-3 X 10^5 per ug of DNA. We have also been able to obtain transformation frequencies of about 1 X 10^6 per ug of DNA with *Lactococcus lactis* LM 2302 which will be used as a recipient for pMD136- pNZ12 co-
transformation experiments.

During the past year we have redoubled our efforts toward isolating active Pediocin A. Various media formulations were evaluated in both deferred antagonism and supernatant assays. We still have been unable to make significant progress in isolating enough active Pediocin A in sufficient quantities for use in plasmid transformation experiments. It appears that Pediocin A may be a very fragile protein in terms of maintaining bioactivity. We believe that physical manipulations may accelerate loss of activity. During the remainder of the project period (till January 1, 1992) we will explore other isolation and concentration procedures that minimize physical stresses to proteins.

Impact of Research:

Modern genetics has provided the tools for constructing lactic acid bacteria with the ability to enhance the quality of fermented dairy foods and to provide a higher degree of preservation. Genetic transfer systems such as transformation, transduction and conjugation have been demonstrated in lactic acid bacteria and are currently being used by researchers in dairy starter cultures for strain improvement. These technologies will allow us to transfer into dairy cultures the genes that encode for antagonistic substances (bacteriocins) that are active against a variety of undesirable spoilage and pathogenic microorganisms that occur in fermented dairy foods. The inhibition of undesirable microorganisms will allow for enhanced product quality and safety which in the long run will enhance milk utilization.

Of current concern to cheese processors are the occurrence of microorganisms which cause blowing faults (Clostridia tyrobutyricum) in Swiss-type cheese and cheeses. Bacteriocin producing starter cultures is one approach for controlling the incidence of such microorganisms in fermented dairy foods. Certain bacteriocins from non-dairy lactic acid bacteria have been shown to inhibit clostridia and Listeria as well as other pathogens such as Staphylococcus aureus.

The acquisition of bacteriocin producing ability by dairy starter cultures through genetic biotechnology may allow the development of strains superior to those presently available.

Publication and Abstracts:

None

Patents:

None
Project Title: Prediction and Determination of the Efficacy of Nisin in Dairy Foods

Personnel:
Mark A. Daeschel, Principal Investigator, Dept. of Food Science and Technology, Oregon State University
Floyd W. Bodyfelt, Principal Investigator, Dept. of Food Science and Technology, Oregon State University
Dong-Sun Jung, Graduate Research Assistant, Dept. of Food Science and Technology, Oregon State University

Funding Sources: Western Dairy Foods Research Center

Objectives:
1. To determine what molecular components of milk can interact with nisin and affect its activity.
2. To use the information gained from achieving the first objective to predict and determine the efficacy of nisin as a preservation agent in novel dairy foods such as carbonated milk beverages.
3. To determine the efficacy of nisin on the inhibition of Listeria and psychrotrophic bacilli in selected milk and milk products and the subsequent impact on safety and keeping quality.
4. Documentation of how food grade emulsifiers enhance nisin activity against Listeria monocytogenes in high fat containing fluid milk.

Results:
Our previous report provided information on the effect of dairy proteins on nisin activity. Beta-lactoglobulin was observed to provide a protective effect to bioassay indicator bacteria when exposed to nisin in the presence of the protein. It is hypothesized that Beta-lactoglobulin may reduce the activity of nisin by binding it and hence preventing it from inhibiting microbial cells.

More experiments were conducted to determine the effects that various components of milk have on the efficacy of nisin. Two approaches were used. 1) Determination of nisin activity after exposure of nisin to different types and concentration of dairy proteins and fats. A quantitative bioassay based on well diffusion was employed. 2) Effect of different dairy components on the ability of nisin to inhibit Listeria monocytogenes in fluid milk. The most significant effect observed was the reduction in nisin activity as the fat concentration was increased in fluid milk. Nisin activity as determined by bioassay was decreased by
more than 90°/0 when added to high fat (11.5%) fluid milk. Concurrently, it was observed that nisin was less effective in inhibiting *Listeria* as fat concentration increased. A representative experiment is portrayed in the following figure. (Fig. 1)

Figure 1. Effect of % milkfat content of fluid milk on the ability of nisin to inhibit *L. monocytogenes*. Sterile milks containing 0, 10, and 50 U/ml nisin were inoculated with *L. monocytogenes* Jalisco at levels $[\log_{10} 7.15 \text{ cfu/ml for 0 U/ml, 7.57 for 1 U/ml and 7.25 for 50 U/ml}]$.

The newly identified project objective; "Documentation of how food grade emulsifiers enhance nisin activity against *Listeria monocytogenes* in high fat containing fluid milk" was formed on our experimental observation that the emulsifier Tween 80 appeared to "restore" nisin activity in milks containing high amounts of milkfat (4-13%) Fig. 2 and Table 1. Our working hypothesis to explain this observation is: We believe that nisin can bind to the surface of milkfat globules and when this occurs, nisin is no longer active or available to inhibit bacteria. The higher the fat content of milk, the less active or available to inhibit bacteria. The higher the fat content of milk, the less active nisin is. We believe the addition of the emulsifier Tween 80 to milk acts to displace proteins (nisin) from the milkfat globule surface resulting in a restoration of nisin activity.
TABLE 1. Effect of emulsifiers on nisin activity in half-and-half cream and skim milk

<table>
<thead>
<tr>
<th>Sample treatment (all contain 50 U/ml nisin)</th>
<th>Inhibition zone dia. (mm)</th>
<th>% nisin activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>skim milk</td>
<td>24.0</td>
<td>82.7</td>
</tr>
<tr>
<td>skim milk+Tween 80</td>
<td>24.8</td>
<td>100</td>
</tr>
<tr>
<td>skim milk+lecithin</td>
<td>23.8</td>
<td>78.9</td>
</tr>
<tr>
<td>half+half cream (h+h)</td>
<td>16.2</td>
<td>19.6</td>
</tr>
<tr>
<td>(h+h)+Tween 80</td>
<td>21.0</td>
<td>43.4</td>
</tr>
<tr>
<td>(h+h)+lecithin</td>
<td>16.0</td>
<td>19.1</td>
</tr>
<tr>
<td>nisin 50 U/ml</td>
<td>24.8</td>
<td>100</td>
</tr>
</tbody>
</table>

(contROLS) do not contain nisin

<table>
<thead>
<tr>
<th>Sample treatment (all contain 50 U/ml nisin)</th>
<th>Inhibition zone dia. (mm)</th>
<th>% nisin activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>skim milk</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>(h+h)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Tween 80</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>lecithin</td>
<td>0</td>
<td>n/a</td>
</tr>
</tbody>
</table>

n/a=not applicable

Figure 2. Effect of Tween 80 (0.2% vol/vol) on the efficacy of nisin (50 U/ml) in milks with varying fat content. Sterile milks were inoculated with *L. monocytogenes* Jalisco at levels [log$_{10}$ 7.20 cfu/ml for treatments without Tween 80 and log$_{10}$ 7.60 cfu/ml with Tween 80].
The importance or implications of these observations to the dairy industry are both relevant and practical. Nisin has been approved as a GRAS additive in cheese spreads and will likely be approved for use in other dairy products. An understanding of how nisin interacts with dairy food components is paramount in order to optimize its efficacy and application in dairy foods.

We plan to experimentally prove (or disprove) our hypothesis by documenting the adsorption or binding to the milkfat globule and its subsequent desorption. This will be accomplished by labeling the nisin molecule with a fluorescent probe and microscopically visualizing its adsorption to milkfat globules. Desorption by Tween 80 will be documented in the same manner. This information will corroborate our existing data on nisin activity measurements as affected by milkfat and Tween 80.

**Impact of Research:**

Nisin is a member of a group of potent antibacterial substances which are bacteriocins. It has been shown to be effective in inhibiting certain gram positive species but not gram negative bacteria, yeasts or fungi. Recent investigations has indicated that nisin and/or nisin producing streptococci are inhibitory toward *Listeria monocytogenes*, a food-borne pathogen of emerging concern.

Nisin, after 25 years of safe use in many European countries was recently affirmed by the Food and Drug Administration (Federal Register, April 6, 1988) as GRAS for use as an antimicrobial agent to inhibit the outgrowth of *Clostridium botulinum* spores and toxin formation in certain pasteurized cheese spreads. The approval of nisin will justify increased research efforts of both an applied and basic nature on the antimicrobial properties of bacteriocins. It is this author's opinion that nisin will eventually be approved for use in other types of dairy foods once a sufficient body of scientific knowledge has been accumulated to justify approval. The use of nisin as an antimicrobial agent in dairy foods could enhance milk utilization by at least three mechanisms:

1. Inhibition of spoilage microorganisms in dairy foods could minimize economic losses due to spoilage.

2. Inhibition of pathogenic and toxigenic bacteria to provide consistently safe products. Contaminated products (such as with Listeria) can give rise to adverse publicity with subsequent sales loss.

Publications:


Project Title: Generation of Molecular Probes for Bifidobacterial Species

Personnel: Joseph W. Booth, Dept. of Biochemistry and Biophysics, Oregon State University
Janine E. Trempy, Dept. of Microbiology, OSU
William E. Sandine, Dept. of Microbiology, OSU

Funding: Western Dairy Foods Research Center

Objectives:

The popularity of products containing viable bifidobacterial cultures is well known in Europe and Japan and has risen to a certain extent in recent years in such products as yogurt in the U.S. as well. The difficulty with using bifidobacteria as adjuncts in dairy products has been their instability in culture with other lactic acid bacteria. Research into culturing protocols which enable the survival of bifidobacteria when grown with other lactic acid bacteria is in great need but is attenuated by difficulty in quantitating bifidobacteria in culture with other organisms. Attempts to develop a selective plating medium to aid in quantitating bifidobacteria in culture with other organisms have been only marginally successful. We have proposed utilization of modern molecular biology to aid in the difficult problem of quantitating bifidobacteria in culture. The present report updates research into making molecular probes which will be used to tag and identify individual bifidobacterial colonies on colony lifts.

The molecular probes whose generation is sought are (1) an antibody probe against fructose-6-phosphate phosphoketolase--anti-F6PPK, and (2) a DNA probe with a nucleic acid sequence complementary to a portion of the F6PPK gene. The anti-F6PPK probe will quantitate bifidobacterial species by Western blotting colony lifts of plated cultures. The DNA probe will quantitate bifidobacterial species by Southern blots of colony lifts. The F6PPK gene product was chosen as a tag for identification of bifidobacteria because it participates in a unique carbohydrate metabolism pathway known to exist only in bifidobacteria. To generate an antibody probe it is necessary to first purify the F6PPK enzyme. To generate a DNA probe it will be necessary to clone and sequence the gene for F6PPK.

1. Purify the F6PPK enzyme and obtain antisera against the enzyme to use for Western blots.
2. Construct a library of genomic sequences of a *Bifidobacterium* specie; identify a clone which contains the gene for F6PPK using a probe deduced from the peptide sequence of the F6PPK protein; sequence the gene for F6PPK; use the sequence of F6PPK to construct DNA probes which can be used in Southern blots to identify bifidobacterial colonies.

**Results:**

Significant protein purification of the F6PPK enzyme has been achieved using protamine sulfate to fractionate cell-free bifidobacterial extracts. We have found that a 1.474 mg/ml final concentration of protamine sulfate precipitates nucleic acid and unwanted proteins while a concentration of 1.534 mg/ml final concentration of protamine sulfate quantitatively precipitates the F6PPK enzyme.

Used in conjunction with protamine sulfate precipitation, native gel electrophoresis is capable of producing a single band on a gel with F6PPK activity. Native gel electrophoresis will be useful in purifying the F6PPK enzyme.

Experiments are currently underway to generate a library of *Bifidobacterium breve* genomic sequences.

**Impact of Research:**

Generation of molecular probes for the identification of bifidobacterial species will make possible the accurate determination of numbers of viable bifidobacterial cells in mixed cultures containing bifidobacteria and other lactic acid bacteria. This will facilitate marketing a variety of bifidobacterial-containing milk products of uniform quality where numbers of these bacteria are concerned.

**Publications:**

None
Project Title: Characterization of Milk Proteolysis by Lactococcal Starter Culture Strains Using Amino Acid Analysis

Personnel: Rodney J. Brown, Dept. of Nutrition and Food Sciences, Utah State University

Christina Beer, Dept. of Nutrition and Food Sciences, Utah State University

Funding: Western Dairy Foods Research Center

Objectives:

1. Use amino acid analysis to study the interactions of the proteinase genes in Lactococcus lactis ssp. lactis cultures that exhibit different proteinase phenotypes.

2. Stabilize the proteinase gene system in the organism.

3. Find the optimum expression of proteinase genes to improve flavor and texture characteristics in cheese, and to be used for accelerated ripening.

Results:

A new graduate student has begun work on this project. She is becoming familiar with the o-phthaldialdehyde test and amino acid analysis used to characterize proteolysis of milk proteins during growth, the patterns of individual amino acid concentrations used to characterize proteolysis, and cluster analysis for differentiating strains beyond what was possible by visual comparison of the amino acid analysis results. In addition, she is working with the proteinase gene system of Lactococcus lactis ssp. lactis cultures.

Impact of Research:

Proteolysis from the bacterial starter cultures plays a significant role in the physical and organoleptic properties of cheese and other fermented dairy products. Improper proteolysis can result in a wide number of defects, including bitterness, texture, and body problems. We have ways to measure gross proteolysis but are very limited in techniques to profile or characterize proteolysis for individual bacterial strains. The proteinase system in Lactococci is very complex and phenotypic changes relative to genetic manipulation of a culture are impossible to measure with present proteolysis tests. This method would allow us to measure subtle differences in proteolysis as the genetic compliment of a culture is modified. An understanding of the interactions of the various genetic factors would allow for the development and optimizations of cultures used in dairy fermentations. This project has the potential to greatly enhance product quality, allow for the production of products with enhanced properties, and even allow the development of new products by using bacterial strains with different proteolytic abilities. This method would also be very valuable in identifying and characterizing newly developed strains from biotechnological endeavors.
SECTION 1: Project Personnel and Summary

1. Proposal Title:
   1a. For professional interpretation:
       "EVALUATION OF IRON-PROTEIN COMPLEXES IN IRON-FORTIFIED Dairy Products"
   1b. For lay interpretation:
       "CHEMISTRY OF IRON PROTEIN COMPLEXES IN MILK"

2. Principal Investigator:
   2a. Name: Arthur W. Mahoney
   2b. Official Position: Professor
   2c. Mailing Address: Home: 347 North 970 East Logan, UT 84321
      Office: Dept. of Nutrition & Food Sci. Utah State University Logan, UT 84322-8700
   2d. Telephone number:
      Home: (801) 752-9538
      Office: (801) 750-2125

3. Project Duration:
   3a. Number of years: (Circle one) 1 2 3
   3b. Dates of entire Proposed Project:
      From 1 July 1989 through 30 June 1992

4. Funding requested from NDPRB:
   4. Total Amount: $ 41,422
      Year 1: $  
      Year 2: $  
      Year 3: $ 41,422

5. Principal Investigator's Organization: (List exact name of organization that will be the contracting party if project is funded)
   5a. Name of organization: Utah State University
   5b. Send payment to: "N/A"

6. Official Legally Authorized to Sign on Behalf of Investigator's Organization:
   6a. Name: 
   6b. Official Position: "N/A"
   6c. Address: 
   6d. Telephone Number: ( )

7. Administrative Official Responsible for Financial Accountability of Project Funding:
   7a. Name: 
   7b. Official Position: "N/A"
   7c. Address: 
   7d. Telephone Number: ( )
June 3, 1991

Dr. Arthur Mahoney
Dept. Nutrition and Food Sciences
Utah State University
Logan UT 84322-8700

Dear Dr. Mahoney:

The WDFRC’s Technical Advisory Committee (TAC) reviewed your proposal “Evaluation of Iron-Protein Complexes in Iron-Fortified Dairy Products.” The TAC recommends funding of this project by the WDFRC. Clarification of issues delineated below is required by the WDFRC administration prior to the August 10 Operating Advisory Committee meeting. The clarification of these issues can be sent in writing to the WDFRC Director.

1. Describe the “reality” of this issue. Is this a “real” issue and, if so, is the project proposal methodology the appropriate means to address the issue? Are there other issues that should be addressed as part of this study?

2. Is the Research Associate (RA) position justified to conduct this research? Can a graduate student accomplish the proposed research? If a RA is required, will the proposed RA dedicate 100% of his time to this project, or is this RA involved in any of the other research your laboratory is conducting in this line of research?

3. The travel budget should be amended. The NDPRB typically funds $1,000 per project per year for travel considerations. Justification for a travel increase can be presented to the WDFRC. Please make this adjustment clarification and re-submit an amended budget to the WDFRC.

These TAC recommendations regarding project funding and methodology clarification will be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10, 1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and vote for or against the WDFRC’s funding of the project.

I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

Paul A. Savello, Director

c: Janet Williams
Section 1
Project and Personnel Summary

1. Proposed Title
1a. For professional interpretation:
   Identifying Batch of Origin of Finished Cheese Made in Continuous Processes.
1b. For lay interpretation:
   Identifying Batch of Origin of Cheese Exiting From Pressing Towers

2. Principal Investigator
2a. Name
   Lynn V. Ogden
2b. Official Position
   Associate Professor
2c. Home Mailing Address
   1457 East 920 South
   Provo, UT 84606
   Office Mailing Address
   Brigham Young University
   Department of Food Science and Nutrition
   475 WIDB
   Provo, UT 84602
2d. Home Telephone
   (801) 377 0635
   Office Telephone
   (801) 378 6038

3. Project Duration
3a. Number of years
   Two years
3b. Dates of entire Proposed Project
   From July 1, 1991
   Through June 30, 1993

4. Funding requested from NDPRB
   Total Amount
   $48,500
   Year 1  $23,500
   Year 2  $25,000

5. Principal Investigators's Institution
5a. Name of institution
   Brigham Young University
5b. Send payment to:
   Carol Hardman
   Office of Research Administration
   A-261 ASB
   Provo, UT 84602
   79
June 3, 1991

Dr. Lynn Ogden
Dept. Food Science and Nutrition
Brigham Young University
Provo UT 84601

Dear Dr. Ogden:

The WDFRC's Technical Advisory Committee (TAC) reviewed your proposal "Identifying Batches of Origin of Finished Cheese Made in Continuous Processes." The recommendations of the TAC include both budgetary and methodology considerations. These are listed below:

1. The "science" (i.e. "research") part of this project concept had been done in your previous research efforts. This proposal is more "developmental" rather than "research."

2. The TAC realizes that Hunter Labs is contributing "in kind" support of the project by allowing you to use the Qual-Probe instrument at no cost. However, the TAC strongly feels that other interested and beneficiary parties should participate in the funding of the project:
   - A cheese company such as Western Dairymen's should be able to quantify the economic loss that the stated problem addresses and it should be able to justify a significant financial input into the project;
   - Equipment manufacturers of continuous cheese manufacturing (e.g. Stoelting) should be approached to financially participate in the project.

3. The TAC recommends one year support by the WDFRC for $12,000 which represents approximately 1/2 of the requested Year 1 budget. The remainder of the project funds (exclusive of the Hunter Labs "in kind" support) should be obtained from interested and beneficiary parties as suggested above.

4. The project does not fall under NDPRB funding guidelines but suggested support (number 3 above) can be granted by non-NDPRB funds from the WDFRC.

These TAC recommendations regarding project funding and methodology clarification will be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10, 1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and vote for or against the WDFRC's funding of the project.
I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

Paul A. Savello, Director

c: Janet Williams
1. Proposal Title
1a. For professional interpretation: The Influence of Preadsorbed Protein on Adhesion of *Listeria monocytogenes* to Dairy Food Contact Surfaces

1b. For lay interpretation: The Role of Milk Proteins in Microbial Adhesion

2. Principal Investigator
2a. Name Mark A. Daeschel
2b. Official Position Assistant Professor
2c. Home Mailing Address 1874 NW Estaview Drive
   City Corvallis State Oregon Zip 97330
   Office Mailing Address Dept. Food Science and Tech., Oregon State University
   City Corvallis State Oregon Zip 97331
2d. Home Telephone (503) 757-1954
   Office Telephone (503) 737-3463

3. Project Duration
3a. Number of years (Circle one) 1 2 3
3b. Dates of entire Proposed Project From July 1, 1991 Through June 30, 1994

4. Funding Requested from NPDRE Total Amount $89,569
   Year 1 $30,354
   Year 2 $29,671
   Year 3 $29,544

5. Principal Investigator's Institution
5a. Name of institution: (List exact name of institution that will be the contracting party if project is funded)
   Oregon State University
5b. Send payment to: Dept. of Food Science and Technology
   City Corvallis State Oregon Zip 97331

6. Official Legally Authorized to Sign on Behalf of Investigator's Institution
6a. Name Richard A. Scanlan
6b. Official Position Dean of Research
6c. Address Research Office, Oregon State University
   City Corvallis State Oregon Zip 97331
6d. Telephone (503) 737-0663

7. Administrative Official Responsible for Financial Accountability of Project Funding
7a. Name
7b. Official Position Business Services Manager
7c. Address Business Affairs, Oregon State University
   City Corvallis State Oregon Zip 97331
7d. Telephone (503) 737-2595

82
June 3, 1991

Dr. Mark Daeschel  
Dept. Food Science & Technology  
Oregon State University  
Corvallis OR 97331-6602

Dear Dr. Daeschel:

The WDFRC's Technical Advisory Committee (TAC) reviewed your proposal "The Influence of Preadsorbed Protein on Adhesion of Listeria monocytogenes to Dairy Contact Surfaces." The recommendations of the TAC include both funding source and methodology considerations. These are listed below:

1. The proposed research appears to duplicate research currently underway at both the Wisconsin Center for Dairy Research and the Northeast Dairy Foods Research Center. Dr. Amy Wong at the University of Wisconsin Food Research Institute is presently conducting research in bacterial adhesion to biofilms on stainless steel material. How does your proposed research differ in hypothesis, methodology, and anticipated use from those other currently funded projects?

2. Why are silicon wafers used in the proposed research? This material may be experimentally suitable but other "real world" materials (e.g. stainless steel) would be more practical to the dairy industry. Comment on possible revision(s) to the proposal incorporating these other materials.

3. Casein would probably be a more realistic biofilm problem for the dairy industry. Your proposed review of β-lactoglobulin can be conducted but the project should also look at casein-based films.

4. Should other pathogens be researched as well as Listeria? Listeria may be the most tenaciously adhering microorganism to biofilms but other pathogens important to the dairy industry can also be investigated.

5. The proposal does not address what to do about the problem once research information is obtained. Do you have ideas/suggestions about "remedy" of the problem?

6. Is the surface to be investigated a cold or hot surface? Will both cold and hot surfaces be investigated? Which is more important to the dairy industry?

These TAC recommendations regarding project funding and methodology clarification will be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10, 1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and vote for or against the WDFRC's funding of the project.
I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

[Signature]
Paul A. Savello, Director

c: Janet Williams
WESTERN DAIRY FOODS RESEARCH CENTER
RESEARCH PROPOSAL

Section 1

Project and Personnel Summary

1. Proposal Title:
   1a. For professional interpretation:
       Using Whey For Improvement of Exposed Subsoils and Sodic and Saline-Sodic Soils
   1b. For lay interpretation:
       Controlled Land Application of Whey to Improve Crop Yield and Reclaim Soils Damaged By Nature or Man

2. Principal Investigator
   2a. Name: Conly L. Hansen
   2b. Official Position: Associate Professor
   2c. Home Mailing Address: 1310 E. 3100 N.
      Logan, Utah 84321
      Office Mailing Address: Nutrition and Food Sciences
      Utah State University
      Logan, Utah 84322-8700
   2d. Home Telephone: (801) 752-2192
      Office Telephone: (801) 750-2999

3. Project Duration
   3a. Number of Months: 20
   3b. Dates of proposed project: 3/1/1991 to 10/30/1992

4. Funding requested from WDFRC
   Total Amount $35,000
   Year 1 $21,000

5. Principle Investigator’s Institution
   5a. Name of Institution: Utah State University
June 3, 1991

Dr. Conly Hansen
Dept. Nutrition and Food Sciences
Utah State University
Logan UT 84322-8700

Dear Dr. Hansen:

The WDFRC's Technical Advisory Committee (TAC) reviewed your proposal “Using Whey for Improvement of Exposed Subsoils and Sodic and Saline-Sodic Soils.” The TAC recommended funding of this project by the WDFRC. Clarification of issues delineated below is required by the WDFRC prior to the August 10 Operating Advisory Committee (OAC) meeting. The clarification of these issues can be sent in writing to the WDFRC Director.

1. The proposal did not address the effect(s) on microflora in soils. This issue does not have to be specifically addressed in this proposal but mention of it should be made. The TAC suggests that you write your thoughts on this microflora issue and your awareness of it – possibly for future project funding.

2. Budget line items need to be clarified. The budget you presented in the proposal appear to “lump” together Year 1 and the additional eight (8) months. You can clarify these budget line items in either of two (2) ways:
   • If you want to pursue the investigation over a twenty (20) month time period, clarify all budget line items as “Year 1” and “Eight Months of Year 2.”
   • If you so desire, the TAC recommended that the requested funds could be spread over twenty four (24) months (rather than the proposed 20 months). If you want to pursue the investigation over the twenty four (24) months time period, clarify all budget line items as “Year 1” and “Year 2.” Please understand that no funding increase was suggested or recommended by the TAC if you decide to make the proposed project a twenty four (24) month project.

3. The TAC requests more specific information about the objective “Determine maximum safe whey land application rate.” How will this safe rate be determined (measured)? How will you decide what is “safe”?

4. Describe the attempt(s) that will be made to quantitate the “odor problem.”

5. Funding by the WDFRC will be $21,000 for Year 1 of the project.

These TAC recommendations regarding project funding and methodology clarification will be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10, 1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and vote for or against the WDFRC’s funding of the project.
I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

[Signature]

Paul A. Savello, Director

c: Janet Williams
Proposal Title

1. For professional interpretation:

Comparative effects of whey protein concentrate (WPC), lactose, salt, phosphate and pH on cooked yield, bind and acceptability of turkey rolls and boneless hams.

1b. For lay interpretation:

Evaluation of whey protein concentrate as a binding agent in low salt turkey rolls and ham with or without phosphate.

Principal Investigator

2a. Name: Daren Cornforth
2b. Official Position: Associate Professor
2c. Home Mailing Address: 56 W. 200 N.
   City: Providence State: Utah Zip: 84332
   Office Mailing Address: Nutrition & Food Sciences, Utah State University
   City: Logan State: Utah Zip: 84322-8700
2d. Home Telephone: (801) 743-0979
   Office Telephone: (801) 750-2114

Project Duration

3a. Number of Years: 2
3b. Dates of entire Proposed Project From: 7-1-91 Through: 6-30-93

Funding Requested from NDPRB

Total Amount $48,810.00
Year 1 $23,990.00
Year 2 $24,820.00

Principal Investigator's Institution

5a. Name of Institution: Utah State University
5b. Send payment to: Department of Nutrition & Food Sciences
   City: Logan State: Utah Zip: 84322-8700

Official Legally Authorized to Sign on Behalf of Investigator's Institution

6a. Name: M.K. Jeppesen
6b. Official Position: Director, Contracts and Grants
6c. Address: Contracts and Grants Office, Utah State University
   City: Logan State: Utah Zip: 84322-1415
6d. Telephone: (801) 750-1227

Administrative Official Responsible for Financial Accountability of Project Funding

7a. Name: Lyn Janes
7b. Official Position: Controller
7c. Address: Controllers Office, Utah State University
   City: Logan State: Utah Zip: 84322-2400
7d. Telephone: (801) 750-1051
June 3, 1991

Dr. Daren Cornforth
Dept. Nutrition and Food Sciences
Utah State University
Logan UT 84322-8700

Dear Dr. Cornforth:

The WDFRC's Technical Advisory Committee (TAC) reviewed your proposal
"Comparative Effects of Whey Protein Concentrate (WPC), Lactose, Salt, Phosphate, and
pH on Cooked Yield, Bind, andAcceptability of Turkey Rolls and Boneless Hams." The
TAC recommended partial funding of this project by the WDFRC. Clarification of issues
delineated below is required by the WDFRC prior to the August 10 Operating Advisory
Committee (OAC) meeting. The clarification of these issues can be sent in writing to the
WDFRC Director.

1. Does this proposed study provide more information than has been demonstrated in
terms of potential to the meat industry for usefulness than is already available from the
previously funded WDFRC project? A clearer description of this proposed project's
impact beyond previous research efforts is required.

2. The TAC suggested that this project may not require a full two (2) years. Processing
technology of this type has been worked out in earlier work and the TAC views this
present proposal as an "ingredient" project only. Clarify this issue if you intend the
project to be more than an "ingredient" project.

3. The TAC recommended that the WDFRC fund Year 1 of the project for the requested
amount ($23,990). If the project idea is good, matching funds from industry should be
obtained for the second year if such additional time is needed.

These TAC recommendations regarding project funding and methodology clarification will
be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10,
1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and
vote for or against the WDFRC's funding of the project.
I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

Paul A. Savello, Director

c: Janet Williams
Dr. Paul Savello  
Western Dairy Foods Research Center  
Dept. of Nutrition and Food Sciences  
Utah State University  
Logan, Utah  
84322-8700

2/28/91

Dear Dr. Savello

This is a request to modify an extension request (original request attached) for the WDFRC project entitled "Prediction and determination of the Efficacy of Nisin in Dairy Foods" USU#207. We request that the extension be for an additional 6 months from Oct 31, 1991 to March 31, 1992. The original extension request was in the amount of 13,983. I wish to change this to $8240 which reflects subtracting the current balance in the account ($5743).

Sincerely,

Mark A. Daeschel  
Assist. Professor  
Food Science and Tech.
Request for extension of existing WDFRC project

Project: Prediction and Determination of the Efficacy of Nisin in Dairy Foods

Principal Investigators: Mark A. Daeschel and Floyd Bodyfelt, Dept. of Food Science and Technology, Oregon State University

Project History: This project was approved for two years of funding for the period of July 1, 1988 to June 30, 1990. However, the project was not initiated until March 31, 1989 at which time the actual funding was available. In light of this, a no cost extension was approved for a 9 month period. The project is now scheduled to terminate March 31, 1991. Ms. Dong-Sun Jung a Ph.D., a candidate within the Food Science and Technology Dept. has been supported by this project since March 31, 1989 and has made this project the basis of her Ph.D. thesis.

Justification for Project Extension: The primary reason for this extension request is to provide Ms. Jung with a continued source of support so she will be able to complete her Ph.D. and write up the project experimental results for publication. We have one manuscript in preparation and envision at least one more. We believe our extension request is further justified on the basis of the findings obtained thus far from the project (see attached project report). Very recently, Ms. Jung has discovered that certain surface active agents (emulsifiers, stabilizers) have an enhancement effect of nisin in dairy foods. These observations promise to provide new strategies for the effective use of nisin and other protein antimicrobials in dairy foods.

Funding Request: Our request is for one year's additional funding to provide support for Ms. Jung.

Graduate Research Assistantship @ .50 FTE for 12 months $ 9,261
OPE at 5% $ 463
Tuition $ 4,258
Total $13,983

Attachments: Original project proposal and 1990 project report.
June 3, 1991

Dr. Mark Daeschel
Dept. Food Science & Technology
Oregon State University
Corvallis OR 97331-6602

Dear Dr. Daeschel:

The WDFRC's Technical Advisory Committee (TAC) reviewed your request for project extension of project "Prediction and Determination of the Efficacy of Nisin in Dairy Foods." The TAC recommended funding of this project by the WDFRC. Clarification of issues delineated below is required by the WDFRC prior to the August 10 Operating Advisory Committee (OAC) meeting. The clarification of these issues can be sent in writing to the WDFRC Director.

1. The results of the originally WDFRC-funded project (same project title) must be reported to the WDFRC. The originally approved objectives (three) should be completed as part of the original study and within the budget.

2. The newly identified objective (the effect of emulsifying agent on nisin activity) should be clearly stated. How is this new objective important to the dairy industry? What methodologies will be used to investigate this new objective?

3. The additional funding requested should be targeted to the investigation of the newly identified objective. You must present to the WDFRC a line item budget clearly indicating how these additional funds will be spent in this research effort.

4. The project completion (of the newly identified objective) will be March 31, 1992. No project extension will be granted beyond that termination date.

These TAC recommendations regarding project funding and methodology clarification will be presented to the Operating Advisory Committee (OAC) of the WDFRC at the August 10, 1991 meeting in Logan, Utah. The OAC will consider these TAC recommendations and vote for or against the WDFRC's funding of the project.
I will be on a business trip to Boston on June 5-13. If you have any specific questions regarding these TAC recommendations, please contact me after June 13.

Respectfully,

[Signature]

Paul A. Savello, Director

c: Janet Williams
The following are the minutes of the Operational Advisory Committee (OAC) Meeting of the Western Dairy Foods Research Center held on August 10, 1991 on the campus of Utah State University.

Committee members in attendance:

Janet Williams (NDPRB)
Rodney Brown (USU)
W. Lee Reese (out-going Utah Dairy Commission member)
Sheldon Pratt (Oregon Dairy Products Commission)
Jeff Strandholm (Kraft General Foods, Inc.)
Raj Narasimmon (Schreiber Foods, Inc.)
Gale Moser (United Dairymen of Idaho)
Douglas Wilrett (Marchall-Rhone Poulenc, Inc.)
Boyd Gardner (in-coming Utah Dairy Commission member)
Harry Papageorge (NDPRB Research Committee)
Clint Warby (Utah Dairy Commission)
Paul Savello (Director, WDFRC)

Visitors were also in attendance and asked to participate in discussions during the meeting.

1. Five (5) new project recommendations (from the Technical Advisory Committee meeting of June 3, 1991) were considered by the OAC. Actions were taken by the OAC on these recommendations as follows:

   1. Project Title: Evaluation of iron-protein complexes in iron-fortified dairy products.
      PI: Arthur Mahoney (USU)
      Motion: By Gale Moser. OAC direct Director Savello to accept further explanation on approved project and to act on his discretion accordingly provided budget presented is not exceeded.
      Second: Boyd Gardner
      Action: Passed.

   2. Project Title: Identifying batch of origin of finished cheese made in continuous processes.
      PI: Lynn Ogden (BYU)
      Motion: By Rodney Brown. Approve project providing matching industry funds and others being obtained and TAC recommendations (of June 3, 1991) being met.
      Second: Lee Reese
      Action: Passed
3. Project Title: The influence of preadsorbed protein on adhesion of *Listeria monocytogenes* of dairy food contact surfaces.
PI: Mark Daeschel (OSU)

Motion: By Floyd Bodyfelt. Approve contingent upon notification from NDPRB that project is not duplication of other work in similar research area.

Second: Sheldon Pratt

Action: Passed with one (1) dissenting vote

4. Project Title: Using whey for improvement of exposed subsoils and sodic and saline–sodic soils.
PI: Conly Hansen (USU)

Motion: By Gale Moser. Approve project as submitted.

Second: Rodney Brown

Action: Passed

5. Project Title: Comparative effects of whey protein concentrate (WPC), lactose, salt, phosphate and pH on cooked yield, bind and acceptability of turkey rolls and boneless hams.
PI: Daren Cornforth (USU)

Motion: By Rodney Brown. Approve project contingent upon recommendations of TAC (June 3, 1991) being met at discretion of Director.

Second: Gale Moser

Action: Passed

II. One (1) recommendation (from Technical Advisory Committee meeting of June 3, 1991) to approve increased funding level for existing WDFRC project was considered. The action taken on this recommendation by the OAC was as follows:

1. Project Title: Prediction and determination of the efficacy of nisin in dairy foods.
PI: Mark Daeschel (OSU)

Motion: By Rodney Brown. Approve project extension and additional funding as requested.

Second: Floyd Bodyfelt

Action: Passed
III. The future focus and direction of the WDFRC was discussed. Director Savello presented slides describing the strengths of the WDFRC. Savello explained that the NDPRB is urging the Dairy Centers to carefully determine the focused research area(s) that each Center will pursue in the future (extension) years of Center activities. Input regarding the focused research area(s) of the WDFRC from OAC members (and from visitors) was solicited by Director.

Warby: The people (i.e. researchers/staff) at the WDFRC should be evaluated to help determine what the focused research area(s) should be.

Williams: The Center needs to know who will be doing what type of research.

C.A. Ernstrom (visitor): The quality/experiences of the people in the Center determine the Center’s focus. The personnel of the WDFRC has changed since its inception in 1987. These changes must be considered in determining the Center’s focused research area(s).

Brown: A strength of the WDFRC is its research activities in milk proteins and how these proteins apply to dairy products.

Williams: The Center should carefully select a “main focus” and augment this with ancillary/support research areas.

Wilrett: Focusing research can also be accomplished by making project proposals within the Center more directed.

Motion: By Clint Warby. To have the WDFRC consider the focused research area by “milk proteins” with research conducted in thermal processing, microbiology, cheese, and new opportunities for use of milk products and to have Director prepare revised proposal to be submitted to NDPRB and OAC with focused area (or 2 to 3 areas) of research.

Second: A second was presented.

Action: Passed

IV. The OAC set the dates July 9 and 10, 1992 for the 1992 Western Dairy Foods Research Center Annual Meeting, to be held in Corvallis, Oregon on the campus of Oregon State University.