Report and Planning Meeting

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WESTERN DAIRY FOODS RESEARCH CENTER

REPORT AND PLANNING MEETING

Eccles Conference Center
Rooms 307-309

Utah State University

July 20-21, 1988
Figure 1. Major and supporting research objectives of the Western Dairy Foods Research Center.
WESTERN DAIRY FOODS RESEARCH CENTER
REPORT AND PLANNING MEETING

Eccles Conference Center  Rooms 307-309
Utah State University

July 20-21, 1988

AGENDA

July 20

8:30 am    WELCOME................................................................. Richardson

8:40 am    A. NDPRB PROJECT REPORTS  (15 min. max. each)

Research Area: Microbiology of Starter Cultures

<table>
<thead>
<tr>
<th>Project Title</th>
<th>Description</th>
<th>Investigators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of Omega-3 Fatty Acids in Whey Permeate</td>
<td>To use <em>S. parasitica</em> to produce long chain polyunsaturated fatty acids in whey permeate as an alternative to Omega-3 fatty acids.</td>
<td>Bodyfelt, Sandine, Selivonchick, Beattie</td>
</tr>
<tr>
<td>Cloning Nisin / Other Genes into <em>Leuconostoc</em></td>
<td>To introduce the inhibitory properties of nisin into <em>Leuconostoc</em> organisms to eliminate selected pathogens in fermented dairy foods.</td>
<td>Sandine, Kondo, Broadbent</td>
</tr>
<tr>
<td>Characterization of Bacteriophage Receptor Sites</td>
<td>To establish a mechanism to prevent bacteriophage from attacking lactic organisms during cheesemaking to improve cheese quality.</td>
<td>Sandine, Geller</td>
</tr>
<tr>
<td>Growth and Survival of Bifidobacterium in Milk</td>
<td>To improve the growth and survival of bifidobacterium in milk to improve its nutritional quality.</td>
<td>Sandine, Ayres</td>
</tr>
<tr>
<td>Purification &amp; Genetic Transfer of Bacteriocin from <em>Pediococcus pentosaceus</em></td>
<td>To purify Pediocin A and transfer its plasmid into dairy fermentation strains to control Clostridia and Listeria.</td>
<td>Dacschel</td>
</tr>
</tbody>
</table>

10:00 am    Refreshment Break
10:15 am

**Temperate Phages as Potential Genetic Sources for Lytic Bacteriophages**

To relate temperate phages to known phage infection and develop a mechanism to alter the infectivity and prevent quality defects in cheeses.

Kondo, Salih

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**Research Area: Curd Formation / Cheese Technology**

- **Improving Mozzarella Properties using Proteinase Negative Cultures**
  - To increase the storage stability of Mozzarella cheese by eliminating body softening due to proteolysis.
  - Richardson, Oberg, Moyes, Wang

- **Effect of Milk Clotting Enzymes on Curing / Quality of Cheddar Cheese**
  - To reduce bitterness in Cheddar cheese by eliminating residual enzyme activity.
  - Emstrom, Yiadom-Farkye

- **Interactions of Protein & Polysaccharides in chymosin and Acid Coagulation of Milk**
  - To incorporate more of the whey proteins and water in cheese during the make process resulting in a cheese with better nutrition and softer texture.
  - Olsen, Muncy, Broadhead, Warner

- **Improved Cheese Manufacture Through Vat Monitoring**
  - To improve the cheesemaking process by monitoring the critical changes during the make procedure.
  - Richardson, LeFevre

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**Research Area: Ultrafiltration and Reverse Osmosis**

- **Production of Cottage Cheese from UF Retentate**
  - To develop an enclosed continuous process for cottage cheese that will increase its shelf-life.
  - Odgen, Lord

**12:00 noon**

**Luncheon Taggart Student Center**

(Members of Operational Advisory Committee, Investigators and Graduate Students)

**1:30 pm**

**Research Area: Product Quality**

- **Rapid Assay for Heat Resistant Proteases in Milk**
  - To develop a simple test for enzyme activity in heat treated milk related to product quality.
  - Bodyfelt, Griffiths

- **Milk Proteins as Whitening Agents in Meats**
  - To develop a new use for milk proteins to lighten the color of veal and poultry rolls making them more acceptable to the consumer.
  - Cornforth, Dobson
<table>
<thead>
<tr>
<th>Topic</th>
<th>Description</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Fortification of Cheese Curd</td>
<td>To develop dairy foods as significant sources of iron for consumers seeking this benefit.</td>
<td>Mahoney, Zhang</td>
</tr>
<tr>
<td>Control of Spore and Adhesion to Dairy Products and Packaging Surfaces</td>
<td>To establish conditions that resist the adsorption of spores and bacteria to dairy product and packaging surfaces, improving product storage stability.</td>
<td>McGuire</td>
</tr>
<tr>
<td>Cheddar Cooling: Effect of Cheese Composition and Cooling Method</td>
<td>To establish the cooling rates that result in flavor and color defects in block Cheddar cheese.</td>
<td>Torres</td>
</tr>
<tr>
<td>Application of Fourier Transform IR to Milk and Dairy Foods</td>
<td>To develop a rapid method to detect vegetable fat in imitation or adulterated dairy foods.</td>
<td>Brown</td>
</tr>
</tbody>
</table>

3:00 pm Refreshment Break

3:15 pm B. Non-NDPRB PROJECT REPORTS (10 min. each)

<table>
<thead>
<tr>
<th>Topic</th>
<th>Description</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability of Proteins During Acid Coagulation of Milk</td>
<td>To determine changes that occur in milk during acidification. To model the coagulation process. To determine the means of controlling acid coagulation.</td>
<td>McMahon</td>
</tr>
<tr>
<td>An Edible Film from Whey Proteins</td>
<td>To fabricate an impermeable film for coating foods.</td>
<td>Savello</td>
</tr>
<tr>
<td>UHT Treatments</td>
<td>To improve milk stability upon high heat treatment.</td>
<td>Savello</td>
</tr>
<tr>
<td>Cheese Markers</td>
<td>To identify different lots of cheese in continuous plant operations.</td>
<td>Ogden</td>
</tr>
<tr>
<td>Biogas</td>
<td>To develop a new technology for cheese whey permeate which can provide additional income to milk procedures and processors.</td>
<td>Hansen</td>
</tr>
<tr>
<td>American Style Cheese from UF Retentate</td>
<td>To increase yield and to obtain low moisture in UF cheese.</td>
<td>Orme, Enstrom, McMahan</td>
</tr>
<tr>
<td>Lactic Culture Selection</td>
<td>Prevention of bitterness in Cheddar Cheese</td>
<td>Richardson, Oberg, Glover</td>
</tr>
</tbody>
</table>
The following abstracts are of papers recently presented at the annual ADSA meetings in Edmonton, Canada. They will be reviewed, in abbreviated versions, by the authors. Those in bold type will have been covered in the earlier reports.


5:00 pm  Break

6:00 pm  Steak Fry, Jensen Historical Farm
          (Operational Advisory Committee, Investigators and Graduate Students)

July 21

8:30 am  B. Finish Reports

C. OPERATIONAL ADVISORY COMMITTEE MEETING

1. Minutes

2. Modifications of present projects

3. New Projects / Directions

4. Approve budget 1988-89

5. Schedule next meeting, 18-19 July 1989? Bear Lake Training Center

D. TECHNICAL ADVISORY COMMITTEE MEETING
Not all streets or roads are named on maps. Construction of streets and roads may be in progress in certain areas.
1. Borden Foods, Refrigerated Products
   Division of Borden, Inc.
   Mr. Bob Crawford, Director, Research and New Product Development
   802 South Street
   Plymouth, Wisconsin 53703

   Dr. John K. Wright
   Director, Marketing and Sales
   Galloway West Co.
   P.O. Box 987
   Fond Du Lac, WI 54935
   Office (414) 922-0600
   Orders (800) 558-9714

   Dr. Mostafa Galal
   Syracuse Research Center
   600 North Franklin Street
   Syracuse, New York 13204

2. Dr. Tom Holzinger
   Borden, Inc.
   960 Kingsmill Parkway
   Columbus, OH 43229
   (614) 431-6623

3. Kraft, Inc.
   Research and Development Division
   Dr. Don W. Mather, Vice Pres., Product Development Dairy Products
   Dr. James Moran
   801 Waukegan Road
   Glenview, Illinois 60025
   (312) 998-3717

4. L. D. Schreiber Cheese Co., Inc.
   Dr. Rajagopalan Narasimhan, Director, Product Development
   P. O. Box 610
   425 Pine Street
   Green Bay, Wisconsin 54305
   (414) 437-7601

5. Intermountain Milk Producers
   Mr. Douglas Larsen
   Cache Valley Dairy
   Smithfield, UT 84335
   (801) 563-3281
6. Damrow Company
Mr. George Manninen
1919 S. Stoughion Road
Madison, WI 53716

Mr. Al Pittelko
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Fond Du Lac, WI 54935
(414) 922-1500

7. Mr. Paul Bjerre, Director of Research
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2 Europaplads
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AArkus, C. Denmark
+45-6-12 41 55

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De Danske Sukkerfabrikker
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DK-1001 Copenhagen K
Denmark

8. Chr. Hansen's Laboratory, Inc.
Dr. Robert Sellars
Dr. Clint Washam
9015 W. Maple St.
Milwaukee, WI 53214
(414) 476-3630

9. Miles Labs
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Logan, UT 84321
(801) 752-6820

Dr. Randy Thunell
Mr. Gary Burningham

10. Utah Dairy Commission
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(801) 487--9976

Mr. W. Lee Reese
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(801) 752-3572
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12. Mr. Cliff Eidemiller
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(208) 337-3083

Mr. Don Papenberg
Administrator, United Dairymen of Idaho
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Boise, ID 837006
(208) 334-4316

13. Mr. Sheldon Pratt
Oregon Dairy Products Commission
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Portland, OR 97219
(503) 229--5033

14. Mr. Ray Focht
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Landier, WY 82520
(307) 332-3123

Mr. Thomas C. Jenkinson
General Manager
Western Dairy Farmers Promotion Association
12450 N. Washington
P. O. Box 33120
Thorton, CO 80233-0120
(303) 451-7721

15. Mr. Greg Rowley
Production Manager
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16. Dr. Robert Lamb
USDA, ARS
Utah State University
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Logan, UT 84322-4815
(801) 750-2159

17. Dean Doyle J. Matthews
Dean, College of Agriculture
Utah State University
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18. "Dr. Richard A. Scanlan, Head
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19. "Dr. Charles C. Hunt
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20. "Dr. Gary H. Richardson
Western Dairy Foods Research Center
Acting Director
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(801) 750-2120

21. "Dr. Rodney J. Brown
Department Head
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Dept. of Nutrition and Food Sciences, UMC 8700
Logan, UT 84322-8700
(801) 750-2126

22. "Dr. Alan R. Huggins
Vice President
Dairy Research Foundation
6300 North River Road
Rosemont, IL 60018-4289

23. "Floyd W. Bodyfelt
422 Nash Hall
Department of Microbiology
Oregon State University
Corvallis, OR 97331

24. Mr. Harry Papageorge
1616 W. Farr-West Drive
Ogden, UT 84404
(801) 782-9383
WESTERN DAIRY FOODS RESEARCH CENTER

REPORT TO OPERATIONAL ADVISORY COMMITTEE

Inclusive dates 4/88 - 7/88

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

Project Number:

Principal Investigator: Floyd W. Bodyfelt

Research and Graduate Assistants: Sam Beattie

Project Objectives: See Attached

Summary of Results During the Year: See Attached

Papers, Abstracts, Technical Reports:
(These can be listed here by literature reference only and complete copies made available at the OAC meeting for those who want the more detailed report).
Project Objectives:

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

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

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

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

The objectives of this work are 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, we are concentrating on cloning lactose metabolizing genes into *S. parasitica*.

Initial studies have examined lipid profiles of *S. parasitica* grown in media limiting the carbon and nitrogen sources. The defined medium that showed optimum omega-3 fatty acid (Eicosapentenoic acid, [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 24.69% EPA. When a casein hydrolysate was used as sole nitrogen source, EPA production averaged 18.4% of the total fatty acids. Limiting the nitrogen source adversely affected the growth and EPA production of *S. parasitica*, but increased total lipid production to 4.0% (weight basis). The information provided by this data provides a baseline for further studies using genetically altered *S. parasitica*.

Since *S. parasitica* does not contain enzymes necessary for lactose metabolism, it is necessary to genetically transform the organism. The first step to this is formation of stable protoplasts that are able to regenerate cell walls and form normal hyphae. Stable protoplasts of *S. parasitica* have been generated using a combination of enzymes (Driselase [fluka] - on extract from Irpex and cellulase) and stabilized by 0.5 M KCl and 1 M sorbitol. With this method, approximately 20% of the protoplasts regenerate and current work is focused on increasing regeneration percentages.

Initial plans were to use a mitochondrial plasmid from *S. parasitica* as a portion of a vector plasmid that would contain the genes for lactose metabolism. Based on electrophoresis, no mitochondrial plasmids have been found thus far. Therefore, we intend to employ a "shot gun" approach by using chromosomal DNA fragmented by restriction enzymes.
I. Project Title: Cloning nisin and other genes of lactic streptococci into Leuconostoc species and amplification of nisin production.

II. Personnel
Principal Investigators:
J. K. Kondo, Department of Nutrition and Food Sciences, USU
W. E. Sandine, Department of Microbiology, OSU

Graduate Students (OSU):
Herb Wycoff
Hamdy Mahmoud
Noreddine Benkerroum

III. Objectives
1. To produce and characterize lactose positive Leuconostoc transconjugants obtained by conjugal matings between S. 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 (e.g. S. 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 S. 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 spore formers 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.

IV. Progress Summary

Leuconostoc strains were examined for proteolysis, aldolase, β-galactosidase, phospho-β-galactosidase, acid and diacetyl production in milk. Wild type strains produced pH values from 5.1 to 6.2 when incubated at 30°C for 48 hours. Specific activity of β-galactosidase in 13 strains ranged from 0 to 339 units. Units of phospho-β-galactosidase ranged from 4 to 58. All strains were aldolase negative and produced gas from glucose. The plasmid content varied from 1 to 5. L. cremoris 19254 was transformed by electroporation using chromosomal DNA obtained from a derivative of S. lactis
C2. Colonies with elevated lactose utilizing ability were selected from M17 agar plates containing vancomycin and bromcresol purple. Transformants were aldolase negative and resistant to phage c2. They showed enhanced acid production in milk; e.g. the parent strain yielded a pH of 6.2 after 48 hours at 30°C in nonfat milk while transformed strains yielded pH values of 5.6. Transformants also had elevated β-galactosidase and phospho-β-galactosidase activities, with the former being elevated the most.

V. Publications


I. **Project Title:** Characterization of bacteriophage receptor sites of lactic streptococci.

II. **Personnel**

Principal Investigators:
Bruce Geller and W. E. Sandine
Department of Microbiology, OSU
Graduate Student
Rudy Valyasevi

III. **Objectives**

1. To investigate the molecular basis of phage infection in lactic streptococci (*S. lactis, S. cremoris, S. diacetylactis*).

2. To develop an assay to quantitate phage receptor activity.

3. To study the effects of chemical modifications and perturbations on phage receptor activity.

4. To fractionate cell membrane and cell walls into individual types of components and test each for receptor activity.

5. To identify the bacterial components responsible for receptor activity and to determine identity variations within species and between strains.

6. To examine phage resistant mutants of lactic streptococci (nonphage-absorbing mutants) to determine the mechanism of resistance with the objective to ultimately produce permanently altered strains unable to be attacked by bacteriophages.

IV. **Progress Summary:** Phage for *Streptococcus lactis, Streptococcus cremoris* and *Streptococcus diacetylactis* are being studied to determine the nature of the phage receptor sites for their different hosts. Number of phage receptor sites per cell have been found to range from $1 \times 10^6$ to $3 \times 10^4$ for *S. lactis* C2 and *S. diacetylactis* 18-16, respectively. A procedure was developed to isolate cell fragments which would adsorb phages. Fragments were perturbed with sodium dodecyl sulfate and mutanolysin to determine the location of the phage receptor sites. Data indicate that host receptor sites are located on the cell wall rather than the cell membrane.

V. **Publications:** None.
I. Project Title: Studies on growth and survival of Bifidobacterium species in milk.

II. Personnel

Principal Investigators:
J. W. Ayres, School of Pharmacy, OSU
W. E. Sandine, Department of Microbiology, OSU

Graduate Student:
Ronshan Cheng

Visiting Scholar:
Xintian Ming

III. Objectives

1. To study the requirements of bifidobacterial species for growth and stability in milk.

2. To use the oxygen consuming membrane fraction (oxyrase) of E. coli to provide anaerobiosis during the growth of bifidobacteria in milk.

3. To use oxyrase in improving plating conditions for enumeration of bifidobacteria in various products.

4. To use newly acquired customized state of the art fermentation equipment to optimize growth parameters to maximize cell numbers and stability.

5. To determine the best delivery system for bifidobacterial cells (lyophilized or frozen concentrates) to produce a sweet bifidus milk.

6. To study the associative growth of bifidobacterial species with other organisms used in dairy fermentations such as lactobacilli, streptococci and Leuconostoc.

IV. Progress Summary: Seven bifidobacterial ATCC strains and two human infant fecal isolates are included in the study. All grow well in reinforced clostridial medium incubated anaerobically (10^8 to 10^9 cfu/ml) but growth in milk is impaired. Bifidobacterium longum, for example, declines at least one logarithm (10^8 to 10^7 cfu/ml) when inoculated into nonfat milk and incubated at 37°C. Growth was improved in milk by addition of 0.05% cysteine; counts of 10^8 cfu/ml were attained by 12 hours at 37°C but declined thereafter to less than 10^5 cfu/ml by 72 hours. Buffering cysteine-containing milk with trimagnesium phosphate minimized cell death and stabilized counts at 10^3 cfu/ml.

An oxyrase-containing whey based medium (whey, 7%; yeast extract, 0.5%; cysteine, 0.05% and oxyrase, 20 µl/ml) shows promise for growing bifidobacteria on an industrial scale. Oxyrase and cysteine together offer better growth and a shorter lag period than either of these supplements alone. Maintenance of the pH with ammonium hydroxide is necessary because the optimum pH for oxyrase to consume dissolved oxygen is above 7.0. Cost consideration, however, may preclude use of oxyrase.

V. Publications: None; a review of recent literature on bifidobacteria is attached.
Bifidobacteria are present in the intestinal tract of humans and various animals, and are also found in the human vagina and mouth (1). They are thought to play a significant health role in the intestine of infants. Bifidobacteria are predominant in the intestine and faeces of breast-fed newborn infants (2,3,4,5). Lower morbidity and mortality are seen in breast-fed infants than in bottle-fed infants which has lead many people to study the health role and ecology of bifidobacteria (2,3,5,6,7).

These bacteria are strictly anaerobic or microaerophilic in the presence of carbon dioxide. Some strains, extremely sensitive to oxygen, require a low oxidation reduction potential for growth and fermentation. However, the need for anaerobiosis is reported to be different for different strains (1,4,8).

Morphology

Bifidobacteria are gram-positive rods but often stain irregularly, especially in older cultures. Freshly isolated strains may be branched, bifurcated Y and V, or spatulate shapes, but may become straight or curved rods of various width and length, and may break resembling branching (1,4). Sodium chloride and chlorides of other univalent cations have been reported to induce pleomorphism. Reversal of this induction by calcium chloride was reported by Koima et al. (9). They reported that branching in bifidobacteria was principally due to inability to form cross walls when grown in a medium deficient in calcium ions. The exact mechanism of branching in bifidobacteria is still unknown.
It has also been reported that microaerophilic, branched bifid bacteria abruptly change to straight rods capable of aerobic growth. Coccoid granules in branched forms were thought to give rise to straight rods (10). This effect has not been explained as either a mutation or contamination (4).

**Taxonomy and Identification**

Classification of bifidobacteria is controversial. Before 1957, they were included in the genus *Lactobacillus* as *Lactobacillus bifidus*, but now they are classified in the genus *Bifidobacterium* on the basis of carbohydrate fermentation, physiological characteristics, and DNA-homology patterns (1,4,11).

Bifidobacteria are classified into eleven species. Differentiation from closely resembled genera of *Lactobacillus*, *Actinomyces*, *Propionibacterium*, and *Eubacterium* can be achieved by biochemical tests. Species differentiation within the genus is based on carbohydrate fermentation (1,11).

The DMS rapid CH strip has 49 different carbohydrate fermentation tests which may be used for identification of bifidobacteria (8). Another system, An-Ident Strip, is also available for evaluation of carbohydrate metabolism. This strip consists of 20 microcouples containing dehydrated substrates. It was designed for rapid carbohydrate utilization and enzymatic tests.

All bifidobacteria ferment glucose, galactose, and fructose. All strains found in humans ferment lactose, lactulose, and N-acetylglucosamine, except *B. adolescentis* and *B. longum* which do not ferment N-acetylglucosamine (1,12).
For bifidobacterial taxonomy, DNA is important. Guanine plus cytosine (G+C) content of DNA varies among different genera of bacteria. The G+C content of Bifidobacterium is about 60.1% (57.2 - 64.5 moles%). However, this general DNA-base composition information can not be used to differentiate bifidobacterial species. DNA homology patterns are required.

Bifidobacterial Species Commonly Found in the Human Gastrointestinal Tract

Bifidobacteria constitute a major part of the fecal flora of healthy humans. They are the predominant microorganisms in the feces of breast-fed infants and increase rapidly with initiation of breast-feeding (2,3). The most prevalent species of bifidobacteria in infants were reported to be B. bifidum type b, B. infantis ss. infantis, B. breve ss. breve, B. breve ss. parvulorum, and B. longum ss. longum type b(11). In children, adults, and senile men, B. adolescentis type a and b, and B. longum were found in high numbers in the intestine. Bifidobacterium bifidum and B. breve occur in both infants and adults (1). Bifidobacterium infantis is believed to be the dominant organism in breast-fed infants and has been isolated most frequently from the feces of babies (1,5,11). However, in a study by Benno et al., B. breve was found to be the most predominant species in both breast-fed and bottle-fed babies (5). Bifidobacterium adolescentis, B. bifidum, and B. longum were often recovered from the feces of babies, but B. infantis was not isolated. They suggested that this might be due to the environment surrounding the babies. Also, the Bifidobacterium strains were suggested be able to be transmitted from infant to infant by the hands of the nurse (11).
Variations occurred within the *Bifidobacterium* species in different hospitals. Infants in the same hospital harbored similar predominating strains.

The bifidus flora of infants was also studied by Beerens et al. (16). The distribution of different *Bifidobacterium* species in the feces of formula-fed infants was reported to be: 60% *B. longum* and 18% *B. infantis*; minor species included *B. bifidum* (13%), *B. breve* (8%) and *B. adolescentis* (1%). In breast-fed infants, 72% were *B. bifidum* and 28% were *B. longum*. *Bifidobacterium infantis* was not the predominant species in either group (6). It should be noted that *B. infantis* was reported to be a synonym of *B. longum* (17).

**Development of Bifidobacteria in Humans and the Influence of Breast-Feeding**

Bifidobacteria appear in stools of breast-fed infants 2 to 5 days old. They become predominant in the feces and increase to represent 99% of the bacteria present (1,3). Development of the intestinal flora of 13 healthy newborn infants was studied by daily culture of the feces (2). Bifidobacteria were the most prevalent organisms in both breast-fed and bottle-fed infants one month old, but the bifidus bacteria in the bottle-fed infant feces were only one tenth as many as for breast-fed infants (2). Also, bifidobacteria were in larger numbers than enterobacteria in the stool of breast-fed infants by a ratio of 1,000:1, but enterobacteria exceeded bifidobacteria by 10.1 in bottle-fed infants at day 6 (3). In breast-fed infants, the pH of the feces was within the range for prevention of growth of *Clostridium* and *Escherichia coli*. The feces had higher counts of bifidobacteria and *S. faecium* compared to *E. coli* (15).
From one month until solid foods are given, breast-fed babies have a simpler intestinal flora, consisting mostly of bifidobacteria and fewer enterobacteria and enterococci than bottle-fed babies (3,5). The number of enterobacteria and enterococci in the large bowel of breast-fed babies rises sharply after introduction of solid foods. Thereafter, the anaerobic bacteria in the large bowel of both groups resemble those of adults (1,3,6). The ratio of anaerobes to aerobes has been reported to be a useful index of intestinal populations (3,5).

The importance of human breast milk to bifidobacterial growth was studied (6,14,15,16). Human milk is high in lactose, low in protein, and relatively low in buffer capacity. The pH is maintained at acid levels (pH=5.0) which inhibits the growth of Bacteroides, Clostridium, and E. coli (6). It was originally believed that the bifidus flora of breast-fed infants inhibited the growth of coliforms directly, but later acetic acid and acetate buffer at pH of 5-6 were found more important for inhibition of enteric pathogens (1,14,15).

Bifidus growth factors in human milk have been described (6,14,17). Human K-casein has bifidus growth-promoting activity and becomes much more effective after treatment with chymosin or pepsin, when human glycomacropeptide (GMP) is released. Both the sugar portion and polypeptide portion of human GMP may play significant roles as bifidus factors (1,17). Substances proposed to be growth promoting include:

1. Lactulose, a disaccharide composed of galactose and fructose, which is not present in a free state in human milk (1,18).

2. N-acetyl glucosamine-containing saccharide. It is used by bifidobacteria for cell-wall synthesis and is present in human milk (1,6).
3. Derivatives of pantothenic acid. Pantethine is required for the growth of B. bifidum var. pennsylvanicus (1,6).

The most frequent pathogens of neonatal infections are coliform organisms and streptococci. Since human milk promotes the growth of bifidobacteria, suppresses the growth of coliform and other pathogenic bacteria and confers resistance to gastro-intestinal infections in neonates, breast-feeding may be considered beneficial to health (2,14,16). It seems likely, therefore, that much benefit can be attributed to increases in bifidobacteria which occur during breast feeding. Also, B. longum has been reported to develop a 'barrier effect, in a germ free rat mode, against pathogenic E. coli.

Mortality of these rats was absent when E. coli was given after B. longum. When E. coli was given alone or before B. longum, mortality was 20% (19).

Selective Isolation and Pure Culture Techniques

Selective isolation of bifidobacteria when they are outnumbered by other anaerobes is difficult (8). Pevely's selective medium for bifidobacteria has been improved (called MPH) by adding riboflavin, nucleic acids, pyruvic acid, and nalidixic acid. It was reported that when used under strictly anaerobic conditions for isolation of bifidobacteria from feces, this medium exhibited selective and high viable counts close to those for a nonselective medium (19).

Antibiotics such as neomycin, kanamycin, or nalidixic acid have been used as the primary selective agent to suppress almost all non-Bifidobacterium strains while inhibiting the growth of bifidobacteria least. Most bifidobacteria are resistant to kanamycin,
nalidixic acid, gentamicin and metronidazole (20). Kanamycin was suggested for selection at a concentration of 80 µg/ml. Nalidixic acid showed a low activity against bifidobacteria at 10 µg/ml but inhibited 90% of bifidobacteria at 344-777 µg/ml (21). Also, when forty strains of bifidobacteria arbitrarily selected were tested at 200 µg/ml of nalidixic acid, polymixin B, or neomycin, then the positive growth of 31 (77.5%), 23 (57.5%) and 6 (15.0%) strains was observed, respectively (19). Therefore, nalidixic acid was used in the modified selective medium (MPN). Further study revealed that nalidixic acid at a concentration of 100 µg/ml in MPN medium suppressed almost all non-bifidobacterium strains. It also was noted that Bifidobacterium species can grow abundantly with ammonia as the sole nitrogen source if strictly anaerobic conditions are provided.

Bifidobacteria with their peculiar morphogenesis, anaerobiosis, biochemical characteristics, and complicated nutritional requirements are cumbersome to handle in systematic studies and fermentations. It is difficult to isolate pure cultures of anaerobic bifidobacteria. A method of isolating single cells was described by Ueda et al. (8). They reported that after training in this method, one could isolate single cells from strains they used with up to 50% success. A drop of melted clean agar is put on a cover-slip and spread immediately to make a thin layer. This is cut into agar blocks on the cover-slip and dried. B bifidum E in a complete medium is diluted serially (9). Tiny droplets are touched one by one on each of the small agar films with a capillary pipette.

Then the cover slip is placed upside-down on a specially constructed carbon dioxide - flow chamber on a slide glass. The cells
in each separate agar film are scanned with a phase contrast microscope. The cover slip containing agar film with a single cell is cut with a diamond pen and placed onto the surface of a complete agar plate. Finally, the colony is picked and cultured further (8). This method for anaerobic isolation of single cells without using a micromanipulator is reported to be simple and exact.

Growth of four strains of Bifidobacterium has recently been studied by Collins and Hall (21). Addition of 0.2% modified deMan, Rogosa, Sharp MRS broth permitted growth of these four species in either 10 or 12% nonfat dry milk. Cysteine (0.05%) plus either ascorbic acid (0.02%) or pyruvic acid (0.5%) replaced addition of the modified MRS broth for B. longum and B. bifidum. B. longum grew well with added cysteine. Two other species, B. adolescentis and B. infantis, could be grown in nonfat milk plus 2% modified MRS broth, but the broth could not be replaced with these compounds. Differences among species of this genus were observed but not explained.

Use Preparations

Therapeutic Aspects

The preponderance of bifidobacteria in the large intestine of breast-fed infants and the low pH of the contents there, are possible factors responsible for resistance to gastroenteritis (1,2,3,5,6,7). Bifidobacteria produce organic acids which act against many organisms both in vitro and in vivo. More acetic acid is produced from the fermentable carbohydrates than lactic acid. Acetate-buffer is believed to be strongly bacteriostatic (15,16).

Freeze dried B. bifidum (10^7 cells/day) have been added to the diet of formula-fed infants or used as supplementary treatment in some
enteric infections (1). Use of *B. bifidum* together with *Lactobacillus acidophilus* was reported to be preferable to use of bifidobacteria alone.

*B. longum* was reported to develop a 'barrier effect' against pathogenic *E. coli* (7). It is contained in a commercially available freeze dried preparation, OMNIFLORA (1). Seventy-eight *Bifidobacterium* strains out of 52 tested were able to hydrolyze the conjugated bile acids, sodium taurocholate and glycocholate (23). Therefore, it was suggested that bifidobacteria play an important role in transformation of bile acids in the intestine. They may accentuate the capacity of bile salts to inhibit susceptible bacteria (1,23).

Bifidus milk containing viable *B. bifidum* produced an improvement in liver cirrhosis patients associated with a decrease in ammonia, free phenols, and fecal pH along with an increase in feces bifidus content. Ammonia produced in the intestine is the main contributor to hepatic coma (1). Lactulose administered with bifidus milk has been proposed to treat liver cirrhosis (1).

**Nutritional Aspects**

Bifidobacteria constitute a major portion of human intestinal bacteria. Their characteristics have been reported to be important for vitamin synthesis in humans (24). It was reported that many strains of *Bifidobacterium* investigated may synthesize thiamine, nicotinic acid, folic acid, pyridoxine and vitamin B$_{12}$ when grown in a semi-synthetic medium (24). The amount of these vitamins produced varied among different species and strains. *Bifidobacterium infantis* and *B. bifidum*, in which the biosynthesis of vitamins is insensitive to feedback control, are believed to be good vitamin
producers in the human intestinal tract. Bifidobacteria fermented milk has a specific flavor, which can be described as clean, mildly sour to slightly acetic. Cultured milks are more easily digested by humans than non-cultured milk, especially by lactose-intolerant individuals.

**Epidemiological Studies**

Bifidobacteria have been suggested as ideal indicator organisms of fecal pollution (25, 26). They may be used to distinguish between human and animal pollution. Sorbitol-fermenting bifidobacteria were suggested to be specific indicators of human fecal pollution of waters and wastewaters (27). These sorbitol-fermenting strains were identified as human *B. breve* or *B. adolescentis*. The use of bifidobacteria as indicators of fecal pollution is limited to non-chlorinated sources (26).

**Cancer Agents**

Nonpathogenic, anaerobic *B. bifidum* was found to selectively localize and proliferate in various tissues of tumor-bearing mice (28). None of these same bacteria could be detected in the tissue of healthy organs such as liver, spleen, kidney, lung, blood, muscle and bone marrow 2 to 4 days after IV administration of *B. bifidum*. It is believed that the focal center of necrosis with zones of low oxygen tension occur frequently in cancer lesions and that healthy tissues are rich in oxygen which is supplied by blood flow. Therefore, *B. bifidum* can be used as a tool for diagnosis and perhaps therapy of cancer.

Lipotechoic acid (LTA) was reported to be a common surface antigen within the genus *Bifidobacterium*. Cross-reactivity with lactobacilli
was very low (29). Binding of LTA of \textit{B. bifidum} to human colonic epithelial cells was specific, reversible, and time and cell concentration dependent (30). Protein or glycoprotein with fatty-acid binding sites were proposed to be the receptors in the membrane of colonic epithelial cells. LTA is a weak immunogenic agent but complexation with methylated BSA makes the LTA strongly immunogenic. Antisera to LTA of \textit{B. bifidum} subsp. \textit{pennsylvanicum} were obtained by injecting LTA/methylated BSA complexes into rabbits (29). LTA may serve as a carrier for other antigens and bind them to target tissues.

Since LTA is a surface component of \textit{Bifidobacterium}, they will adsorb antibodies complexed with chemicals. These chemicals may be gathered in tumor cells with low oxygen tension where \textit{B. bifidum} proliferate. Then, the immunocytotoxic reactions related to LTA would occur.
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Sorbitol-fermenting bifidobacteria as specific indicators of human fecal pollution.


Project Title: Improvement of Mozzarella cheese yield and physical properties through proteinase modification of starter cultures.

Project Number: UAES 181

Principal Investigator: G.H. Richardson, Cooperators; C.J. Oberg, L.V. Moyes, and A. Wang

Project Objectives:

1. Obtain proteinase negative cultures for use in Mozzarella cheese manufacture. To see if the present bank of mesophilic cultures can perform adequately at the higher cooking temperatures involved in Mozzarella manufacture.

2. Isolate proteinase negative variants of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.

3. Evaluate curd made from the isolates to see if desirable properties are retained longer than when proteolytic cultures are used exclusively. The properties to measure include meltability, stretchability, and color scorching.

4. Examine curd made from milk containing high levels of plasmins to compare with the curd prepared above.

Summary of Results during the Year:

Numerous isolates were successfully obtained. Cheese has been made using single strains of these isolates and compared with cheese made with direct acidification and proteinase positive strains. Differences have not been measurable except for color and meltability. We have built several devices to measure curd stretch but these have not worked well. Currently, we are evaluating a modification of a helipath Brookfield viscometer system. This was recently reported at the ADSA meetings and appears to do a good job
of estimating curd stretch. If this works well, we should be able to progress rapidly on the evaluation of the above objectives.

Papers, Abstracts, Technical Reports:
Project Title: Improvement of Mozzarella cheese yield and physical properties through proteinase modification of starter cultures

Project Number: UAES 181

Principal Investigator: Gary H. Richardson

Research and Graduate Assistants: Craig Oberg, Lynn Moyes, and Amos

Project Objectives: The major objective of this project is to find proteinase negative or deficient cultures that can be used in the manufacture of Mozzarella cheese to increase yield and improve the physical properties of curd without causing adverse flavor reactions. The first objective is to carefully screen the available proteinase negative strains of S. cremoris for acid production and survival at the Mozzarella cooking temperatures, a range from 41 to 48°C depending on the desired finished moisture. The cultures will also need to produce sufficient acid during the 42°C cheddaring step utilized in some production methods.

The next objective will be to isolate and characterize proteinase negative strains of Streptococcus thermophilus and Lactobacillus bulgaricus for casein proteolysis and acid production. Trial production yield studies will follow using direct acid Mozzarella curd as the control.

A third objective will be to obtain current physical testing procedures from Pizza Hut and other Mozzarella cheese buyers, select representative methods, and quantitate changes in the cheese resulting from the different proteinase systems involved. The physical properties to be measured will include, but not be limited to, stretchability, cook color, blister development, and meltability. Additional properties will be added upon suggestions from cheese buyers.

Summary of Results During the Previous Year:

Activity tests were run at 37, 42, 44, and 46°C on the following proteinase negative strains of Streptococcus cremoris; UC 320+, UC 320-, UC 85-, UC 45-, and UC 310- (inoculum levels were .5, 1, 3, 5, 10% in NFDM). Results showed noticeable activity up to 42°C for several strains.

High temperature isolation of Streptococcus cremoris UC Prt- strains was done. Cultures were initially grown at optimum temperatures in M17-lac broth and then transferred to a new tube of M17-lac and grown at a series of temperatures up to 42°C. Results of three trials showed only UC 85 Prt- grew at all the temperatures tested, UC 310 Prt- and UC 161 Prt- grew well at 39°C but not at higher temperatures. Mozzarella cheese could be manufactured at a lower temperature (39°C) and allow these organisms to be used. High temperature incubation of Streptococcus cremoris Prt- cultures at 42°C in M17-lac broth for five days was done to select for cultures that could adapt to that temperature. OD readings were taken of the tubes. Only UC 85 Prt- exhibited growth at this temperature.

Individual and mixed Italian cheese starter cultures were obtained from a number of culture companies. Mixed cultures were separated into individual Streptococcus thermophilus (coci) and Lactobacillus bulgaricus (rods) strains. Lactobacillus bulgaricus was grown on FSDA II and TCMA agar plates to see if the rods would grow on a phosphate-buffered milk media for isolation of Prt-variants. Rods were also grown in M17-lac at 42°C for several days to determine which strains could tolerate the phosphate buffer. No strains grew
on the plates or in the phosphate-buffered tubes. Activity tests were run on eight rods and 5 cocci, all Prt+ strains, at the standard inoculum at 42°C for 6 hours to get a baseline activity curve. There were variations in activity between strains in either the rods or the cocci, but it did not appear that the rods of cocci were clearly more active than the other.

MAG agar isolation of spontaneous Prt- variants of S. thermophilus was attempted with 12 strains. A two tube coagulation test (at 40°C) was used to screen all potential Prt- isolates. Thirty-one potential isolates were selected based on colony size from the screening procedures. There were very few small colonies that appeared on the plates even though large numbers of plates are used. Only 3 of these isolates appeared to be Prt- when examined with the coagulation test. MAG agar isolation of five strains of S. thermophilus that were grown under high temperature stress (48-50°C) for Prt- variants was then done and potential Prt- isolates screened. Twenty three isolates were obtained that exhibited the small colony size and 11 appeared to be Prt- . A second round of testing with the coagulation test showed that only 3 were Prt-. Induced mutation with novobisin of four strains of S. thermophilus attempting to produce Prt- variants. MAG agar was used to screen for the resulting mutants. Poor results were obtained with very few colonies on the plates and none that were Prt-. We are presently characterizing the 6 Prt- variants obtained and are working on isolating addition Prt- variants.

A two tube coagulation test is run on 28 strains of L. bulgaricus and 3 strains of L. helviticus to look for possible Prt- variants. One Prt- strain was found and we are know characterizing this strain.

All possible Prt- cocci and rods isolated were tested for the ability to ferment lactose and galactose to see if they were exhibiting slow coagulation readings due to an inability to ferment milk sugars. Both Prt+ and Prt- thermophilic cocci were lactose positive and galactose negative. Most Prt+ rods were both lactose and galactose positive, with the possible Prt- strain being weakly lactose positive and galactose negative.

UV induced mutation of L. bulgaricus with the cultures either plated on basal agar or milk agar has yielded a number of promising isolates that exhibit a deficiency of proteinase activity. Activity tests and the OPA proteolysis test are presently being used to evaluate these cultures.

Four liter vats of experimental Mozzarella cheese is now being manufactured to start examining the effect different cultures have on the physical properties of the cheese with time. Experimental vats of cheese made by direct acidification are made as controls. The cheese is being examined at 1, 7, 14, and 28 days of age. Browning during cooking is being evaluated with the colorimeter. Presently the melting properties are being monitored by the Schreiber petrie plate test, but the Olson-Price tube method is being tried to see if the differences can be more clearly discerned. Several attempts have been made to develop a test suitable to measure stretch and now we are going to use the helical viscometer with testing starting this week.

Initial data indicates that the physical properties of the cheese are affected by changes in the proteolytic ability of the starter cultures.
Effects of strain of starter bacteria and residual milk-clotting enzymes on proteolysis and quality of Cheddar cheese during ripening

by

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1987-1988
ABSTRACT

The effects of strain of starter bacteria and residual milk-clotting enzyme (chymosin, bovine pepsin, porcine pepsin, *Mucor miehei* protease and *Mucor pusillus* protease) on proteolysis and quality of Cheddar cheese during ripening were evaluated. Residual milk-clotting enzyme activities (rennin units per kilogram of cheese) in fresh cheese made from normal milk (pH 6.6) were 8.5 for chymosin, 0 for porcine pepsin, 1.2 for bovine pepsin, 2.9 for *M. miehei* protease, and 2.6 for *M. pusillus* protease. Intact αs1-casein in cheese after 9 months of ripening were 9%, 7%, 40%, 13% and 8% respectively for cheese made milk chymosin, bovine pepsin, porcine pepsin, *M. miehei* protease and *M. pusillus* protease. Electrophoretic patterns of cheese made with the same enzyme but different strains of starter bacteria were identical. Residual milk-clotting enzyme in cheese assist but are not needed for development of Cheddar cheese flavor.
Project Title: Interaction of Protein and Polysaccharides in Chymosin Coagulation of Milk

Project Number: UTA186

Principle Investigator: R.L. Olsen

Research Technicians: D.N. Warner, C.A. Muncy

Project Objectives:

1. To investigate the effects of commercially available gums on rennet and acid coagulation and syneresis of milk.

2. To investigate the effects of extracellular polysaccharide from lactic acid bacteria on rennet and acid coagulation and syneresis of milk.

3. To continue through the cheesemaking process and determine the properties of the finished products.

The first and part of the second objective were examined during this time period. A variety of commercially available polysaccharides were studied in a reconstituted nonfat dry milk and calcium chloride mixture. Clotting time and gel formation characteristics using a Formagraph and Rolling Bottle apparatus were recorded. Several measures of syneresis and protein destabilization were also examined. The polysaccharides used were cross-linked modified corn starch, xanthan, pectins, carrageenans, cultured milk solids, and a high polysaccharide extract of cultured milk solids.

Except for modified starch, interactions with the polysaccharides were observed. Preheating to 72°C and cooling to 32°C did not affect coagulation properties for xanthan, iota carrageenan or modified starch.

Low methoxyl (LM) pectin had little effect on the firming rate as measured by the Formagraph. An increase in gel strength followed by a decrease was observed. The clotting time decreased with increasing LM pectin concentration. These effects were still observed after neutralization of the mixtures to compensate for the acidic properties of the LM pectin. Selective precipitation of kappa casein and para kappa casein at 5°C using an acetate buffer showed an increased rate of hydrolysis with LM pectin.

With xanthan the clotting time as measured by the Formagraph was constant. The Rolling Bottle clotting time decreased with increased xanthan concentration. The actual firming rate and the curd firmness decreased with increased xanthan concentration. Xanthan rapidly destabilized the milk.
proteins forming a two phase mixture with poor coagulation properties. The Rolling Bottle method was more sensitive to this destabilization than was the Formagraph.

Much variation was observed for curd firmness with kappa and iota carrageenan. The cultured milk solids gave results similar to xanthan. The high polysaccharide extract gave results similar to LM pectin.

Syneresis decreased with increasing LM pectin concentration during small-scale cheesemaking trials.

Polysaccharide extracts are currently being characterized and optimal growth parameters established.

Project Title: Improved control of cheese manufacture through vat monitoring.

Project Number: UAES 187

Principal Investigator: G.H. Richardson

Project Objectives:

1. Evaluate the control of curd strength during cottage cheese manufacture.

2. Evaluate the control of curd strength during Cheddar cheese manufacture. Determine the lower limits of curd strength that would cause significant product losses. Determine the higher limits that would prevent proper moisture removal. Add these data to an expert system base and program the expert system to optimize coagulation parameters.

3. Determine abilities of chymosin, calcium chloride, and lactic culture in milk for Cheddar cheese to overcome the inability of milk from cows in late lactation to coagulate.

4. Compare the performance of proteinase negative lactic cultures when monitored by the system. Determine if they can perform at constant high cooking temperatures and if they confirm the observations of Linklater and Hall that culture volume is more important than temperature in pH control.

Summary of Results during the Year:

We were not successful in keeping the Technician/PhD candidate to initiate the project. We were able to attract MS-candidate Michael LaFevre to conduct the project. He has assembled most of the modules required to interface with the laboratory Zymark robot. The robot will remove milk from iced storage, warm
it up, add chymosin and reagents, measure the three parameters desired, analyze the results, clean the electrodes and start the next sample automatically.

We negotiated for over six months to obtain permission to work with the Hot Wire instrument for measurement of milk coagulation. Technology on the CEM 1000 was not released back to USU even though marketing has ceased. The permission was granted and Dr. Shiinoki will spend from 14 Aug through 3 Sep getting the system going for our research. In the meantime, Remy Grappin delivered a hot wire system to us that is fabricated in France! I indicted that patent rights might preclude marketing the French instrument in the USA but we would be able to evaluate the instrument. This will make it possible for us to make more rapid progress on the project.

Papers, Abstracts, Technical Reports:
PROGRESS REPORT

Project Title: Continuous Production of Cottage Cheese from U.F. Skim Milk Retentate.

Investigators: Lynn Ogden and Rick Lord

Objectives:

1. To learn to make cottage cheese in a continuous process from ultrafiltered skim milk retentate.

2. Improve the yield of cottage cheese by using diafiltration to reduce the lactose so that washing may be reduced or eliminated.

3. Determine to what extent cooking can be accelerated and design a curd cooker that will cook the curd in minimum time.

Status:

Experiments were conducted to determine the effect of acidification pH, concentration, and hold time after acidification on curd quality. Curd was formed in quiescent tubes.

In the pH range of 4.4-4.8 curd is softer at lower pHs, mealiness is minimum in the 4.5-4.6 range, and matting is minimum at high concentrations and generally lower pHs. Acidifications below 4.6 sometimes resulted in premature coagulation before warming which resulted in a small, soft, matted curd.

Concentrated retentate produces more firm curd with less tendency toward matting. High concentrations seem to aggravate mealiness but pHs between 4.5-4.6 with careful cold acidification minimize it.

Holding up to 100 minutes after cold acidification has little effect on firmness and mealiness.

Next Steps:

The effect of retentate preheating temperatures up to 80°C for 15 minutes, curd cooking times from 20 minutes to 2 hours, and curd cooking temperatures from 48 to 70°C will be investigated. Retentate of 9.2% protein will be cold acidified to pH 4.6. Curd will be formed in quiescent tubes by heating retentate to 38°C.
Macropeptides were isolated from a 2.5% solution of whole casein incubated with crystalline chymosin (1 mg rennin/200 ml solution). Three different concentrations of trichloroacetic acid (2, 8 or 12%) were added to precipitate the proteins and to inactivate the enzyme after 5, 15, 30 or 60 min of incubation. The filtrate was exhaustively dialyzed (3500 MW cut off) against distilled water for one week to remove the acid and small molecules. The dialyzate was lyophilized and stored at -20°C. These macropeptides were compared using reverse phase HPLC on a C3 column with purified macropeptides isolated by the same method (8% trichloroacetic acid, 15 min incubation time) from pure κ-casein.

Whole casein was separated into individual proteins by reverse phase HPLC on a C3 column after denaturation with mercaptoethanol and urea to allow dissociation of casein micelles. A linear gradient was used to separate the proteins. One solvent was 15M sodium chloride and triethylamine at pH 2.5, and the other was 40% acetonitrile. The gradient was begun with 40% of the first solvent and 60% of the second and continued to 100% of the second. The order of elution was κ-casein, αs-casein then β-casein. Protein peaks were collected and analyzed for purity and identification by electrophoresis. Analysis was standardized to measure amino acids in milk by digesting in 6N HCL at 145°C for 4 h. They were also collected as they eluted from the column, dried and hydrolyzed in 6 N HCl at 145°C for 4 h then analyzed for amino acid composition. Mixtures of the three purified proteins were analyzed and estimated their individual concentrations in the mixtures were made based on their amino acid compositions.

Meltability of pasteurized process cheese food made from UF curd was evaluated after processing at 66, 68, 70, 72, 76, 78, 80, and 82°C. Meltability decreased with increasing processing temperature which was believed to be caused by heat denaturation of whey proteins, and disulfide interaction with κ-casein. Soluble nitrogen at pH 4.6 was determined as an estimate of undenatured whey protein in process cheese food. Denatured whey protein precipitates at pH 4.6, although the native proteins remain in solution. Soluble nitrogen at pH 4.6 decreased from 15.8% of total nitrogen in processed cheese food cooked at 66°C to 7.48% for that cooked at 82°C. Soluble nitrogen at pH 4.6 correlated positively with meltability (correlation coefficient, R = .95). UF curd was blended in proportions of 0, 16, 33, 50, 66, 83, and 100% with Cheddar cheese to make process cheese food. Cheddar cheese was first heated to 82°C, then water and sodium citrate were added with UF curd for processing at 68 or 72°C. Blends of up to 66% UF curd were made with acceptable meltability when processed at 68°C.
Casein inhibition of bovine plasmin. E. D. Bastian*, R. J. Brown, and C. A. Emstrom,
Utah State University, Logan.

Bovine plasmin (MW 89,000) activity was measured on valyl-leucyl-lysyl-p-nitroanilide in the
presence of casein by following absorbance at 405 nm. Initial rates of reactions with all
combinations of .1, 1, and 10 × Km substrate concentrations and .1, 1, and 10 × KI casein
concentrations were measured. Steady state kinetic parameters Vmax, Km, KI, and KI' were
determined by nonlinear least squares fitting of the data to the equation:

\[ V_o = \frac{V_{max}}{1 + \frac{K_m}{S} + \frac{I}{K_I} + \frac{K_m \times I}{K_I \times S}} \]

Bovine plasmin is competitively inhibited by casein (\( \frac{I}{K_I} \rightarrow 0 \)) and has a Vmax of
0.424 A405/min, Km of 0.107 mM, and KI of 8.5 × 10⁻⁴ g/ml toward the peptide substrate. Bovine
plasmin can be measured directly in bovine milk without interference from casein if substrate
concentration is high.

Cross reactivity of antibodies against Mucor miehei and Mucor pusillus rennets. C. I.
Osuala*, and R. J. Brown, Utah State University, Logan.

Although their actions in milk differ, proteolytic enzymes from Mucor miehei and Mucor
pusillus have both been used as calf rennet substitutes in cheese manufacture. Antibodies were
produced by intramuscular injection of M. miehei and M. pusillus milk clotting preparations
emulsified with Freund's complete adjuvant into New Zealand White rabbits. Serum was heated at
57°C for 30 min to inactivate complement factors and contaminating proteins then centrifuged at
1700 × g for 30 min. Both ring test and Ouchterlony methods were used to test for cross
reactivity. Antibodies against M. miehei reacted with M. pusillus antigen and M. pusillus
antibodies reacted with M. miehei antigen. Differences in the characteristics of these two enzyme
preparations exist, even though they are indistinguishable immunologically. At least one antigenic
factor is common to the two enzyme preparations.

The future of dairy testing. Rodney J. Brown*, Utah State University, Logan.

Soon we will replace the methods now used for testing milk and dairy products with better
methods, just as those we now use replaced their predecessors. Methods that are now available
only in research laboratories will become available to the dairy industry. Stability of instruments
will improve so that time between calibrations can increase. Factors that affect concentration
measurements, such as saturated:unsaturated fat ratios and lipolysis of milk fat, will no longer be
problems. Components will be measured at a more fundamental level (individual proteins vs.
total protein) and things will be measured that are not now measured. Tests will be made
throughout dairy plants and the information obtained will be used to automatically control
processing parameters for optimum profitability. Addition of processing plants to the traditional
market for analytical instruments will help keep instrument prices from rising unreasonably.

Detection of added casein or whey protein in nonfat dry milk and goats' milk in whole
cows' milk. M. K. Walsh* and R. J. Brown, Utah State University, Logan.

Mixtures of casein or whey protein with nonfat-dry milk were made with ratios of concentrations
from 1:9 to 10:0. Similar mixtures of defatted goats' milk defatted cows' milk were prepared.
Analysis was standardized to measure amino acids in milk by digesting in 6N HCL for 1, 4, 20,
and 70 h. Samples were hydrolyzed in 6N HCL at 145°C for 4 h. Multiple regression equations
were derived to estimate concentrations of whey protein or casein added to nonfat-dry milk and
goat's milk added to cows' milk using amino acid profiles of whey protein, casein, nonfat milk,
cows' milk and goats' milk. R² values for these equations were all > .99.
A main objective of pricing milk is that the price paid (received) for milk reflects as accurately as possible the amount and value of the products made from it. A number of pricing formulae were examined for their ability to relate prices for milks of varied compositions to the milk cost per unit of products produced: "fat differential plus constant", the current system in North America, gave least inequities for fluid milk; fat + SNF or fat + protein + lactose + minerals gave least inequities for butter and skim milk powder; fat + protein gave least inequities for cheese. None of these pricing formulae were satisfactory for all three product classes; they yielded inequities in other product categories that would be economically very important to plants receiving milk differing by as little as .1% fat or .1% protein. Pooling or blending the appropriate pricing formulae, depending on proportions of products produced, resulted in the least inequities; such a product-yield pricing system is indicated for milk.

The previous paper indicated that inequities occur in cost of milk per unit of product produced when one pricing system is applied to all products. The amount of inequity depends on the magnitude of variations in the composition of milk - between and within plants. A survey was made of the monthly and annual average composition of milk received by plants. Economically significant variations were found in terms of seasonal and interplant inequities in cost of milk per unit of product produced. For example, with the current system of fat-differential-plus-constant, a plant receiving milk containing 3.6% fat and 3.1% protein would pay approximately 1.3% more for milk per unit of product produced than a plant receiving milk containing 3.6% fat and 3.2% protein. This 1.3% represents a large part of operating costs and a much larger part of profit.

Development of gene transfer and host-vector systems in lactic streptococci have made it possible to study the genetics and plasmid biology of these industrially significant bacteria. Basically, four methods of gene transfer exist: transduction, conjugation, protoplast fusion, and transformation/transfection. Transduction of chromosomal genes using virulent bacteriophages and of both chromosomal and plasmid-coded genes using temperate phages have been described. Conjugal transfer of genetic information occurs by solid-surface matings. High-frequency conjugation systems involving cell aggregation and cointegrate formation are well documented. Insertion sequences mediate cointegrate formation and may be involved in conduction and integration of genes during gene transfer. There are few reports of protoplast fusion but this technique may be a powerful tool in recombining genes from two organisms. Whole cell and protoplast transformation procedures and electroporation techniques permit gene cloning and expression analysis in lactic streptococci. Shuttle vector systems for cloning and analysis of lactic streptococcal genes in Escherichia coli, Bacillus subtilis, and Streptococcus sanguis allow for detailed molecular analysis of genes and gene products. Use of these gene transfer and cloning systems in basic and applied research is vital for the success of strain improvement of lactic streptococci for dairy fermentations.

Ice cream mix was fermented with yogurt cultures of Streptococcus thermophilus and Lactobacillus bulgaricus to four different pH's then frozen in a batch ice cream freezer. A consumer panel of 120 people tasted samples of strawberry flavored product with pH's of 4.4, 4.7, 5.1, and 5.7 and commercial frozen yogurt as a standard. Results from the panel were used to predict a preferred pH of 4.9. Another panel of 181 people compared product at pH 4.9 with 10, 15 and 20% strawberry flavoring. There was not a statistically significant difference among levels of flavoring. Starter culture populations and lactose activity were monitored for one month in yogurt and in the frozen fermented ice cream mix.
**D155** Measurement of abnormal milk with reflectance colorimetry robotics. T.C. Yuan*1, R. Grappin2, and G.H. Richardson1. Utah State University, Logan, and Station Experimentale Laitiere, I.N.R.A., Poligny, France

Mastitic milk was assayed using (1) a modified Mohr test for NaCl and (2) an N-acetyl-β-D-glucosamidase (NAGase) test. Color changes were quantitated using an Omnispec reflectance colorimeter. In (1) diluted raw milk (1/100), 0.3 mL were added to microtiter plate wells containing 3 μL mixture of 1% NaOH and 5% K2Cr2O7, then added 4.5 μL 0.1 M AgNO3, and b* values determined w/o incubation. Test correlation with somatic cell counts, R = 0.98. In (2) 50 μL of 10 mM substrate soln. were added to 0.1 mL milk in well, after incubation at 50 C for 15 min, 0.15 mL of 3.33 M glycine were added, and b* values measured; R = 0.86 and data range was wider than in (1). No incubation time on instrument allows device to test abnormal milk samples during day and other assays overnight.

**D163** Sensory quality of iron fortified fresh cheddar cheese. D. Zhang and A.W. Mahoney*, Utah State University, Logan, UT

Dairy products are rich in most nutrients but not in iron. To determine the effects of iron fortification on oxidized flavor and thiobarbituric acid (TBA) values, cheese was prepared from 8.0 L of Pasteurized milk. Starter culture, iron (10 mg Fe/L of milk), color and rennet were added, mixed and allowed to sit 30 min for coagulation. The curd was cut, cooked for 1 h at 39°C (102°F), drained, matted at 32°C (90°F), and pressed overnight. Depending on the iron source added, 52-81% of the iron was recovered in the cheese; and the cheeses contained 60-101 ppm iron. In 7-day curd, iron caused slightly higher TBA and oxidized flavor values. However when aged for 30 days, oxidized flavor was not detected in most of the fortified cheeses; and TBA values were reduced. At 7 and 30 days, oxidized and cheese flavor in most cheeses fortified with iron protein complex were not different (P<.05) from unfortified product. Iron did not affect cheese color.
D20 The effect of natural fat globule membrane material on light stability of added and indigenous vitamin A in milk. B. P. Bartholomew* and L. V. Ogden, Brigham Young University, Provo, Utah.

Butter oil and a buttermilk were harvested from 38% cream by churning then warming to melt the fat to facilitate separation. The fat was divided into four lots. Two lots were fortified with vitamin A. Two lots remained unfortified. Emulsifier was added to one lot of each prior to reemulsification into skim milk. The other two lots were reemulsified into skim milk containing the harvested buttermilk. The resulting milk was 1% butterfat. These samples and normal fortified and unfortified 1% milk were exposed to light (400 ft. candle) for 0, 24, 4, 8, 16, and 32 hours at 4°C. Superior stability of indigenous over fortified vitamin A was not observed. Vitamin A in reformed fat globules was less sensitive than either indigenous or added vitamin A in the unfortified and fortified 1% milk. No difference in stability was observed due to type of surface active material used in the reformed fat globules.


Thermophilic starters of L. bulgaricus and S. thermophilus were grown in an internally-buffered medium (IP4) and tested for number of cells present as well as acid-producing activity. Tests were done at zero time and after storage at ambient (25°C), refrigeration (5°C) and freezer (−40°C) temperatures for up to 30 days unfrozen and for 8 weeks frozen. Unfrozen cultures at 5°C retained most of their activity for 30 days in IP4 and for 7 days at 25°C. Small scale yogurt could be satisfactorily made using cultures stored 30 days at 5°C in IP4 and also when held 5 days at 25°C followed by 13 days under refrigeration. Cultures grown in IP4 were supplemented with 6% malt extract lost no activity when stored at −40°C for 8 weeks. Data suggest thermophiles may be grown in IP4, canned, shipped at ambient temperatures and stored in dairy for up to 30 days at 5°C and used over this time for direct set yogurt manufacture.


Blueberry flavored milk beverages, carbonated and noncarbonated, were evaluated to examine the effect of sweetener source on other sensory qualities and consumer acceptability. The effect of carbonation on sensory qualities was also studied. There were eight treatments in the test design, four sweeteners (sucrose, high fructose corn syrup, pear concentrate and aspartame), each prepared with and without carbonation. Both carbonated and noncarbonated products were evaluated by a trained panel based on a split plot design. Carbonation enhanced the sensory ratings of overall flavor intensity, sweetness and blueberry flavor (P < .05). The sweetener source significantly affected the panel rating of viscosity, but not those of the other three sensory attributes. The perceived viscosity of the pear concentrate sweetened product was highest, while that of the sucrose sweetened product was lowest. A consumer panel also evaluated the carbonated beverages. Carbonation and sweetness levels were close to an optimum level, but the intensity of blueberry flavor was too low. In this particular beverage formulation, the sucrose and HFCS appeared to be more highly acceptable sweeteners than were aspartame or pear concentrate.


Microgard extended the shelf life of cottage cheese, functioning optimally at pH 5.30 and below. The antimicrobial principle(s) were particularly inhibitory for Pseudomonas and related psychrotrophic genera. Salmonella and Yersinia also were inhibited as measured by incorporation of Microgard into agar and performing pour plate assays at pH 5.3. Gram positive bacteria were not inhibited, though Listeria monocytogenes gave variable results. Microgard was stable to boiling and autoclaving up to pH 11.0. Chymotrypsin destroyed and Albumin antagonized the inhibition by Microgard. Treatment with Trypsin at higher pH enhanced the inhibition. While Microgard offered variable results for inhibition of L. monocytogenes, depending on the assay medium and procedures. Pediococcus cerevisiae metabolite(s) inhibited this and other pathogens. Data suggest these inhibitory activities maybe due to bacteriocins, though in some cases the activity is bacteriostatic rather than bacteriocidal.
Minutes:

The first meeting of the Center Operational Advisory Committee (OAC) of the Western Dairy Foods Research Center was called to order at 3:10 p.m. by acting chairman, Gary H. Richardson. The chairman reviewed the history of the contract with the National Dairy Board (NDB) and the fact that the AOC was replacing the intended "Board of Directors". He indicated that the directorship of the Center was to be decided by the Dean of the College of Agriculture at Utah State University. This represented a change from previous correspondence which indicated that the "Board of Directors" would select the director of the Center. The chairman requested that consideration be given to officers of the OAC to include at least a chairman and a secretary.

Considerable discussion ensued with respect to the operation and staffing of the Center. It was agreed that the research plan prepared for submission to the NDPRB each year by 1 July be submitted to each member of the OAC in June, thus allowing time for the consideration prior to the annual meetings. Additionally, the annual report prepared for the NDB would also be submitted to the OAC following the annual meeting in July. Quarterly reports will additionally be forwarded to the OAC including the titles and project proposals that have been approved by the Technical Advisory Committee (TAC).

The director of the Center was asked to serve as the Chairman of the OAC and to request that the committee secretary be appointed from the secretarial staff of the Department of Nutrition and Food Sciences.

A copy of the proposed 1987-88 budget has been incorporated in the report meeting booklet. It was also projected on the wall and explained to the OAC. It was moved, seconded, and passed that the director be given authority to adjust the budgetary values within the constraints of the contracting agencies. It was further generally agreed that there should be a minimum of subdivision of these funds from a central pool thus allowing maximum flexibility in the research program. The procedure incorporated in the contract was accepted for project approval. Thus, the TAC composed of three members from the Center and one from the NDB would pass scientific judgment on each proposal. Approved projects would be signed by the director and submitted to the USU Agricultural Experiment Station for project management. Those projects approved by the TAC for management at Oregon State University would be submitted to the Oregon State University Research Vice-President for management.

The motion was made, seconded and passed to retain the name of the center as the Western Dairy Foods Research Center.
Roy Focht offered to host the next meeting in Lander, WY. This offer will certainly be investigated for future potential. However, the concern over financing maximum student participation prompted a motion that was seconded and passed that the next meeting be held July 20-21 in Logan. It was agreed that one day would be required to complete the reporting and discussion process and that the second day would be used for the OAC and the TAC meetings.

The Chairman expressed appreciation for the continued support of the former Dairy Research Advisory Board members and their willingness to retain the financial base for the Center.

The meeting adjourned at 4:50 p.m.
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WESTERN DAIRY FOODS RESEARCH CENTER

Report to Operational Advisory Committee
Inclusive dates: 9/1/87 - 6/30/88

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

Project Number: 30-262-9796

Principal Investigator: Floyd W. Bodyfelt

Research and Graduate Assistants: Ralph R. Meer (Indirectly prior to 9/15/87)
Anthony Gatherum (After 8/1/88)

Project Objectives:

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

2. Determine the appropriate casein fraction and the optimal buffering and suspension systems for the substrate matrix for conduct of the linear diffusion/precipitation test.

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

4. Develop a method for monitoring populations of Bacilli sp. in selected milk samples by a combination of preliminary incubation and a dye reduction test. This approach should help achieve test prerequisites of being: (1) rapid, (2) sensitive (3) accurate, (4) simple (convenient) and (5) economically feasible.
Summary of Results During the Year:

A survey and analytical results provided useful information on the prevalence and characteristics of Bacilli sp. in one Oregon raw milk supply, and the subsequent commercially pasteurized milk products. Results reflect that 83% of 59 farm bulk tanks were positive for psychrotrophic sporeformers and 12 different Bacilli sp. were isolated from this milk source. Additionally, 3/4 of the pasteurized products were positive for sporeformers. The proteolytic and lipolytic properties and some sensory characteristics of these Bacilli sp. isolates were determined.

During the course of a sabbatical leave with the Microbiology group at the Hannah Research Institute in Ayr, Scotland, the principal investigator conducted studies relevant to several objectives of this project. A reassessment of the optimum temperature and time parameters for milk sample pre-treatment were undertaken. However, inconclusive results were obtained. Apparently, different strains of Bacilli exhibit varied degrees of heat resistance or sensitivity and respond accordingly to the heat pretreatment(s) employed. Experiments indicated that $\beta$-casein is the substrate of choice for assessing the proteinase activity of Bacillus sp. Other test parameters evaluated were: pH optima, sample "loading" criteria, reaction time, measurement of casein precipitation bands and a comparison of vertical vs. horizontal dispersions of casein and agar. Development and evaluation of an effective microbial growth media for enhancing the sporulation rates of Bacilli was also studied. Brief trials were also undertaken to consider the applicability of Petri-film, microwave oven heating of samples and the usefulness of sporulation agents for improving test performance. Initial trials have indicated potential for a combined preliminary incubation of milk samples and a simultaneous dye reduction test for effectively monitoring heat resistant sporeforming psychrotrophs in raw or pasteurized milk.

Papers, Abstracts, Technical Reports:
Project Title: Evaluation of milk proteins as whitening agents in processed meats and poultry products

Project Number: UAES 184

Principal Investigator: D.P. Cornforth

Funding for this project began October, 1987. A graduate student for this project was not able to begin until June 6, 1988. Thus, the funds allocated for the first year were not spent. It was requested and granted that this project receive a one year extension.

Preliminary experiments found that sodium caseinate is a superior agent in the pre-emulsification of pork fat for later incorporation into beef bologna-type product. No whitening effect was observed in this product, due to the low level of fat (18%) used. Literature review indicates that the whitening effect is more pronounced in pork and poultry products, which have less red heme pigment. Preliminary results suggest that milk proteins lighten meat products due to their superior fat emulsification properties. It is the opaque fat droplets which cause the lighter color of emulsified meat products.
Project Title: Iron Fortification of Cheese Curd

Project Number: UTA00182

Principle Investigators: Arthur W. Mahoney and Dejia Zhang

Project Objectives:

1. To identify potential iron fortification sources that will not reduce cheese quality in laboratory batches of Cheddar cheese.

2. To evaluate the bioavailability of these iron sources in cheese curd.

Practical Applications:

Iron deficient is prevalent in the United States which is hard to correct by diet manipulation. Dairy products contribute about 15% of energy and lots of nutrients to American people without contribution of iron. If iron fortification in cheese can be successfully done, it can promote the quality of cheese which benefits both dairy industry and American people.

Summary of Results During the Year:

In three experiments, we prepared two-pound batches of Cheddar cheese fortified with nothing (unfortified, negative control), ferric chloride (positive control), ferric citrate, iron casein complex (with different iron concentrations), and ferripolyphosphate whey protein complex. Iron was added to the unclotted milk to provide 20 mg per 1000 kcal cheese. Each of the iron-fortified cheeses were prepared with or without coloring. The products were evaluated for iron content, TBA number, oxidized flavor, cheese flavor and cheese color 7, 30, and 90 days after preparation.

From 52 to 81 percent of the fortification iron added to the milk was recovered in the curd. The fortified cheeses contained from 40 to 169 ppm iron in this study.

Oxidized flavor was not increased in cheese fortified with iron-casein complex or ferric chloride compared with unfortified cheese after aging 30 or 90 days. Ferric citrate consistently increased oxidized flavor in fortified cheese. Adding cheese coloring reduced oxidized flavor. Oxidized flavor was not increased with duration of aging of fortified cheeses.
Although the TBA values were low in all cheeses, each of the iron sources increased TBA values slightly. TBA values did not consistently increase with duration of aging. Adding cheese coloring reduced TBA values.

Cheese flavor was not adversely affected (P>.05) by fortification with iron-casein complex or ferric chloride after aging 30 or 90 days. However, ferric citrate and ferric polyphosphate whey protein complex consistently caused flavor deterioration in fortified cheese.

Cheese color was not affected by iron fortification.

Medium scale Cheddar cheeses (22 - 25 pounds per batch) were made with fortification of iron from Fe-casein, ferric polyphosphate-whey protein (FIP-WP), Fe-whey protein and FeCl3. These cheeses contained about 40 mg Fe/kg as a result of fortification. More than 2/3 of added iron was recovered in all iron fortified cheeses. TBA number was not detectable in all iron fortified cheese after aging 7 days and 30 days. Cheese flavor was not affected by fortification of iron (P>0.05) at 15th day after cheese making. Oxidized flavor score of the cheese fortified with Fe-whey protein was not significantly different from that of unfortified control, but the cheese fortified with FIP-WP, Fe-casein or FeCl3 had higher oxidized flavor score than unfortified control after aging 15 days. After aging 30 days, oxidized flavor scores were not statistically different among the treatment groups. Cheese fortified with FIP-WP had cheese flavor score similar to the cheese fortified with FeCl3 or Fe-WP and higher than the control after aging 30 days.

Seventeen diets were preparing for bioavailability evaluation of iron from fortified cheeses and iron dairy protein complexes. Iron depleted growing rats and iron repleted adult rats will be used to test maximum and practical iron bioavailability.

Publications:


June 29, 1988

Dr. Gary Richardson, Director
Western Dairy Foods Research Center
Department of Nutrition and Food Sciences
Utah State University
Logan, Utah 84322-8700

Gary:

Enclosed is the annual report for my project entitled, "Characterization of the Post-adsorptive Behavior of β-lactoglobulin for Control of Spore and Microbial Adhesion to Dairy Product Processing and Packaging Surfaces". Please let me know if any further information or clarification is required. I look forward to seeing you later this month.

Sincerely,

Joseph McGuire
Assistant Professor of Food Engineering
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.

Project Number:
88-087

Principal Investigator:
Joseph McGuire, Departments of Food Science & Technology and Agricultural Engineering, OSU

Research and Graduate Assistants:
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Project Objectives:
The project objectives, as originally stated for the first year are
1. to develop the mathematics required for a theoretically sound analysis of contact angle data for solid surface tension determination; and
2. to use contact angle methods to evaluate the dispersive and polar components of solid surface tension for various materials.

Summary of Results During the Year:
Currently, there is no universally accepted, reproducible method for dairy fluid contact surface characterization, i.e., the measurement of solid surface energy, $\gamma_s$. Such methodology would be useful as this variable may be incorporated into simple methods for prediction of any one of many contact surface-dairy fluid constituent interactions. Measurement of this property can be accomplished by evaluation and addition of the dispersive and nondispersive (polar) force contributions exhibited by a solid surface upon liquid contact (i.e., $\gamma_s = \gamma_s^d + \gamma_s^p$). This is commonly accomplished with an analysis of contact angle data provided by a variety of diagnostic chemicals. To perform this analysis, evaluation of the dispersive and polar components of the work of adhesion between a diagnostic liquid and the solid surface is required. The work of adhesion, $W_a$, is the free energy change per unit area associated with separation of an interface. The dispersive component of $W_a$ is related to the geometric
mean of the dispersive force contributions of the solid and liquid; the evaluation of $\gamma_s^d$ is therefore straightforward with a sufficient amount of contact angle data. However, methods used to determine the polar component of $W_a$, leading to determination of $\gamma_s^P$, have no theoretical basis. A first step toward both a useful and theoretically sound surface quantification is evaluation of the relationship between the polar contribution to $W_a$ and the polar force contribution of the diagnostic liquid surface tension, i.e., the relationship between $W_a^P$ and $\gamma_L^P$. It is elucidation of this relationship, and its use in surface characterization of materials targeted for exposure to dairy fluids which are currently under investigation.

Development of a method for rapid, unambiguous evaluation of $\gamma_s$ for dairy product contact materials is in progress. We are using a contact angle goniometer equipped with an environmental chamber, and a series of aqueous ethanol solutions and aqueous methanol solutions as diagnostic liquids. A water-saturated surface is required to assure both equilibrium between the drop and the surface and the validity of the mathematics applied to the system. With the environmental chamber, we can evaluate surface properties at conditions of 100% humidity. The diagnostic liquids have been characterized with respect to their polar and dispersive components of surface tension, and several dairy contact materials have been acquired and machined to a form suitable for study. The materials include several types of polyethylene, polypropylene, nylon, teflon, and an acetal; we are studying both pure polymeric materials targeted for dairy product contact and actual packaging surfaces. The dispersive component of surface tension for each of the materials has been evaluated. The contact angle methodology developed may now be introduced to evaluate the influence of $\gamma_L^P$ on $W_a^P$. Once this relationship is determined, a suitable protocol for determination of $\gamma_s$ will be documented and used for the remaining phase of the project, which focuses on ellipsometry to monitor the influence of surface properties on the post-adsorptive behavior of $\beta$-lactoglobulin.

Papers, Abstracts, Technical Reports:

directly relevant work (unsponsored):

sponsored by WDFRC:
CHEDDAR CHEESE BLOCK COOLING: EFFECT OF COOLING CONDITIONS AND CHEESE COMPOSITION

GOAL:

A CONTROLLED AND OPTIMIZED COOLING OF THE CHEDDAR CHEESE BLOCK (BEFORE MATURATION).

OPTIMIZED? TO ACHIEVE A BALANCE BETWEEN ADDITIONAL GROWTH OF STARTER CULTURE AND THAT OF ADVENTITIOUS MICROORGANISMS.
CONSEQUENCES:

1. TO MAINTAIN pH OF THE YOUNG CHEDDAR CHEESE WITHIN A MORE DESIRABLE RANGE.

2. TO REDUCE THE OCCURRENCE OF HIGH ACID (SOUR) AND BITTER OFF-FLAVOR.

3. TO REDUCE OCCURRENCE OF MANY BODY AND TEXTURE DEFECTS (SHORT, CRUMBLY, GRAINY AND PASTY).

4. TO REDUCE OCCURRENCE OF SEVERAL COLOR AND APPEARANCE DEFECTS (ACID CUT, MOTTLED AND UNDESIRABLE WHITE DEPOSITS).
MATHEMATICAL MODELS:
QUANTIFICATION AND PREDICTION OF THE EFFECT OF COOLING RATE.

1. HEAT TRANSFER MODEL TO PREDICT THE TEMPERATURE IN THE BLOCK AS A FUNCTION OF THE COOLING METHOD AND THE CHEESE THERMAL AND PHYSICAL PROPERTIES.

2. MICROBIAL GROWTH RATE MODELS TO PREDICT CELL NUMBERS AS A FUNCTION OF INITIAL VALUES AND TEMPERATURE AT EACH CHEESE LOCATION AS PREDICTED BY THE HEAT TRANSFER MODEL.
EXPERIMENTAL:

1. CONSTANT TEMPERATURE STUDIES. SLICES STORED AT VARIOUS CONSTANT TEMPERATURES AND SAMPLED AT REGULAR INTERVALS.

2. COOLING OF BLOCKS AT VARIOUS RATES AND SAMPLED AT REGULAR INTERVALS.

3. EXPERIMENTAL DETERMINATIONS:

ACID PROFILE  TOTAL PROTEIN  TRAINED SENSORY
LACTOBACILLI  FAT
ENTEROCOCCI  SALT
PEDIOCOCCI  MOISTURE
SOL. NITROGEN  pH

ANALYSIS
PROJECT SUMMARY

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

Investigator: Rodney J. Brown

Background

This new project was requested by one of the parties contributing to the funding of the Western Dairy Foods Research Center. They want a fast method for detecting non-dairy products, particularly fat, in products labeled as dairy products. The project will also provide an improved method for measuring fat, protein, lactose and water content in all dairy products. This should assist the dairy industry in use of milk components in all foods becomes a larger share of total milk utilization. Most methods that have been adapted from laboratory configurations to industrial applications are based on very simple, usually old, technology. This is true of instruments used for testing milk and dairy products, most of which were state of the art thirty years ago.

Emphasis on instrument design has recently shifted toward intelligent instruments. Many previous limitations are now overcome by mathematical algorithms. Computer capabilities are built into instruments that match those found in multi-user mainframe computers ten years ago. Microcomputers have changed the way instruments are built and the way they are used. Before computers, it was necessary to use a separate sensor specific for each constituent measured. Much effort went into making sure that sensors gave linear responses. These sensors could not be influenced by anything other than their specific target. This has all changed with addition of computers to instruments. Measurements still depend on sensors, and better sensors make better measurements, but many of the shortcomings of sensors are now overcome in the data processing step. Sensors can now be used that are not specific for only one component in a mixture because the ability to interpret their signals can be provided. If each sensor responds differently to at least two components in the sample mixture, then the concentration of each component can be determined. The ideal situation for obtaining maximum information from an instrument is a large group of nonselective sensors attached to one computer. Even more information is available from large arrays of sensors if pattern recognition, or fingerprint, methods are used. This requires the computer to "learn" the combination of responses from all the sensors caused by each possible component in a mixture.

Fixed filter spectroscopy has been the leading method for measuring fat, protein and lactose in milk for many years. Improvements over the years have not overcome the limitations imposed by the small number of filter bands available with such instruments. Nor have they overcome interference by changing levels of saturation in milk fat from one sample to the next or the effects of lipolysis on milk fat.

Fourier Transform Infrared (FTIR) has many advantages over fixed filter methods. Fixed filter instruments are limited to a few (usually not more than four) pairs of filters. These must be rotated into the light path for measurements to be made. With FTIR, measurements at hundreds of different wavelengths could be made almost instantaneously. FTIR measurements are very narrow bands of the spectrum instead of the broad bands of filter measurements. With additional wavelengths available, measurements can be made more accurately. The large number of measurements possible in a short time allows much more powerful data processing methods to be used. Any number or combination of readings can be used to measure any component. Handling of this large amount of data is no longer a problem with computers built into all new instruments.

Some major factors that now interfere with accurate measurements would be eliminated. An FTIR instrument can consider variables such as saturation level of the fat, lipolysis of fat, etc. so they do not interfere with accurate measurements. Calibration of the instruments would be much less frequent, perhaps monthly rather than weekly.
Objectives and Procedures

1. **Find a set of wavelengths that respond to changes in fat concentration.**
   Spectra will be recorded for several samples prepared with different levels of fat concentration. These spectra will then be statistically analyzed with a computer to see which wavelengths are most affected by variation in fat concentration.

2. **Find a set of wavelengths that respond to changes in protein concentration.**
   Spectra will be recorded for several samples prepared with different levels of protein concentration. These spectra will then be statistically analyzed with a computer to see which wavelengths are most affected by variation in protein concentration.

3. **Find a set of wavelengths that respond to changes in lactose concentration.**
   Spectra will be recorded for several samples prepared with different levels of lactose concentration. These spectra will then be statistically analyzed with a computer to see which wavelengths are most affected by variation in lactose concentration.

4. **Find a set of wavelengths that do not respond to changes in saturation level and chain length of fatty acids.**
   Spectra will be recorded for several different fatty acids such as 16:0, 16:1, 16:2, 18:0, 18:1, and 18:3. These spectra will then be statistically analyzed with a computer to see which wavelengths are least affected by variation in saturation level and in chain length.

5. **Find a set of wavelengths that do not respond to changes in free fatty acid levels.**
   Spectra will be recorded over time as lipase enzyme is allowed to act on triglycerides. These spectra will then be statistically analyzed with a computer to see which wavelengths are least affected by changes in free fatty acid levels.

6. **Combine these (procedures 1–5) to make a robust set of wavelengths common to all constraints.**
   Results of the first five procedures will be combined into a set of wavelengths that respond to changes in fat, protein and lactose, but are not affected by type of fatty acid or degree of lipolysis.

- **Robust Sub-set of Wavelengths**
7. From the total infrared spectrum of milk, determine the individual spectra of milk fat, milk protein, and milk lactose.

These three separate spectra will be found by recording the spectra of whole milk, skim milk and a lactose solution with the same lactose concentration as the milk. The fat spectra will then be found by subtracting the skim milk spectra from the milk spectra. The protein spectra will be found by subtracting the lactose solution spectra from the skim milk spectrum. The lactose spectrum will be measured directly. One additional spectrum, that of the milk salts, will be found by subtracting the fat, protein and lactose spectra from the milk spectrum.

8. Find a set of wavelengths common to milk fat, milk protein, and milk lactose.

This is the same as the left diagram in procedure 6, but with the data from procedure 7.

9. Find a set of wavelengths common to the milk components (procedure 8) and to the robust set (procedure 6).

The areas of the spectrum contained in the robust set of wavelengths (right diagram of procedure 6) will be matched against the results of procedure 8 to find common wavelengths.

10. Statistically calibrate for testing samples of unknown composition using only this set of wavelengths (procedure 9) and milk samples chemically tested for fat, protein, lactose, and moisture.

Independently tested samples will be obtained from Dairy Quality Control Institute, St. Paul, MN for this procedure. The statistics to be used here will initially be multiple regression with all cross reactions included. This will be adequate to complete the objective, but trials with ridge regressions and fingerprint methods will also be done to determine whether there is enough improvement to justify routine use of such methods.

Depending on the wavelengths available from procedure 9, those less than 1600 cm\(^{-1}\) wavenumber will be used if possible to avoid the necessity of homogenizing samples.

11. Establish sample preparation procedures and calibrate to test dairy products other than milk.

A series of procedures will be developed for preparation of sample to be tested. Concentrations of components will be near the ideal range for testing. Solvents will be selected for their ability to solubilize the products and absence of absorbance by the solvents at the wavelengths used.

12. Calibrate to determine saturation level of the fat in dairy products (especially cheese).

The effect of level of fat saturation on the spectrum above 2700 cm\(^{-1}\) will be determined by scanning samples of fatty acids such as 16:0, 16:1, 16:2, 18:0, 18:1, and 18:3. (This procedure can be done at the same time as procedure 4.) These results will then be used to correlate saturation level with substitution of non–dairy fats in dairy products. The sample preparation procedures of procedure 11 and the analysis methods of procedure 10 will be used here. The first dairy food to be considered will be cheese. The next likely product for analysis will be butter/margarine blends.
Lactose use by genetically manipulated Leuconostoc. H. M. A. Mahmoud* and W. E. Sandline, Oregon State University, Corvallis; J. K. Kondo, Utah State University, Logan

Leuconostoc strains were examined for proteolysis, aldolase, ß-galactosidase, phospho-ß-galactosidase, acid and diacetyl production in milk. Wild type strains produced pH values from 5.1 to 6.2 when incubated at 30°C for 48 hours. Specific activity of ß-galactosidase in 13 strains range from 0 to 339 units. Units of phospho-ß-galactosidase ranged from 4 to 58. All strains were aldolase negative and produced gas from glucose. The plasmid content varied from 1 to 5. L. cremoris 19254 was transformed by electroporation using chromosomal DNA obtained from a derivative of S. lactis C2. Colonies with elevated lactose utilizing ability were selected from M17 agar plates containing vancomycin and brom cresol purple. Transformants were aldolase negative and resistant to phage c2. They showed enhanced acid production in milk; e.g. the parent strain yielded a pH of 6.2 after 48 hours at 30°C in nonfat milk while transformed strains yielded pH values of 5.6. Transformants also had elevated ß-galactosidase and phospho-ß-galactosidase activities, with the former being elevated the most.

Cointegrate formation associated with conjugal transfer of a lactose plasmid in Streptococcus lactis C2O. J. K. Kondo, A. S. Smigielski, K. Gillies*, and M. B. Barnes, Utah State University, Logan.

Streptococcus lactis C2O contains 5 plasmids of 55 (pJK550), 44.8 (pJK448), 43 (pJK430), 3.7 (pJK037), and 2.1 (pJK021) kilobases (kb). Plasmid-curing studies indicate that lactose utilization (Lac) and proteinase activity are coded for by pJK550 and pJK430. Conjugal transfer of Lac to plasmid-cured derivatives results in transconjugants containing pJK550, a new plasmid of 99.8 kb (pAS005), both pJK550 and pAS005, or no detectable plasmids. Restriction endonuclease and DNA hybridization studies indicate that pAS005 is a cointegrate plasmid resulting from fusion of pJK550 and pJK448. In secondary matings, Lac is transferred at frequencies ranging from $10^{-1}$ to less than $10^{-9}$ transconjugants/donor. Formation of cointegrates was recombination independent suggesting fusions were mediated by transposable elements. A 9.0 kb BamHI fragment has been identified which contains the junction fragment of the fused plasmids. We are sequencing the junction fragment to characterize the transposable element and to compare it to other known transposons and insertion sequences.


The effects of commercially available polysaccharides on chymosin coagulation of milk were investigated. Polysaccharide material from lactic acid bacteria was obtained and also examined. Xanthan, carrageenans, guar gum, low methoxyl pectin, low methoxyl, amidated pectin, modified corn starches, dried whole cell preparations, and lactic acid bacteria polysaccharide material were evaluated using the Formagraph and Rolling Bottle methods. Chymosin activity was affected slightly by added xanthan. Clotting time was reduced by all polysaccharides except polar Gel 11 modified starch. The reduction of clotting time was greatest with iota carrageenan. Xanthan caused an increase in clotting time following heat treatments of greater than 75°C.