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1989 Annual Report and Planning Meeting

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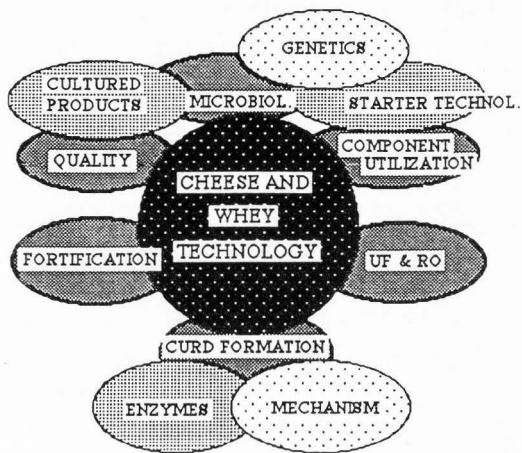


Figure 1. Major and supporting research objectives of the Western Dairy Foods Research Center



UTAH STATE UNIVERSITY • LOGAN, UTAH 84322-8700

Department of Nutrition and Food Sciences
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College of Family Life
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1989

WESTERN DAIRY FOODS RESEARCH CENTER
REPORT AND PLANNING MEETING
AGENDA

July 13

8:30 WELCOME AND INTRODUCTORY COMMENTS

Dr. Jeff Kondo, Acting Director, WDFRC
Dr. H. Paul Rasmussen, Associate Director, Utah Agricultural
Experiment Station
Dr. C. A. Ernstrom, Senior Vice President, National Dairy Promotion
and Research Board

WDFRC PROJECT REPORTS - MICROBIOLOGY

8:50 Cloning the nisin and other genes from Lactococcus into Leuconostoc
species and amplification of nisin production.

Jeffery K. Kondo, Utah State University
William E. Sandine, Oregon State University

9:15 Characterization of bacteriophage receptor sites of Lactococcus
bacteria.

William E. Sandine and Bruce L. Geller, Oregon State University

9:30 Studies on the growth and survival of Bifidobacterium species in
milk.

William E. Sandine and John. W. Ayres, Oregon State University

9:45 Purification of a bacteriocin from Pediococcus pentosaceus and
genetic transfer of the plasmid-borne determinant.

Mark A. Daeschel, Oregon State University

10:00 Prediction and determination of the efficacy of nisin in dairy foods.

Mark A. Daeschel, Oregon State University

10:15 Break

10:30 Production of omega-3 fatty acids by genetically altered fungi and
lactic acid bacteria.

Floyd W. Bodyfelt, William E. Sandine, and Daniel P. Selivonchick,

Oregon State University.

WDFRC PROJECT REPORTS - PRODUCT QUALITY

- 10:45 ✓ **Rapid assay for heat resistant microbial proteases in raw milk by a simple casein denaturation method.**
Floyd W. Bodyfelt, Oregon State University
M. W. Griffiths, Hannah Research Institute, Scotland
- 11:00 ✓ **Application of fourier transform infrared technology to milk and dairy products.**
Rodney J. Brown, Utah State University
- 11:15 ✓ **Estimation of individual milk proteins and genetic variants by multicomponent analysis of amino acid profiles.**
Rodney J. Brown, Utah State University
- 11:30 ✓ **Evaluation of milk proteins as whitening agents in processed meats and poultry products.**
Daren P. Cornforth, Utah State University
- 11:45 ✓ **Iron fortification of cheese curd.**
Arthur W. Mahoney, Utah State University
- 12:00 **Lunch.** West and Center Colony Rooms, Taggart Student Union.
- 1:00 ✓ **Evaluation of iron-protein complexes in iron-fortified dairy products.**
Arthur W. Mahoney, Utah State University
- 1:15 ✓ **Characterization of the post-absorptive behavior of B-lactoglobulin for control of spore and microbial adhesion.**
Joseph McGuire, Oregon State University
- 1:30 ✓ **Method for identifying batch of origin of semi-continuous cheese processes.**
Lynn V. Ogden, Brigham Young University
- 1:45 ✓ **Optimization of the sensory qualities of flavored yogurt.**
Mina R. McDaniel and Floyd W. Bodyfelt, Oregon State University

1989

WDFRC PROJECT REPORTS - ULTRAFILTRATION AND REVERSE OSMOSIS

- 2:00 ✓ Cogeneration of biogas and single cell protein from ultrafiltration permeate and whey.
Conly L. Hansen, Utah State University
- 2:15 ✓ Low moisture cheese from homogenized UF milk.
Donald J. McMahon, Utah State University
- 2:30 ✓ Continuous production of cottage cheese from ultrafiltered skim milk retentate.
Lynn V. Ogden, Brigham Young University
C. A. Ernstrom, Utah State University
- 2:45 ✓ Use of ultrafiltration and different heat treatments on yogurt flavor and physical properties.
Paul A. Savello, Utah State University

3:00 **BREAK**

WDFRC PROJECT REPORTS - CURD FORMATION/CHEESE TECHNOLOGY

- 3:15 ✓ Cooling rate of cheddar cheese: Comparison between 40 and 640 lb blocks and uniform cooling of 640 lb blocks.
J. Antonio Torres and Floyd W. Bodyfelt, Oregon State University
Conly L. Hansen, Utah State University
- 3:30 ✓ Cheddar cheese blocks: effect of cheese composition and cooling method.
J. Antonio Torres and Floyd, W. Bodyfelt, Oregon State University
- 3:45 ✓ A new method for measuring syneresis of renneted gels applied to development of cheese.
Conly L. Hansen, Utah State University
- 4:10 ✓ Interaction of protein and polysaccharides in chymosin and acid coagulation of milk.
Robert L. Olsen, Utah State University
- 4:15 ✓ Improving yield and physical properties of mozzarella cheese.
Gary H. Richardson, Craig Oberg, and Lynn Moyes, Utah State
- 4:10 ✓ Improved control of cheese manufacture through vat monitoring.

4:45 Gary H. Richardson, Utah State University
✓ **Omnispec: new instrumentation for the dairy industry.**

Gary H. Richardson, Utah State University

5:00 **Final Comments**

6:00 **Steak Fry - Logan City Hydro II Park**

July 14

8:30 **Operational Advisory Committee Meeting**

1. Approval of minutes.
2. Director approval.
3. Project and budget approval.
4. Discussion of the operation and direction of the WDFRC.
5. Technical Advisory Committee changes.
6. ADSA symposium - Involvement of the WDFRC in transferring technology to product developers.

How is the potential economic impact of a project assessed prior to funding?

How are the technological needs of the industry that lead to basic research design identified?

How is the industry involved during the life of a project?

What is the mechanism for finally implementing technology in the industry?

How is the communications or industry relations aspect of the program structured?

How is technical backup provided after implementation?

What is our center's approach toward bringing dairy food manufacturers together with commercial users of dairy products?

Adjournment of OAC meeting

Technical Advisory Committee signatures needed for project budget changes

Western Dairy Foods Research Center
Operational Advisory Committee
July 13, 1989

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WESTERN DAIRY FOODS RESEARCH CENTER
ANNUAL PROGRESS REPORT

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

Personnel: Jeffery K. Kondo, Assistant Professor, Department of Nutrition and Food Sciences, Utah State University, Logan.

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

Jeff Broadbent, Graduate Research Fellow, Department of Nutrition and Food Sciences, Utah State University, Logan.

Herb Wyckoff, Graduate Research Assistant, Department of Microbiology, Oregon State University, Corvallis.

Kevin Gillies, Principal Research Technician I, Department of Nutrition and Food Sciences, Utah State University, Logan.

Mary Barnes, Research Technician, Department of Microbiology, Oregon State University, Corvallis.

Funding Sources: Western Dairy Foods Research Center; Utah Agricultural Experiment Station; National Science Foundation; United States Department of Agriculture-Agricultural Research Service National Needs Graduate Fellowship Program; United States Department of Agriculture-Competitive Research Grant Program; Oregon Agricultural Experiment Station.

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.

Results, Utah State University:

Our research for the past year has focused upon objectives 3 and 5 as described above. Studies were initiated to examine the conjugal transfer of nisin production among strains of Lactococcus lactis ssp. lactis (formerly S. lactis, 5). These experiments led to the development of a new, rapid and efficient method for conjugation in these organisms. Using this technique, which we term "direct-plate" conjugation (1), we have been able to more efficiently transfer the nisin production/immunity and sucrose phenotypes (4,6) into a variety of Lactococcus lactis ssp. lactis recipients (Table 1). We have also been able to transfer these traits into Lactococcus lactis ssp. cremoris SW224. Plasmid analysis confirmed the transfer of the nisin genes from L. lactis ssp. lactis 11454 to L. lactis ssp. cremoris SW224 (Figure 1). Nisin assays using the agar overlay technique described by Steele and McKay (6) demonstrated that all transconjugants produce the antibiotic (Fig. 2). We are presently involved in experiments to transfer the nisin genes into Leuconostoc. We plan to use other lactic acid bacteria as recipients in future experiments as we see many applications among dairy, food, and agricultural lactic fermentations.

In related research examining plasmid-encoded proteinase activity in lactococci, we have found that the proteinase genes, located on the lactose plasmid (2,3), are not sufficient for fast-acid production in many strains which acquire the plasmid. Results from conjugation and electro-transformation studies indicate that additional gene(s), located on a 43 Kb plasmid of L. lactis ssp. lactis C20, are required for fast acid production in many lactococcal strains. The additional gene(s) required can be either plasmid or

chromosomally encoded depending on strain. These results support prior data obtained in Dr. Sandine's laboratory where Leuconostoc recipients, electrotransformed with a lactococcal lactose-proteinase (Lac*Prt*) plasmid, only showed a slight increase in proteolytic activity (unpublished results). Experiments are presently underway in Dr. Sandine's laboratory to co-transform Leuconostoc with the Lac*Prt* and Prt factor plasmids to produce Leuconostoc strains with rapid-acid producing capabilities.

Results, Oregon State University:

During the past year we have developed a successful transformation system for Leuconostoc species using electroporation. Various parameters such as voltage, buffer composition, and capacitance levels have been determined for obtaining optimal transformation frequencies. This system has been used to successfully transform Leuconostoc mesenteroides ssp. cremoris, Leuconostoc mesenteroides ssp. dextranicum, and Leuconostoc lactis. Under these conditions, transformation does not alter the resident plasmid DNA content of the recipient cells. Several cloning vectors which replicate in Leuconostoc have also been identified.

With the electro-transformation system developed, transformation frequencies are at sufficient levels to allow for cloning directly into Leuconostoc spp. or to allow the transformation of intact plasmids isolated from suitable donor organisms. Without these possibilities, passage of DNA through other organisms (e.g. Bacillus subtilis, Escherichia coli) would be necessary. These situations would be of relevance when genetically engineered strains are considered for approval by appropriate regulatory agencies.

Future research involving the transformation and cloning of proteinase and lactose utilization genes from lactococci into Leuconostoc is now possible

since the necessary components for a gene cloning system in Leuconostoc spp. have been defined.

Impact of Research - Nisin:

Nisin, a polypeptide antibiotic produced by some strains of L. lactis ssp. lactis, is an effective inhibitor of gram-positive bacteria. It has been used as a food preservative in Europe since 1954 and has recently been approved for use in the United States for certain dairy products.

For many years researchers have been interested in cloning the nisin genes into other organisms used to manufacture fermented foods thus enhancing the shelf-life of these products. Use of conjugation, a natural gene transfer process, is another avenue to achieve this end. New strains constructed via conjugation receive only genes that have come from safe, food-grade lactic organisms. As a result, conjugally manipulated strains will have fewer FDA restrictions than strains developed using recombinant DNA technology.

Using the direct plate conjugation method developed, we were able to transfer the nisin production/immunity and sucrose utilization genes among lactococcal strains. These results suggest that we may be able to conjugally transfer the nisin genes to other genera of lactic acid bacteria. We can envision many applications among dairy, food, and agricultural lactic fermentations for the use of nisin-producing lactic acid bacteria to enhance the shelf-life and safety of foods.

Impact of Research - Rapid acid-producing Leuconostoc spp.

Leuconostoc spp. are slow acid-producers in milk and thus are unable to produce appreciable amounts of diacetyl in pure milk cultures. This has been accredited to the lack of proteolysis and limited lactose utilization. In

mixed cultures with lactococci, the growth of Leuconostoc is often inhibited, resulting in the lack of desirable flavor in cultured products. It is believed that by increasing the levels of proteolysis and lactose utilization of Leuconostoc, flavor defects that commonly occur may be eliminated. Improved strains of Leuconostoc will also allow the manufacture of specialty cheeses similar to varieties now being imported or the development of new fermented dairy products.

The characterization of a second proteinase factor and the development of an efficient transformation system for Leuconostoc now make possible the construction of Leuconostoc strains which grow and rapidly produce acid in milk.

REFERENCES

1. Broadbent, J.R., J.K. Kondo, and W.E. Sandine. 1989. Conjugation among Lactococcus lactis ssp. lactis on selective media. In submission to Appl. Environ. Microbiol. (7/89).
2. Fitzgerald, G.F., and M.J. Gasson. 1988. In vivo gene transfer systems and transposons. Biochimie **70**:489-502.
3. Kok, J., and G. Venema. 1988. Genetics of proteinases of lactic acid bacteria. Biochimie **70**:475-488.
4. Murphy, M.C., J.L. Steele, C. Daly, and L.L. McKay. 1988. Concomitant conjugal transfer of reduced-bacteriophage-sensitivity mechanisms with lactose- and sucrose-fermenting ability in lactic streptococci. Appl. Environ. Microbiol. **54**:1951-1956.
5. Schleifer, K.H. 1987. Recent changes in the taxonomy of lactic acid bacteria. FEMS Microbiol. Rev. **46**:201-203.
6. Steele, J.M., and L.L. McKay. 1986. Partial characterization of the genetic basis for sucrose metabolism and nisin production in Streptococcus lactis. Appl. Environ. Microbiol. **51**:57-64.

Table 1. Conjugal transfer frequencies using direct and milk agar plate techniques.

Matrig donor x recipient	Selected phenotype	Technique	Transfer frequency ¹ transconjugants/donor
ML3 x LM306	Lac ⁺ Ery ^r	Milk agar; RP ²	2.2 x 10 ⁻⁹
ML3 x LM306	Lac ⁺ Ery ^r	Milk agar; harvest ³	1.1 x 10 ⁻⁷
ML3 x LM306	Lac ⁺ Ery ^r	Direct-plate	1.1 x 10 ⁻⁷
ML3 filtrate x LM2306	Lac ⁺ Ery ^r	Direct-plate	0
ML3 + DNase x LM2306 + DNase	Lac ⁺ Ery ^r	Direct-plate	6.2 x 10 ⁻⁸
Non-viable ML3 ⁴ x LM2306	Lac ⁺ Ery ^r	Direct-plate	0
ATCC 114:4 x LM2306	Suc ⁺ Ery ^r	Milk agar; RP ²	2.4 x 10 ⁻⁸
ATCC 114:4 x LM2306	Suc ⁺ Ery ^r	Milk agar; harvest ³	1.9 x 10 ⁻⁶
ATCC 114:4 x LM2306	Suc ⁺ Ery ^r	Direct plate	1.3 x 10 ⁻⁵
ATCC 114:4 filtrate x LM2306	Suc ⁺ Ery ^r	Direct-plate	0
ATCC 114:4 + DNase x LM2306 + DNase	Suc ⁺ Ery ^r	Direct-plate	2.7 x 10 ⁻⁷
Non-viable ⁴ x LM2306 ATCC 114:4 ⁴	Suc ⁺ Ery ^r	Direct-plate	0

¹Donor C.U. was calculated using initial donor counts for direct and replica-plated milk agar plates. Frequencies for harvested milk agar plates were determined using modified donor counts (see text).

²Transconjugants obtained by replica-plating onto selective media (2).

³Conjugal mixture harvested from milk agar using 1 ml 0.85% saline and then plating cells on selective media (8).

⁴Donors heat-killed (55°C for 1h) or chlorox treated (6).

1. Plasmid Profiles

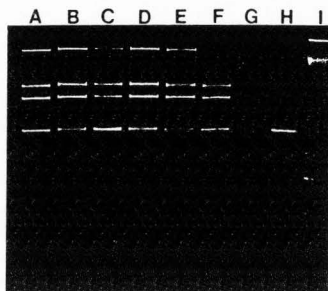


Figure 1. Plasmid profiles of *Lactococcus lactis* ssp. *cremoris* SW224 nisin-producing transconjugants. Lanes A-F, Nisin-producing SW224 transconjugants; Lane G, SW224; Lane H, JB0213 recipient strain containing pGK13; Lane I, *E. coli* V517 reference mobility plasmids.

2. Nisin Assays

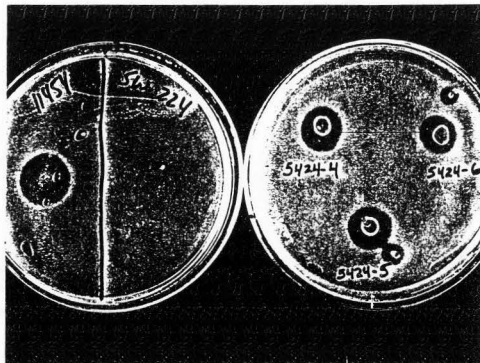


Figure 2. Nisin assays of transconjugants. Left plate, 11454 nisin-producing donor strain showing inhibition of indicator strain (*L. lactis* ssp.

New Visions

Joint Project: Nordica International, Inc., Sioux Falls, S.D.
Utah State University, Department of Nutrition and Food Sciences,
Logan, UT (J. Kondo)

We are fortunate to have such a wide variety of fermented dairy foods to choose from in North America. From the literally hundreds of cheese varieties to the many fruit flavors of yogurt, the consumer has a wide selection of interesting yet nutritionally good foods to purchase. New Visions, this issue, looks at opportunities for the manufacture of new products as well as discusses some of the practical aspects of producing traditional fermented dairy foods. We hope that as readers you will find these articles both informative and of some benefit to you as manufacturers of fermented dairy products.

COTTAGE CHEESE + YOGURT = NEW PRODUCT

Cottage cheese is a well established cultured dairy product in the United States. Approximately one billion pounds of cottage cheese are consumed annually. It is a soft, unripened cheese with a delicately mild flavor. Nutritionally, cottage cheese has many things going for it. It is a good source of protein and meets today's growing consumer demand for dairy foods with lower fat.

Why then have sales of cottage cheese been essentially flat for the past 20 years (Figure 1)? One of the reasons might be that cottage cheese is too bland or even downright boring. Maybe it has been marketed too much as a diet food. For some reason, it is difficult for the American consumer to imagine that a food can be good for you and still taste good. Or maybe the way in which cottage cheese is packaged has not kept pace with changing lifestyles. Cottage cheese is most frequently packaged in family portions, rarely as single servings.

Then on the other hand, let's look at yogurt. Only a decade ago, yogurt sales in the United States were roughly half that of the cottage cheese volume; while today yogurt sales surpass that of cottage cheese and continue to grow and expand into new areas.

Like cottage cheese, yogurt is considered a diet-conscious food; but unlike cottage cheese, sales of yogurt have not been limited to only something you eat when on a diet. Most of the yogurt in the United States is flavored with various fruits. Yogurt is eaten for breakfast, lunch, dessert, snacks and even on occasions to ward off illness. Yogurt has also capitalized on the single serving package to great success.

Wait a minute. Why then doesn't every-

one eat yogurt if it is such a perfect food? Estimates vary, but the majority of Americans do not eat yogurt on a regular basis and many have not even tried it. One of the most common criticisms of yogurt is that it tastes too sour. Let's face it. Americans have not developed the European palate for strongly flavored dairy products. How many people do you know that eat goat's milk cheese and actually like it?

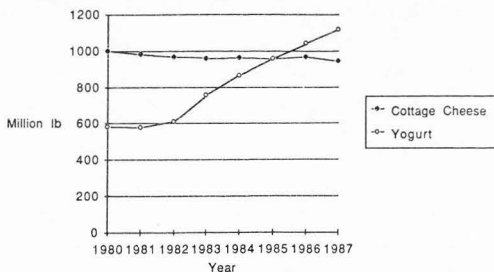
If cottage cheese is boring without enough flavor and yogurt is too sour, what then? Just add fruit to cottage cheese. What a great idea.

Wait a minute. That's already being done. Why then don't we see more fruited cottage cheese? Some think it is a marketing problem that if promoted long enough would eventually sell. Look how long it took to get drinkable yogurt going in Europe. Others point out some technical problems such as the incompatibility of the low pH fruit with the higher pH cheese. Yet others say once again it is a packaging problem. In family size packages, consumers want to mix in their own fresh fruit upon serving. With today's changing lifestyles, how many people want to carry a jar of fruit and a 16 ounce container of cottage cheese in their lunch bag? However, cottage cheese being a very perishable product, does not lend itself well to single serving packaging.

Continues on page 6

By:
Douglas Wilmet, Ph.D.
Vice President
Marketing

Cottage Cheese and Yogurt Sales in the United States



NEW PRODUCT- continued from page 3

Now I've got it. We need the mild taste of cottage cheese with the fruit varieties of yogurt that has better shelf life and comes in single servings. That's exactly what happened when we took the best of cottage cheese and combined it with the best of yogurt. We got a totally new product that we call Yo cheese.

Yo cheese is a yogurt cheese. Cottage cheese style curd is made using thermophilic yogurt bacteria. The washed curd is then mixed with a cultured dressing resulting in a finished cheese that looks and has a mouthfeel similar to cottage cheese, but it has the pH and shelf life of yogurt. And to our delight, when interfaced with fruit on the bottom, does not result in hardening of the curd.

Other unique properties of this product include the fact that it is a live culture product like yogurt. Because the starter bacteria are thermophilic, they survive the cheese cook. In fact, the curd-making process actually concentrates the bacteria somewhat resulting in higher cell numbers than in yogurt. This is in sharp contrast to cottage cheese where the starter bacteria are pretty much killed by the cooking process.

Yo cheese has nearly twice the protein of yogurt with considerably less lactose. It can be made as a one percent fat product with no more calories than most low fat yogurts. The curd is mild but has a distinctly more cultured flavor than cottage cheese curd because of the live culture. It is softer than most cottage cheese curd but this proves to be an advantage when interfacing with the fruit layer. Through the use of select strains of bacteria, Yo cheese possesses a very mild yogurt flavor without the harshness of most yogurts. In addition, the acidity is less because a major portion has been removed during the washing of the curd. The net result is a low pH product that requires less added sweeteners without the acid bite.

Because Yo cheese contains live yogurt bacteria, it also serves as a rich source of microbial lactase, the enzyme responsible for hydrolyzing the milk sugar lactose. As is the case with yogurt but not with cottage cheese, Yo cheese could be consumed by those people who are lactose intolerant.

From the manufacturing side, Yo cheese could be made in any cultured products plant that has the facilities to manufacture cottage cheese and yogurt. No new equipment would be required.

Yo cheese is just one example of new product technology that is possible by combining the best of existing products into one new product. Cottage cheese has too many good things about it to just let it stagnate and die. By combining it with the strengths of yogurt along with the right marketing, just maybe new consumers that previously did not enjoy cottage cheese or yogurt could be attracted to cultured dairy products.

PROGRESS REPORT FOR WESTERN DAIRY FOODS RESEARCH CONFERENCE JULY 1989.

Project Title: Characterization of Bacteriophage Receptor Sites of
Lactococcus Bacteria

Project No : WDFRC #

Principal Investigators: B.L. Geller and W.E. Sandine

Graduate Student: R. Valyasevi

Research Objectives:

The overall objective is to understand the molecular mechanism of phage adsorption. Specifically, the objectives are;

1. To identify the bacterial component, cell wall versus cell membrane, responsible for receptor activity.
2. To define the phage receptor at the molecular level..
3. To better understand the mechanism of resistance to binding and use this information in combination with other mechanisms of resistance ultimately to produce permanently altered strains unable to be attacked by bacteriophages

Introduction

In our last progress report (1987-1988), we have shown that the receptor sites of all phages studied were located on the cell wall rather than the cell membrane. The bacterial hosts used were *Lc lactis* C2 and ML3, *Lc cremoris* KH and 205 and *Lc diactylactis* 18-16. Subsequently we have found that the group-specific carbohydrate is the phage receptor for all of the 21 phages to 5 hosts that we have studied. We are currently collecting and analyzing data

that will more narrowly define the molecular determinant of the receptor in *Lc cremoris* KH. Our strategies in pursuing this goal are: 1. to analyze and identify the cell wall components in *Lc cremoris* KH; 2. to isolate phage binding resistant mutants and determine the changes in their cell wall components from the wild type; 3. to define the determinant of binding using lectins with different specificities; 4. to study the interaction of phage to the determinant.

Results

The cell wall of *Lc cremoris* KH was analyzed by Gas Chromatography (GC) and Mass Spectrometry (MS). The components found on the cell wall were rhamnose, galactose, glucose, N-acetylglucosamine and muramic acid (fig. 1 and 2). The ratio of rhamnose, galactose and glucose per unit dry weight of cell wall is approximately 6.8:1:1.6 respectively. As a first step to define the determinant of phage binding, the resistant mutants were isolated and cells with mutations in the phage binding were screened using four different phages infective to *Lc cremoris* KH. The cell walls of three selected resistant mutants were analyzed by GC. In the resistant mutant 1 (RMKH 1), reductions in percentages of binding of all four phages were found (table 1). RMKH 1 had 38% less galactose than the wild type (table 2). This suggested that galactose was an important component in phage binding. The RMKH 2 cells exhibited a total loss in binding to both phages kh and ml 3 and a small reduction in binding to phage 643 (table 1). RMKH 2 had 57% less rhamnose and 46% less galactose molecules than the wild type (table 2). This suggested that in addition to galactose, rhamnose was an important component in the binding of phages kh and ml 3. In RMKH 3 cells, the cell wall was found to contain 86% less galactose, 32% more glucose and 17% more rhamnose molecules than the wild type (table 2). An 86% loss of galactose in the cell wall of RMKH 3 contributed to a significant loss in binding of all four phages (table 1). Therefore the data suggest that galactose is essential to the binding of all four phages and both the rhamnose and the galactose are necessary for the binding of phages kh and ml 3. However it is

difficult to judge from these results the specificity of binding of each of this phage.

Further evidence of the importance of each of these components to the binding of phage kh was obtained from lectin binding studies. Lectin from *Momordica charantia* (specific for galactose) inhibited the binding of phage kh to the cell wall whereas lectin from *Vicia faba* (specific to glucose) did not (fig. 3). The addition of soluble galactose to the binding assay reversed the *M. charantia* lectin inhibition of phage binding to the cell wall (fig. 4). This indicated that the lectin inhibition of binding of phage kh to the cell wall was a result of the binding of *M. charantia* lectin to galactose on the cell wall.

Although the lectin studies suggested that phage kh bound to the galactose on the cell wall, incubation of phage with D (+) galactose was not inhibitory to the phage infection (fig. 5). However, phage kh binding was inhibited up to 99 % when the phage was incubated with 0.6 M α -L (+) rhamnose. The inhibition was specific because D (+) glucose and both α -D (+) and α -L (-) fucoses, the stereoisomers of rhamnose, were not inhibitory (fig. 6). Further evidence that rhamnose is inhibitory to phage kh is that cells of *Lc cremoris* KH grown in the presence of 0.4 M rhamnose were protected from lysis whereas cells grown in the presence of 0.1 M rhamnose or without rhamnose were lysed by phage kh (fig. 7). When cells grown in the presence of 0.4 M rhamnose were washed free from rhamnose and resuspended in M17 broth in the presence of phage, lysis of cells occurred (fig. 8). This indicates that protection of the bacterial host cells is due to phage inhibition by rhamnose rather than the emergence of a phage resistant mutant. All of these suggest that the major determinant of binding for phage kh is rhamnose and the configuration of the receptor molecule is important to phage binding

Preliminary evidence (not shown) indicates that antisera directed against the oligo-rhamnose group B specific carbohydrate from *Streptococcus pyogenes* inhibits phage kh binding to *Lc*

cremosis KH . This suggests that rhamnose is an important determinant of the phage kh receptor.

Summary

Galactose plays an important role in determining the phage receptor. This is apparent from the loss of galactose in all mutants analyzed, even though rhamnose was fully present in at least one of the mutants. Rhamnose also plays a crucial role in phage binding, as shown by the ability of soluble rhamnose to prevent phage infection, and the difference in loss of phage kh and ml 3 binding between RMKH 1 and RMKH 2 . We speculate that galactose may determine the conformation of an oligo-rhamnose phage binding site. Alternatively, both carbohydrates may be necessary for an optimal receptor.

Impact of Research

Bacteriophage adsorption to the bacterial host cell is the first step of infection. If phage cannot bind to its host, it cannot infect the cell. Our findings are particularly significant in that; phage binding to the cell wall fragment (non viable cells) is practically irreversible and rhamnose, the determinant of phage kh binding, is inhibitory to phage infection. Addition of a soluble receptor, i.e., rhamnose, to a growing culture in the presence of phage irreversibly inhibits phage infection. This indicates that phages can be inactivated using mimic receptors. A strategy using mimic receptors and possibly in combination with other mechanisms of resistance needs further investigations to develop into a comprehensive phage prevention program.

Fig. 1 Gas Chromatography of Cell Wall components of *Lc cremoris* KH

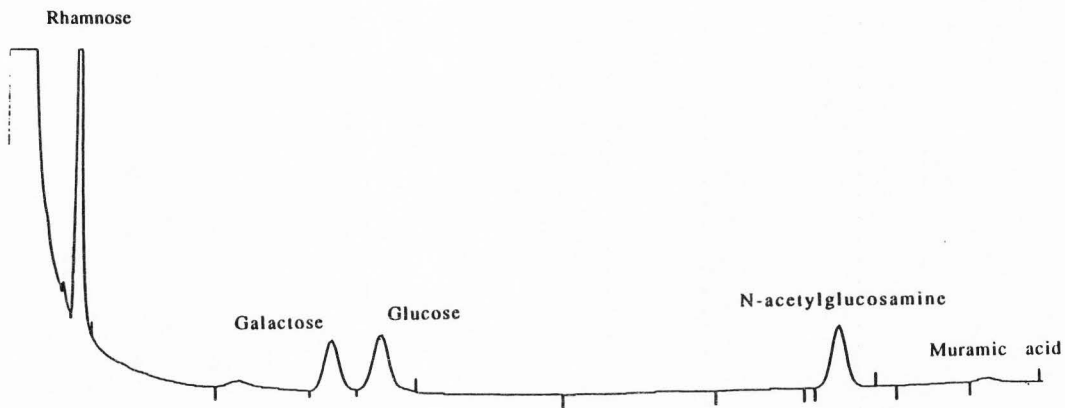
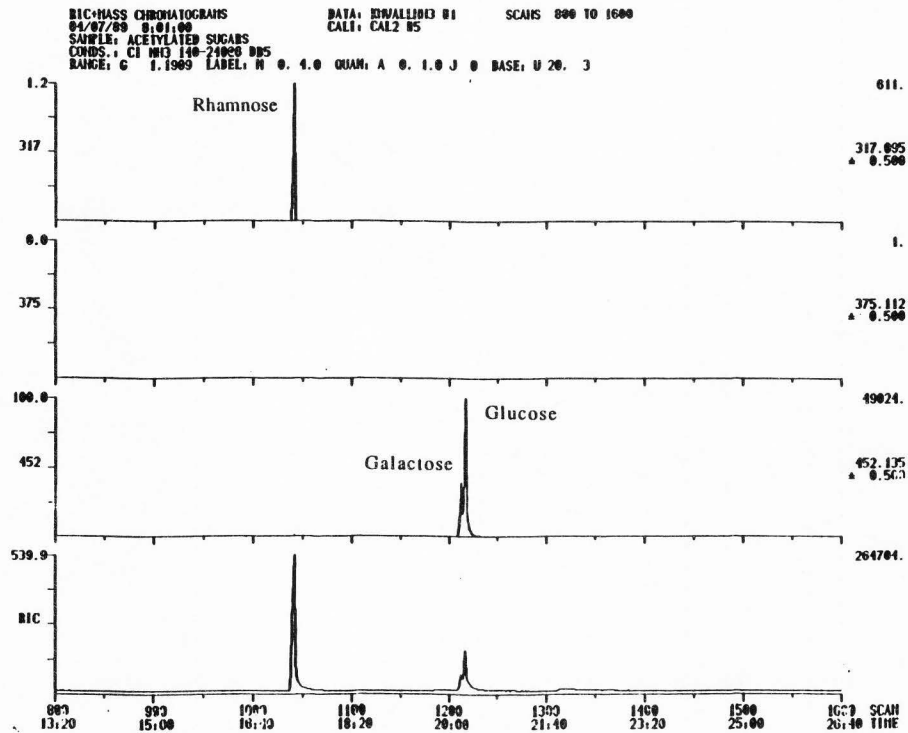


Fig. 2 Mass Spectrum Identification of Cell Wall Components of *Lc cremoris* KH.



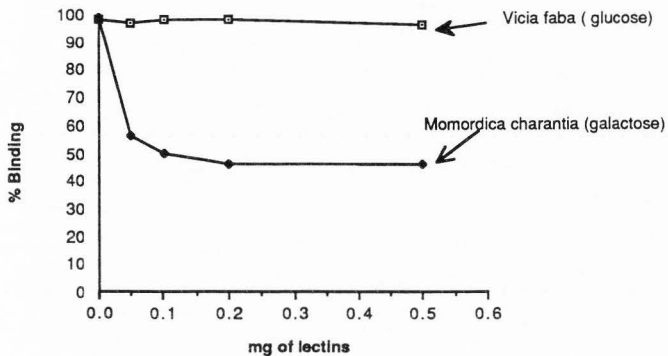


Fig. 3 Inhibition of Phage kh Binding by Lectins from Vicia faba (glucose) and Momordica charantia (galactose).

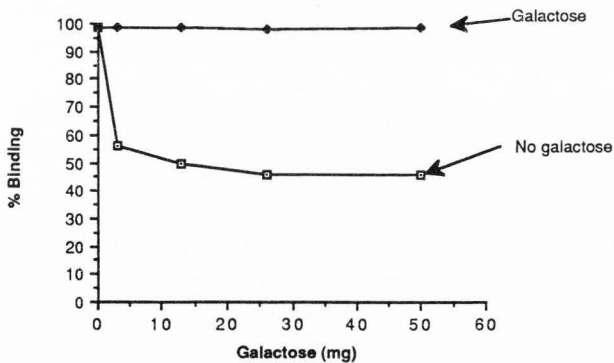


Fig. 4 Reverse of M. charantia Inhibition to Phage kh Binding by Galactose Molecules.

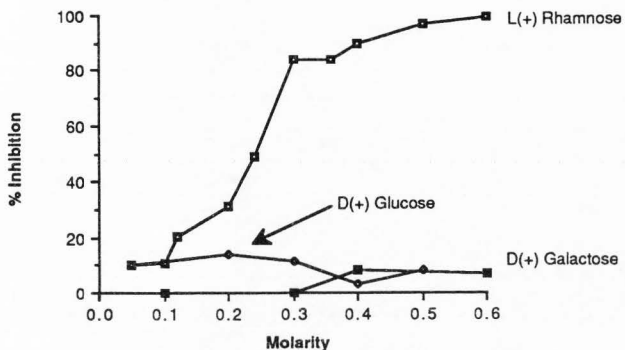


Fig. 5 Inhibition of Phage by L(+) Rhamnose, D(+) Galactose and D(+) Glucose.

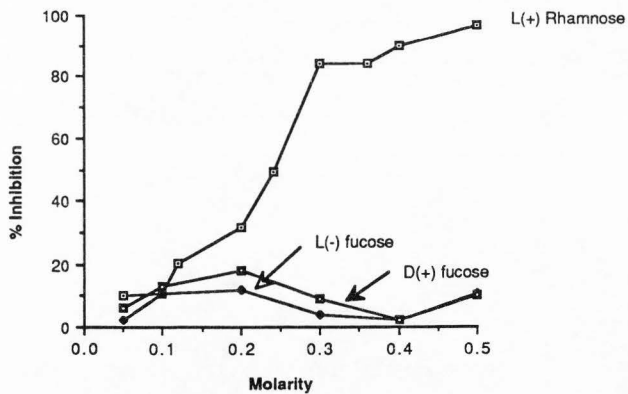


Fig. 6 Inhibition of Phage kh by L (+) Rhamnose, L (-)Fucose, and D (+) Fucose.

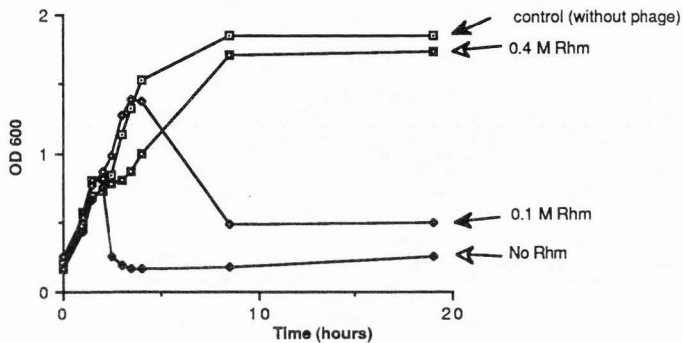


Fig. 7 Inhibition of Phage kh Infection by Rhamnose in Culture.

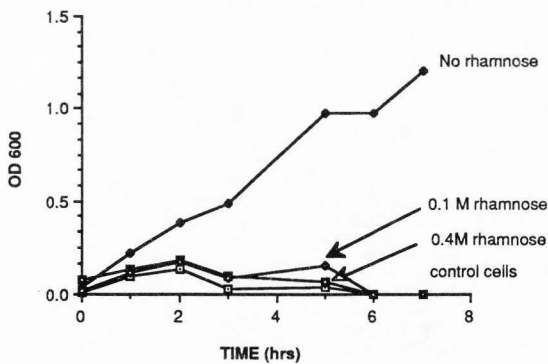


Fig. 8 Phage kh Infection of Resuspended Cells

Table 1. The Percentages of Phage Binding to Cell Walls of *Lc cremoris* KH and the Resistant Mutants.

Phage	Wall(mg)	KHWT	RMKH 1 % Binding	RMKH2	RMKH3
k h k h	0.5	99-98	68-65	12-2	0
643	5	84	71-40	75-66	16-0
m13	0.5	98	71-69	0	37-0
c 2	10	84	41-40	96-95	38-0

Table 2. The Concentrations of Hexose Molecules in the Cell Walls of *Lc cremoris* KH Wild Type and the Phage Resistant Mutants and Their Percentage Changes Over the Wild Type.

	Rhamnose		Galactose		Glucose	
	μg*	% change	μg*	% change	μg*	% change
KH WT	159		24		38	
RM KH 1	138	-13	15	-38	41	+8
RM KH 2	68	-57	13	-46	34	-10
RM KH 3	186	+17	3.3	-86	50	+32

+ increase

- reduction

* expressed in equal dry weight of cell wall hydrolyzed

WESTERN DAIRY FOODS RESEARCH CENTER
PROGRESS REPORT
JULY 13, 1989

I. Project Title: Studies on growth and survival of Bifidobacterium species in milk.

II. Project number: WDFRC # 88-083

III. Personnel:

Principal Investigators:

J. W. Ayres, School of Pharmacy, OSU

W. E. Sandine, Department of Microbiology, OSU

Graduate Student:

Ronshan Cheng

IV. Objectives:

1. To study the requirements of bifidobacteria 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 condition 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 bifidobacteria 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, lactococci, and leuconostoc.

V. Progress summary:

A whey-based medium(7% sweet whey, 0.05% cysteine and 0.3% yeast extract, WCY-0.3) was found satisfactory for growth of a variety of Bifidobacterium species without using anaerobic conditions. Freshly pasteurized(90°C ,45 min) and cooled(37°C) WCY-0.3 was inoculated with 0.2% 10^6 to 10^7 CFU/ml) of the following active Bifidobacterium: B. bifidum 1569, B. breve 15700, B. longum 15707, B. breve 15698, B. longum L10 , B. longum L12, and B. longum 3J. Following incubation for 12 hr , most strains reached maximum cell densities of 10^9 to 5×10^9 CFU/ml, except B. bifidum

15696 and B. longum 3J. Addition of the oxyrase to the WCY(7% sweet whey and 0.05% cysteine with any level of yeast extract) at 0.03 unit/ml (WCOY) reduced the lag phase of all strains, allowing maximum populations to be achieved more quickly. Boiled oxyrase(0.3 unit/ml, 100°C for 10 min) was found to be a growth stimulant for strain 15707, but not for the others. Two to seven times of population densities could be reached in the WCOY-0.3 medium of four tested strains 15696, 15700, 15707, L10 by incorporating of 1.9% disodium glycerophosphate or 1.9% trimagnesium phosphate before 12 hr incubation at 37°C . The viability of these strains was retained throughout a 24-hr incubation period, in contrast to rapid death of cells without the neutralizer. Direct inoculation with 10^7 to 10^8 CFU/ml of frozen concentrates stored at -40°C for three months in either WCY-0.3 or WCOY-0.3 yielded no differences in cell numbers for strains 15700, 15707, 15698, and T10, except for strain 15696 , which still grew much better in the oxyrase-containing medium. However B. longum L10 , whose initial inoculum was 5×10^6 CFU/ml , did show an extended lag period in the medium without oxyrase. Stability of WCOY-0.3 grown bifidobacteria, when resuspended in pasteurized skim milk and refrigerated at 4°C was strain dependent and enhanced by the presence of 0.05% cysteine. Stability of WCOY-0.3 grown bifidobacteria resuspended into WCY-0.3 with 15% glycerol during six months storage at -40°C was strain dependent. Strains 15696, 15700, 15707, 15698, and L12 did not lose their viability; however strain L6 lost about 50% viability and strains L10, T10, and T2 lost almost 90% of their population densities during the frozen storage period. A total 35 strains of bifidobacteria were also surveyed for their plasmid profiles and only one strain, B. breve 15698, harbored a 5.8 Kb plasmid.

VI. Impact of Research:

Recent research has indicated consumption of bifidobacteria is beneficial for human health of all ages. Because the growth requirements of bifidobacteria on an industrial scale are poorly understood, the objective of this present study was to define these growth needs more precisely and to develop an inexpensive growth medium. This is essential to more wide spread utilization of these bacteria in cultured dairy products. Data indicated that whey-based medium can be used industrially to prepare concentrates of bifidobacteria for use in manufacture of new fermented dairy products.

VII. Publication:

Growth characteristics of Bifidobacterium species in a whey-based medium. R. Cheng, and W. E. Sandine. 1989. J. Dairy Sci. 72: Supplement 1, abstract accepted.

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

Personnel: Mark A. Daeschel, Assistant Professor, Food Science and Tecnology, Oregon State University. Ms. Xintian Ming, Graduate Student, Food Science and Technology

Funding Sources: Western Dairy Foods Research Center, Oregon Agrcultural Experiment Station.

Objectives: The primary objectives of this study are twofold.

1. To purify the bacteriocin, Pediocin A, using protein purification mehodology to a purity suitable for the production of polyclonal antibodies.
2. To genetically transfer the pediocin A plasmid (pMD136) into dairy fermentation strains via the current state of the genetic transfer systems that have been demostrated with lactic acid bacteria.

Results:

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

A concurrent approach to developing a selection system for recivering Pediocin A transformants is to utilize the antagonistic effect

Pediocin A transformants would have on untransformed recipients. Previous data (Daeschel, unpublished) has shown the frequency of (bac-) cells within populations harboring pMcd 136 ranged from .001% to 99.9% depending on temperature of cultivation. Populations that were predominantly (bac-) after cultivation at 42 C became predominantly (bac+) after several growth transfers at 37 C. This may reflect the "self selecting" of the bacteriocin-immunity genotype where plasmid (pMD136) cured cells may be vulnerable to the bacteriocin. Our next experimental approach will be to utilize these observations to develop a selection system for Pediocin A transformants based on their ability to predominate over untransformed cells after several growth cycles.

Economic Impact:

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

Of current concern to cheese processors are the occurrence of microorganisms which cause blowing faults (Clostridia tyrobutyricum) in Swiss-type cheese and Listeria monocytogenes, a pathogen which can contaminate milk and soft cheeses. Bacteriocin producing starter cultures is one approach for controlling the incidence of such microorganisms in fermented dairy foods. Certain bacteriocins from non-dairy lactic acid bacteria have been shown to inhibit Clostridia and Listeria as well as other pathogens such as Staphylococcus aureus. The acquisition of bacteriocin producing ability by dairy starter cultures through genetic biotechnology may allow the development of strains superior to those presently available.

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

Personnel: M.A. Daeschel, Assisitant Professor, F.W. Bodyfelt, Professor, Nancy Eid, Student and Student Worker, and Randy Bender, Research Assiitant, Dept. of Food Science and Technology, Oregon State University

Funing Sources: Western Dairy Foods Research Center; Oregon Agriultural Experiment Station

Objectives:

1. To determine what molecular components of milk can interact with nisin and affect its activity.
2. To use the information gained from achieving the first objective to predict and determine the efficacy of nisin as a preservation agent in novel dairy foods such as carbonated milk beverages
3. To determine the efficacy of nisin on the inhibition of Bacilli sp. in selected milk and milk products and the subsequent impact on keeping quality

Results: Experiments were conducted to determine what specific components of dairy foods could enhance or diminish the activity of nisin. Nisir has been reported to be adsorbed to protein components of certain food. We examined a series of proteins including the milk protein, Beta-lactoglobulin for their effect on nisin activity in aqueous solution. Fig 1. (c) shows a protective effect of Beta-lactogobulin on nisin sensitive bactria when exposed to nisin. This is evidenced by the growth of the seered bacteria in the area where Beta-lactogobulin has diffused from the vell into the medium. The outer ring of inhibition in well C is due to nisir diffusing faster than Beta-lactogobulin.

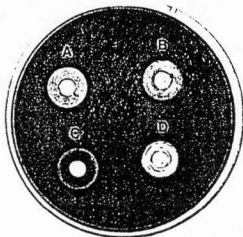


Fig.1 Effect of Beta-lactogobulin of the ability of nisin to inhibit a nisin sensitive bacterium in a seeded agar assay
A.)0.1mg/ml Beta-lactogobulin + 100 U nisin/ml
B.) 1.0 mg/ml " " " "
C.) 10 mg/ml " " " "
D.)100Unisin/ml

Experiments were also conducted to test the effect of milk fat on the activity of nisin. Nisin was added to non-fat milk, 4% milk and heavy cream and then dialyzed against pH 6.5 buffer in membrane bags and then assayed for activity at 24 hour intervals. The activity in the dialysates was greatest with non-fat milk and the least with heavy cream. This suggests that milk fat can reduce nisin activity by a binding type of mechanism.

Impact of Research:

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

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

- 1) Inhibition of spoilage microorganisms in dairy foods could minimize economic losses due to spoilage.
- 2) Inhibition of pathogenic and toxigenic bacteria to provide consistently safe products. Contaminated products (such as with *Listeria*) can give rise to adverse publicity with subsequent sales loss.
- 3) Extension of the shelf-life of perishable dairy products.

WESTERN DAIRY FOODS RESEARCH CENTER
REPORT TO OPERATIONAL ADVISORY COMMITTEE

Inclusive dates 4/88 - 6/89

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

Personel: F.W. Bodyfelt and D.P. Selivonchick, Professors, S.E. Beattie, Graduate Research Assistant, Food Science and Technology, and W.E. Sandine, Professor, Microbiology, Oregon State University.

Funding Sources: Western Dairy Foods Research Center.
Oregon Agricultural Experiment Station

Research Objectives:

1. Examine *Saprolegnia parasitica* for plasmids with the goal of using an indigenous plasmid for cloning purposes.
2. Once genetically altered, determine growth and lipid accumulation 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.
4. Determine scale up economics with an emphasis on optimizing lipid extraction from large scale chemostat production of *S. parasitica*.
5. Begin work towards construction of strains of lactic acid bacteria and yeasts that are able to produce omega-3 fatty acids.

Results:

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 [20:5 Δ 5,8,11,14,17], EPA) production contained adequate carbon and nitrogen as glutamic acid and no glucose. When grown on this medium at 20 °C, the fatty acid profile of *S. parasitica* was 24.69% EPA. When a casein hydrosylate was used as the sole nitrogen source, EPA production averaged 18.4% of the total fatty acid profile. Limiting the nitrogen source adversely affected the total growth and EPA production of *S. parasitica*, but increased total lipid production to 4.0% (wet weight basis). This data 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 have been generated by use of a combination of enzymes and osmotically stabilized with 0.5M KCl or 1M sorbitol. With this method, approximately 20% of the protoplasts regenerate. More recently 0.6M sorbitol was found to stabilize protoplasts and allow higher 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 electrophoretic analysis, no mitochondrial plasmids have been found. Therefore, a 'shotgun' approach has been employed using chromosomal DNA and various plasmids.

Within the last month we have successfully transformed *S. parasitica* to lactose utilization. Plasmid pKR1B-Lac4-1 (kindly supplied by R. Dickson) was used to transform *S. parasitica* by a polyethylene glycol procedure. This plasmid contains the β -galactosidase and lactose permease genes as well as an ARS section of the yeast *Kluyveromyces lactis*. Transformed protoplasts were identified by their ability to grow and produce blue colonies on a lactose based medium that contained the chromogenic indicator X-gal. Relatively high concentrations of plasmid DNA are required to achieve transformation and only 10-15 transformants/10⁶ protoplasts have been obtained. Colony growth is very poor on the regeneration medium currently in use. Work to improve transformant growth and identification of mode of integration of pKR1BLac4-1 into *S. parasitica* are in process. Future experiments will focus on lactose uptake and whether the permease is needed. Development of a whey permeate medium is planned in an effort to optimize lipid profiles.

Economic impact:

While there is some discussion of the health promoting benefits of PUFAs (especially omega-3), it is generally accepted that a diet with a high unsaturated to saturated fat ratio is beneficial. Some health experts encourage the addition of omega-3 fatty acids supplements in the form of fish oil gelcaps (MaxEPA®, Pronega®, etc) to normal diets. Additionally, it has been discovered that some animals when fed omega-3 fatty acids are able to incorporate them into tissue fats. Hens fed fish meal tend to lay eggs high in omega-3 fatty acids. With markets for omega-3 fatty acids increasing, a new source of these fats could have an economic impact within the scope of the dairy industry.

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

WESTERN DAIRY FOODS RESEARCH CENTER

Report to Operational Advisory Committee

Inclusive Dates 4/88-6/89

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

Personnel: F. W. Bodyfelt, Professor and Sergio Feijoo, Graduate Student, Department of Food Science and Technology, Oregon State University; and M. W. Griffiths, Microbiologist in Charge, Department of Food Science, Hannah Research Institute Ayr, Scotland.

Funding sources: Western Dairy Foods Research Center and Oregon Agricultural Experiment Station

Objectives:

1. Develop a diffusion casein-agar test capable of quantitating the proteolytic activity exhibited by heat resistant sporeforming 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 proteinase assay.
3. Determine the optimal conditions for: (a) The initial heat treatment of milk samples, (b) preliminary incubation conditions, and (c) other necessary assay parameters.
4. Develop a method for monitoring populations of Bacilli sp. in selected raw milk samples by a combination of preliminary incubation and a dye reduction test.

Results:

Experiments were conducted to reassess the optimum conditions (temperature and time) for the initial heat treatment of raw milk samples. Results indicated that the use of 75°C for 20 minutes produces higher eventual counts of surviving (and outgrowing) microorganisms than does the standard or conventional 80°C for 10 (or 12) minute treatment (Fig. 1). Furthermore, it was demonstrated that different strains of psychrotrophic Bacilli exhibit varied degrees of heat resistance or sensitivity and respond accordingly to the employed heat pre-treatments. Several experiments demonstrated that β -casein is the preferred substrate for assessing the proteinase activity of Bacillus sp. Some of the optimum test parameters for Bacilli sp. proteinase assays were evaluated

and possibly determined: e.g. pH optima (7.25); sample load onto the casein-agar matrix, minimum reaction or development time and a comparison of vertical vs. horizontal approaches for the conduct of enzyme dispersion through an agar-casein matrix.

An improved microbial growth media for enhanced sporulation rates for Bacilli sp. was formulated. Initial trials indicated the potential for a combined preliminary incubation of heat treated milk samples, and a simultaneous rapid dye reduction (redox) test for a more rapid, simple, reliable and economical method for monitoring heat resistant spore forming psychrotrophs in either raw or pasteurized milk samples.

Surveys conducted of the majority of Grade A milk producers in Oregon (1983-87) indicated that at least 25% of all producers had high counts for heat resistant psychrotrophic sporeformers (e.g. >100 cfu/ml.). More recently (1988), a majority of the pasteurized milk and cream products in another Oregon survey indicated shelf life limitations probably due to Bacilli sp. surviving the pasteurization treatment (Table 2).

Impact of Research:

Three major milk processors in the Pacific Northwest incurred serious and wide-spread consumer complaints related to "sweet curdle" defects in fluid milk and cream products in January-February, 1989. Numerous school milk quality problems stem from "early" curdling and objectionable off-flavors due to proteinase activity of Bacilli sp. derived from raw milk supplies. Cottage cheese and conceivable cheddar cheese yields and product quality can be adversely affected when Bacilli sp. and their associated proteinases prevail in milk supplies. The current test method for heat resistant sporeforming psychrotrophs in raw milk sources requires at least 10-12 days to complete. A more rapid method for determining objectional levels of Bacilli sp. in raw milk supplies would be a most advantageous analytical tool for inclusion in conventional quality bonus or incentive payment programs.

Conservatively, assuming that approximately one fourth of all milk producers incur higher than desirable Bacilli sp. counts, there may be no better way to focus on this ubiquitous milk quality problem than to develop a rapid, routine test method for screening milk samples for this troublesome microflora. Such a reliable and economically feasible analytical tool could be an invaluable step forward for enhancing milk quality at the farm level. An effective tool for rapid and accurate detection of Bacilli sp. could serve as a keystone test within milk quality incentive programs for the U. S. dairy industry.

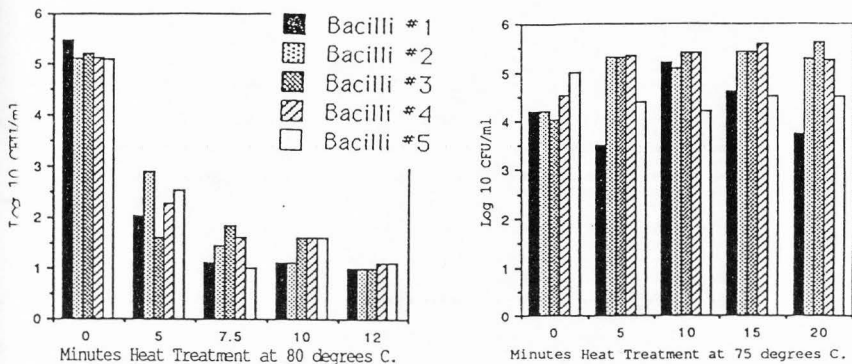


FIG. 1. SURVIVOR SPORE COUNTS FOR 5 STRAINS OF PSYCHROTROPHIC BACILLI

TABLE 1
A SURVEY OF OREGON PASTEURIZED MILK & CREAM PRODUCTS* FOR THE OCCURRENCE OF SPOREFORMERS AND PRODUCT "SPOILAGE POTENTIAL"

No. of Samples	Spore Cts. >10/ml	Psychro Spore Cts. >10/ml	Flavor "Failure" @ 7°C-days	
			10d	14d
59	33	37	35	40
(100)%	(56)	(63)	(59)	(68)

*Skim, Low-Fat, Homog. & H&H

Project Title:

Application of Fourier Transform Infrared Technology to Milk and Dairy Products

Prsonnel:

Rodney J. Brown, Professor, Nutrition and Food Sciences, Utah State University;
Ivan Mendenhall, Senior Research Technician, Nutrition and Food Sciences, Utah State University

Finding Sources:

Western Dairy Foods Research Center; Utah Agricultural Experiment Station

Objectives:

This project has a long series of specific objectives that lead to the ability to rapidly measure fat, protein, lactose, moisture, and fat saturation level in milk and dairy products. These specific objectives must be accomplished in a logical order:

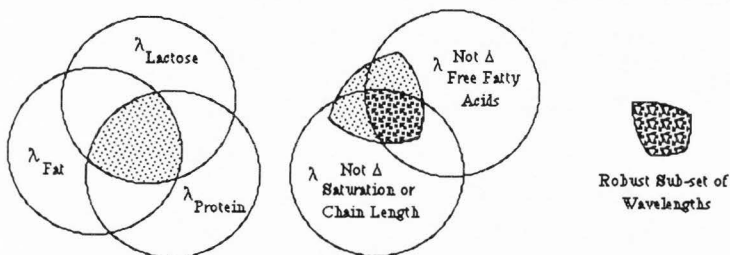
1. Find a set of wavelengths in the infrared spectrum that respond to changes in fat concentration.
2. Find a set of wavelengths in the infrared spectrum that respond to changes in protein concentration.
3. Find a set of wavelengths in the infrared spectrum that respond to changes in lactose concentration.
4. Find a set of wavelengths in the infrared spectrum that do not respond to changes in saturation level and chain length of fatty acids.
5. Find a set of wavelengths in the infrared spectrum that do not respond to changes in free fatty acid levels
6. Combine these (1-5) to make a robust set of wavelengths common to all constraints.
7. From the total infrared spectrum of milk, determine the individual spectra of:
 - Milk fat
 - Milk protein
 - Milk lactose
8. Find a set of wavelengths common to milk fat, milk protein, and milk lactose.
9. Find a set of wavelengths common to the milk components (8) and to the robust set (6).

10. Statistically calibrate for testing samples of unknown composition using only this set of wavelengths (9) and milk samples chemically tested for fat, protein, lactose, and moisture. (Less than 1600 cm^{-1} wavenumber should be used if possible.)
11. Establish sample preparation procedures and calibrate to test dairy products other than milk.
12. Calibrate, using wavenumbers greater than 2700 cm^{-1} , to determine saturation level of the fat in dairy products (especially cheese).

Results:

The total infrared spectrum of milk has been divided into the individual spectra of fat, protein, and lactose. These three separate spectra were 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 was found by subtracting the skim milk spectra from the milk spectra, the protein spectra by subtracting the lactose solution spectra from the skim milk spectrum, and the lactose spectrum was 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.

Sets of wavelengths in the infrared range that respond to changes in fat, protein and lactose concentrations have been found. We are now looking for sets of wavelengths that do not respond to changes in saturation level or chain lengths of fatty acids. These will be statistically analyzed to see which wavelengths are least affected by variations in saturation level and chain length. A set of wavelengths that do not respond to changes in free fatty acid levels will next be found. Results of the



experiments to this point 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.

Milk samples chemically tested for fat, protein, lactose, and moisture have been used to make preliminary statistical calibrations for testing samples of unknown composition. The mathematical procedures work. When the experiments described above are completed, calibration will be done using only wavelengths meeting the requirements they impose.

A series of procedures will be developed for preparation of dairy product samples 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. The first dairy food to be considered will be cheese. The next likely product for analysis will be butter-margarine blends.

Impact of Research:

This project will provide an improved method for measuring fat, protein, lactose and water content in dairy products. Most methods that have been adapted from laboratory configurations to industrial applications are based on very simple, usually old, technology (Brown, 1986). This is true of instruments used for testing milk and dairy products. The instruments we use in dairy testing were state of the art instruments thirty years ago (Hirschfield, et al., 1984). A fast method for detecting non-dairy ingredients, particularly fat, in products labeled or sold as dairy products is needed. Adulterated products sold as dairy products replace real dairy products.

For many years fixed filter spectroscopy has been the most common method for measuring fat, protein and lactose in milk (Goulden, 1956, 1961, 1964). Improvements over the years have not overcome the limitations imposed by the small number of filter bands available in such instruments (Grappin, 1984; Sjaunja and

Anderson, 1985). 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 (Brown, 1986).

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. 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 will be less frequent.

References:

- Brown, R.J., 1986, Modern methods of analysis of milk and milk products: Rapid determination of main components. pp. 507 *In* Milk the Vital Force. Reidel Publ. Co., Dordrecht, The Netherlands.
- Goulden, J.D.S., 1956, *J. Sci Food Agric.* September 7:609.
- Goulden, J.D.S., 1961, *Nature* 191:905.
- Goulden, J.D.S., 1964, *J. Dairy Res.* 31:273.
- Grappin, R., 1984, *Challenges to Contemporary Dairy Analytical Techniques.* The Royal Society of Chemistry, London.
- Hirschfield, T., J.B. Callis and B.R. Kowalski, 1984, *Science* 226:312.
- Sjaunja, L.-O. and I. Anderson, 1985, *Acta Agric. Scand.* 35:345.
- Warner, I.M., E.R. Davidson and G.D. Christian, 1977, *Anal. Chem.* 49:2155.

Project Title:

Estimation of Individual Milk Proteins and Genetic Variants by Multicomponent Analysis of Amino Acid Profiles

Personnel:

Rodney J. Brown, Professor, Nutrition and Food Sciences, Utah State University;
Marie Walsh, Graduate Student, Nutrition and Food Sciences, Utah State University;
Susan Collinge, Research Associate, Utah State University; Carol Hollar, Graduate Student, Nutrition and Food Sciences, Utah State University

Funding Sources:

Western Dairy Foods Research Center, Utah Agricultural Experiment Station

Objectives:

The goal is to determine proportions of specific milk proteins, groups of proteins (such as whey protein or casein), and genetic variants of proteins in milk and other dairy products from information contained in a single amino acid analysis of the sample. The overall objective will be met by completing the following specific objectives:

1. Determine concentrations of groups of proteins in milk such as caseins or whey protein using amino acid analysis.
2. Determine concentrations of specific milk proteins: α_1 , α_2 , β , and κ -caseins, α -lactalbumin, β -lactoglobulin, bovine serum albumin using amino acid analysis.
3. Separate genetic variants of specific milk proteins, and use amino acid analysis to quantify individual variants in a protein mixture.
4. Use techniques developed in 1-3 to analyze milk and other dairy products.
5. Determine mathematical procedures to obtain the most accurate and reproducible methods for estimating milk protein concentrations.

Results:

The basis of this method is the knowledge that each class of milk protein has a different amino acid composition. Five pure milk proteins were prepared and analyzed by amino acid analysis. They were α -, β -, and κ -casein, β -lactoglobulin and α -lactalbumin. Five different mixtures with varying ratios of these five proteins were

made and analyzed for amino acid composition. Concentrations of individual proteins in each of these mixtures were estimated (Walsh and Brown, 1988a, 1988b). One set of estimates is shown in Figure 1.

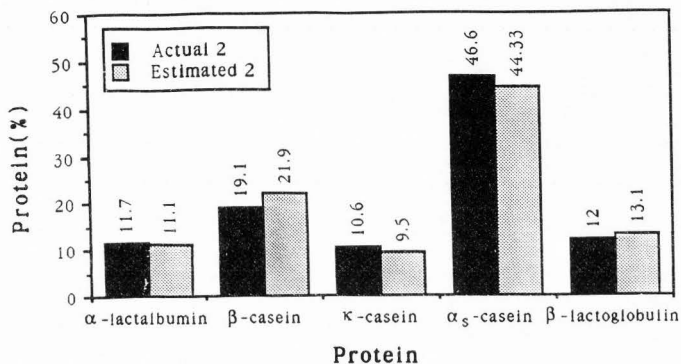


Figure 1. Concentrations of milk proteins in a mixture based on amino acid analysis estimates and measured values.

Milk samples that have been characterized for specific milk proteins and genetic variants have been obtained from Dr. Juan Medrano at University of California, Davis (Medrano and Sharrow, 1989). Information obtained from amino acid analysis of these proteins will be used to determine proportions of specific proteins and genetic variants. Individual proteins will be quantified and compared with estimates from amino acid analysis.

Impact of Research:

The overall goal of our dairy products research program is to develop new dairy products by using new manufacturing processes. To do this, we must be able to measure the individual components in milk without separating them from milk. Methods available to determine casein and whey protein concentrations in milk require precipitation of casein followed by protein measurement of both precipitate and whey.

ANNUAL REPORT
FOR
WESTERN DAIRY FOODS RESEARCH CENTER
(1988-1989)

Project Title:

Iron Fortification of Cheese Curd

Personnel:

Arthur W. Mahoney and DeJia Zhang, Nutrition and Food Sciences, Utah State University

Funding Sources:

Western Dairy Food Research Center; Utah Agriculture Experiment Station

Objectives:

1. To evaluate effects of 12 months aging on quality of iron fortified cheeses prepared in the last few months of the current project.
2. To determine the iron binding characteristics of iron-casein, ferripolyphosphate whey protein (FIP-WP) and FeCl_3 -whey protein (Fe-WP) complexes prepared with different iron concentrations.
3. To determine the effects of iron fortification with 'optimized' iron-protein complexes on cheese quality.
4. To determine the effects of iron fortification on yogurt quality.
5. To determine the bioavailability of iron in the 'optimized' iron-protein complexes as well as yogurt and cheese fortified with them.

Results:

To objectives 1 and 3.

Effect of Iron Fortification on Quality of Cheddar Cheese II. Effect of Aging and Fluorescent Light on Pilot Scale Cheeses. (Manuscript in preparation)

Based on the success of iron fortification of Cheddar cheese at the laboratory level (about 1 kg) in a preliminary study, a medium scale experiment (about 12 kg) was conducted to confirm the results and to obtain more information. An unfortified control cheese and four cheeses fortified with Fe-casein, ferripolyphosphate-whey protein (FIP-WP), Fe-whey protein (Fe-WP), or FeCl_3 were made. Iron levels and recoveries were 40, 39, 39 and 42 mg/kg, and 69, 68, 65 and 70% for cheeses fortified with Fe-casein, FIP-WP, Fe-WP and FeCl_3 , respectively. Cheese yield ranged from 9.1 to 9.7%. Iron fortification did not affect cheese moisture, fat, protein, Na and Ca contents.

TBA number and trained taste panel scores are listed in Table 1. TBA number of iron fortified cheeses and control cheese were undetectable up to 1 mo aging and remained low thereafter through 12 mo of aging. TBA number was not affected ($P>0.05$) by iron fortification. Trained taste panelists judged low oxidized flavor for control and Fe-WP fortified cheeses at 15 d of aging and did not distinguish any differences ($P>0.05$) for oxidized off-flavor among the cheeses thereafter. Oxidized off-flavor did not increase ($P>0.05$) with aging up to 12 mo. Trained taste panelists judged better cheese flavor for cheeses fortified with FIP-WP, FeCl_3 or Fe-WP at 1 mo of aging, for cheese fortified with Fe-casein at 9 mo, and did not distinguish differences in cheese flavor at 15 d, 4 mo, 7 mo or 12 mo.

Hedonic scores from the open panel of 66 lay subjects were evaluated. The judges scored all cheeses above average on the preference scale. The panelists did not detect differences ($P>0.05$) in texture among the cheeses. They preferred control cheese and the cheese fortified with Fe-casein over other cheeses for cheese flavor and overall quality.

TBA values of cheeses up to 28 d intensive fluorescent light exposure were determined. Cheeses aged for 6 mo were cut into small blocks, 14 x 10 x 2.5 cm and re-vacuum sealed in polyethylene bags, 18 x 30 cm, of 0.06 mm thickness. Sealed cheese blocks were placed in a ventilated cool room at 10°C (50°F) under fluorescent lighting which was kept on 24 h per day at a light intensity of about 1280 lux. TBA values of

cheese (about 2mm thick of surface towards lights) were determined 0 d, 3 d, 7 d, 14 d and 28 d of light exposure. Intensive light exposure slightly increased TBA number after 7 d which did not differ significantly among the cheeses. TBA numbers of the first 1mm layer of cheese was higher than the second 1 mm layer in all cheeses after 28 d of light exposure, indicating that light exposure does influence the oxidation process of cheese. However, the TBA number of the inner 1 mm cheese layer was not different from cheeses stored in the dark. Compared with unfortified cheese, fortifying iron into cheese with different iron sources did not change TBA number under this intensive light exposure.

To objective 5.

Bioavailability of Iron-milk-protein Complexes and Fortified Cheddar Cheese.

(Manuscript submitted)

Cheddar cheese was fortified with FeCl_3 or iron-casein, FIP-WP and Fe-WP complexes. Bioavailability of iron from iron fortification sources and fortified cheeses was determined by hemoglobin regeneration efficiency (HRE), a determination of percentage dietary iron incorporated into hemoglobin. Maximal iron bioavailability was measured in anemic weanling rats fed low-iron diets (about 22 mg iron/kg) for the iron fortification sources and fortified cheeses. Basal iron bioavailability was determined for iron fortification sources in normal adult rats fed high-iron diets (about 145 mg iron/kg) of iron density (32 mg iron/1000 kcal) found in some high-iron human diets.

HRE values of the anemic rats fed iron-fortified cheeses are listed in Table 2. More than two-thirds of the dietary iron was incorporated into Hb in anemic rats fed on iron-fortified cheese diets. There were no statistically significant differences ($P > .05$) in HRE among the cheese diets. The HRE values of the iron-protein complexes were similar whether they were mixed directly into diets or in fortified cheese and then mixed into diets ($P > .05$). The HRE values were the same for the FeSO_4 and FeCl_3 supplemented diets.

HRE values of adult rats fed high-iron diets (basal iron bioavailability) were much lower than those of anemic growing rats fed low-iron diets (maximal iron bioavailability) (6% versus 77%, $P < 0.01$). Iron status and dietary iron level strongly affected the iron

bioavailability values for all iron sources. However, differences of bioavailability among the iron sources were not significant ($P>.05$) either in anemic rats fed low-iron or in normal rats fed high-iron diets (Table 2). The basal iron bioavailability values were very similar to iron bioavailability values for typical nonheme dietary iron sources reported for normal humans

Feeding the high iron diets to adult rats for 14 d resulted in lower HRE than feeding for 10 d ($P<0.05$); the rats had no further increase in Hb iron during the additional 4 d of feeding indicating that the rats regulate the the absorption of iron from these iron fortification sources well. Longer time determination is needed to further confirm the capability of rats to regulate iron absorption, information which will be necessary in the establishment of the safety of iron fortified cheese. It will be necessary to first establish the most suitable iron sources for cheese manufacture, however.

Objective 2 will be studied in a separate experiment started in July, 1989.

Objective 4 will be started in July, 1989. Instead of yogurt, process cheese fortified with iron will be studied.

Impact of Research:

Iron deficiency anemia is still a major world wide nutritional problem. It is hard to increase dietary iron intake because dairy products, the major sources of dietary calcium which compose a large portion of diets, contain almost no iron. The people who consume high iron density diets (9 or more mg Fe/1000kcal) consume the least dairy products while those with lower iron density diets consume more dairy products. Iron fortified dairy products could be promoted for increasing the iron density of the low-iron diets as well as calcium and vitamin D contents thereby addressing two major nutritional concerns of women and children: i.e., iron deficiency (women, children under age 2 and elderly men) and osteoporosis (middle-aged women, growing children, elderly men).

Iron has not been fortified into dairy products because fortified iron causes oxidative damage. No trial on iron fortification of cheese has been reported. The results

reported here confirmed that Cheddar cheese could be fortified with iron up to 12 months of aging without oxidative damage and iron fortification did not cause oxidative damage when the cheeses displayed under intensive fluorescent light exposure for 28 d. These results indicate the possibility that cheese could be fortified with iron in a large factory scale and iron fortified cheese could be kept in market condition without deterioration. They also indicate that other dairy products could be fortified with iron sources used in this study to avoid oxidative damage.

Iron bioavailability of fortified cheese is important information concerning the value of fortified iron. Although bioavailability of iron in cow's milk is reported to be lower than that of human and goat milks, iron fortified cheese had highly bioavailable iron and could provide sufficient iron to meet human needs.

Food consumption trends of US women has been changing from 1977 to 1985 as an impact of nutritional messages. Consumption of lower-fat milks increased and whole milk decreased over the last decade. The percentage of women consuming higher-fat cheeses has also increased with no change observed in per capita consumption. These changes may be impacted by the nutritional messages, such as low fat and high fiber in diet may decrease the risk of cancer, and high calcium may lower the risk of osteoporosis and hypertension. However, when iron deficiency is the immediate nutritional concern of the people, the consumption of dairy products and other low-iron foods may decrease. Iron fortified cheese may provide the US population a healthier and more nutritional food and may add an extra product to counter for the choice of people concerned with their iron intakes.

TABLE 1. TBA values and trained taste panel scores of iron-fortified cheese

Iron Source	Control	FIP-WP	Fe-casein	Fe-WP	FeCl ₃	LSD ¹
Cheese Fe, µg/g	1	39	40	39	42	---
TBA Number						
7 days	0.00	0.00	0.00	0.00	0.00	---
1 month	0.00	0.00	0.00	0.00	0.00	---
4 months	0.05	0.16	0.16	0.12	0.18	---
7 months	0.00	0.03	0.00	0.03	0.05	---
9 months	0.02	0.03	0.10	0.06	0.07	---
12 months	0.03	0.08	0.07	0.07	0.07	---
Taste panel score ²						
Oxidized flavor						
15 days	1.4a	4.2c	3.6c	2.0ab	3.1bc	1.5
1 month	3.4	3.0	4.8	4.0	2.8	NS
4 months	3.3	3.8	4.2	4.8	4.5	NS
7 months	3.2	3.0	3.8	5.2	5.3	NS
9 months	2.9	3.6	2.4	4.0	3.5	NS
12 months	3.1	3.4	5.6	5.7	4.3	NS
Cheese flavor						
15 days	6.7	4.8	6.0	5.6	5.5	NS
1 month	5.9bc	7.7a	5.5c	6.4abc	7.0ab	1.4
4 months	6.0	4.9	4.4	3.8	4.7	NS
7 months	5.9	6.1	5.8	5.3	4.8	NS
9 months	5.4bc	4.4c	7.2a	4.8bc	5.6b	1.2
12 months	6.5	6.5	5.6	4.0	6.2	NS

a,b,c Means with the same superscripts are not significantly different.

¹ Least significant difference values were calculated when F was larger than F_{0.05}

(P < 0.05).

² Taste panel scores were set from 1 to 10. For oxidized flavor, the higher score indicated a stronger flavor. For cheese flavor, the higher score indicated a better quality.

TABLE 2. Iron bioavailability determined by hemoglobin regeneration efficiency in rats¹

	FeCl ₃	Fe-casein	FIP-WP	Fe-WP	FeSO ₄
<i>Iron fortified cheese</i>					
Rats' iron status	D	D	D	D	
Rats' growing stage	G	G	G	G	
Iron intake	2.05	1.98	2.00	2.09	
Repletion period	10	10	10	10	
HRE	75	66	74	67	
<i>Iron fortification source</i>					
Rats' iron status	D	D	D	D	D
Rats' growing stage	G	G	G	G	G
Iron intake	2.30	2.05	2.15	2.33	2.22
Repletion period	10	10	10	10	10
HRE	85	71	73	72	85

Rats' iron status	N	N	N	N	N
Rats' growing stage	A	A	A	A	A
Iron intake	15.31	14.33	15.07	15.11	15.37
Repletion period	10	10	10	10	10
HRE	5	8	6	7	5
Iron intake	21.04	21.00	20.55	20.96	21.11
Repletion period	14	14	14	14	14
HRE	4	4	3	3	5

¹ Abbreviations: D=iron-deficient; G=growing; N=normal; A=adult; HRE= hemoglobin regeneration efficiency.

ANNUAL REPORT
FOR WESTERN DAIRY FOODS RESEARCH CENTER
(1988-1989)

Project Title:

Evaluation of Iron-Protein Complexes in Iron-Fortified Dairy Products

Personnel:

Artiur W. Mahoney and Mohan I. Reddy, Dept of Nutrition and Food Sciences, Utah State Univ.

Funding Sources:

Western Dairy Foods Research Center; Utah Agricultural Experiment Station.

Objectives:

1. To determine the nature of the basic interaction of individual milk proteins, viz., α S1-casein, β casein, K-casein, β -lactoglobulin, α -lactalbumin and bovine serum albumen with ferrous sulfate ions, in simple buffer systems such as phosphate buffer at pH 6.6. Isolated proteins will be interacted with ferrous ions at pH 6.6 (pH of milk) and room temperature ($\sim 25^{\circ}\text{C}$) to determine the kinetics of interaction, the rate of complex formation and the optimum iron/protein ratios for stable complex formation using equilibrium dialysis.
2. To study the effect of pH, temperature and ionic strength on the iron-protein complex formation for determining the optimum conditions for binding and stable iron-protein complex formation.
3. To determine the effect of iron binding on self-association of individual proteins and/or protein cross-binding. This will be of interest if whey proteins (β -Lg & α -La) are cross-linked to caseins via chelation in terms of cheese yield.
4. To characterize the iron-protein complexes in Jennes-Koops buffer (32) (simulate milk salt buffer) using individual proteins as well as casein micelles and determine their stability. Also, the effect of iron binding on calcium (II) content of casein micelles will be studied.
5. To study the effect of iron binding to K-casein and casein micelles on the rennin hydrolysis of the phe-met bond of K-casein and subsequent coagulation of casein micelles.
6. To test the iron-protein complex formation in milk systems. Required amount of ferrous or ferric iron will be added to the milk system under controlled conditions and the complex formation will be allowed to occur. (This milk will be used for making yogurt and cheese.) The individual iron-protein complexes will be isolated from milk using ultracentrifugation and ion-exchange chromatography and characterized.
7. To determine the catalytic potency of iron-protein complexes on oxidative damage to model lipids and to lipids in milk, yogurt and cheese systems.

Results:

This project was funded 6 October 1988, but Dr. Mohan I. Reddy was not available until 1 July 1989. Therefore, research on this project is just being initiated.

Impact of Research:

Iron deficiency continues to be a major nutritional problem because of inadequate iron intakes. Dairy products contain practically no iron. Furthermore, people who generally have high-iron diets consume relatively small amounts of dairy products while people who consume larger amounts of dairy products have relatively low iron intakes. Iron fortification would increase the iron intakes of people who consume large amounts of dairy products, and it would allow people who are concerned with their iron nutrition to consume larger amounts of dairy products to achieve greater dietary calcium intakes. Thus, dairy products would be even more healthful in the diet if iron-fortified.

This research will provide basic information on the mechanisms of iron binding in dairy products, information essential to industrializing the technology of fortifying dairy products with iron.

**WESTERN DAIRY FOODS RESEARCH CENTER
REPORT TO OPERATIONAL ADVISORY COMMITTEE**

1 July 1988 - 30 June 1989

Project Title:

Characterization of the Post-adsorptive Behavior of β -lactoglobulin for Control of Spore and Microbial Adhesion to Dairy Product Processing and Packaging Surfaces.

Personnel:

Joseph McGuire (PI), Assistant Professor, Agricultural Engineering and Food Science & Technology, OSU

Kaial Al-malah, Graduate Research Assistant, Chemical Engineering, OSU

Sidey A. Kirtley, Graduate Research Assistant, Food Science & Technology, OSU

Vivat Krisdhasima, Graduate Research Assistant, Chemical Engineering, OSU

Euiang Lee, Graduate Research Assistant, Chemical Engineering, OSU

Ja-ael Luey, Graduate Research Assistant, Chemical Engineering, OSU

Robert D. Sproull, Assistant Professor, Chemical Engineering, OSU

Prært Suttiprasit, Graduate Research Assistant, Food Science & Technology, OSU

Kenneth R. Swartzel, Associate Professor, Food Science, NCSU

Fundin Sources:

Western Dairy Foods Research Center; Oregon Agricultural Experiment Station; Oregon Agricultural Research Foundation; AIChE Research Institute for Food Engineering; National Science Foundation

Objectives:

The project objectives, as originally stated are as follows.

1. to develop the mathematics required for a theoretically sound analysis of contact angle data for solid surface energy determination;
2. to use contact angle methods to evaluate the dispersive and polar components of solid surface energy for various materials.
3. use dynamic ellipsometry to continuously measure the changing thickness and refractive index of films adsorbed from an aqueous solution of β -lactoglobulin onto the characterized surfaces;
4. mathematically relate the conformational changes to time and solid surface energy;
5. combine β -lactoglobulin adsorption equilibrium data with the ellipsometric results to determine the unique protein conformation or range of conformations which bring about permanent adhesion of macromolecules, whole cells and spores; and
6. demonstrate the validity of predictive models.

Results

Project objectives 1 and 2 have been completed. We developed a methodology which requires only a simple contact angle goniometer equipped with an environmental chamber, and a series of aqueous ethanol solutions as diagnostic liquids. Several dairy contact materials were acquired and machined to a form suitable for study. The materials include many types of polymers, such as different polyethylenes, polypropylene, nylon, teflon, acetal, and others, along with stainless steel and glass.

The surface characterization methodology was recently published in detail; it can be briefly outlined as follows. Each surface under analysis is kept water-saturated to assure both equilibrium between the drop and the surface and the validity of the mathematics applied to the

system. Diagnostic liquids are characterized with respect to their polar and dispersive components of surface tension, and the dispersive component of surface energy for each of the contact materials is evaluated. Using contact angle data recorded for each material, the polar component of the work of adhesion for each drop is evaluated and plotted against the polar component of the corresponding diagnostic liquid. A straight line always results; the slope of the line and its intercept are recorded, along with the work of adhesion between the solid surface and water, as evaluated using the straight line equation. These parameters, unique for each material, are mathematically combined with the previously determined dispersive component of solid surface energy and a composite surface energy is thereby constructed. Surface energetic parameters used to construct the composite surface energy provide sensitive indices of surface hydrophilicity and hydrophobicity, as well as material cleanliness.

This protocol for determination of contact surface properties will be 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 β -lactoglobulin. Toward this end, cells which allow the continuous *in situ* observation of surface-induced protein conformational changes have been designed and constructed, and experiments are in progress. Also, funding has been received from other sources to study the surface equilibrium behavior of β -lactoglobulin and the surface chemical nature of protein binding - topics not covered under these Dairy Center project objectives, but essential to their successful completion.

Impact of Research:

It is the purpose of this research to quantify the post-adsorptive behavior of β -lactoglobulin on several materials as a function of time and contact surface properties. An understanding of this relationship should provide direction for the control of surface phenomena including biofilm development and associated bacterial colonization.

The danger associated with consuming foods contaminated by some microbial species is well-documented, and dairy foods have received wide attention as sources for such contamination in the food supply. Colonization and growth of microorganisms on dairy processing surfaces naturally presents a serious impediment to consistently providing

wholesome, high quality milk products. Control of this problem seems best attained by focusing attention on the early events which occur at the interface and lead to biofilm formation. Support for this approach stems from the fact that microbial adhesion is suggested to be dependent upon the presence and conformational state of a pre-adsorbed, proteinaceous conditioning film on the contact surface. The conformational state of this pre-adsorbed film is dependent upon measurable properties of the original contact surface. Adsorbed protein which adopts a conformation approximating its native state is suspected to render a surface less susceptible to further film formation and microbial adhesion, whereas surface protein in a sufficiently denatured state invites further biofilm formation. A quantitative understanding of contact material surface properties and their relationship to the initial surface-protein and subsequent protein-microbe interactions associated with biofilm development will provide powerful direction for control of biofilm formation.

It seems clear that milk consumption cannot be enhanced if product quality and safety are not maintained. Moreover, product quality and safety are inseparable; quality can be assured only with active concern for safety of the food through the entire period associated with its production, processing, packaging and storage. Adverse publicity stemming from a listeriosis outbreak, for example - or even a single confirmed case - would likely generate a consumer reaction that is both negative and serious. Controlling the presence of undesired microorganisms in milk will undoubtedly serve to enhance its quality and safety. In the long run, an enhanced consumer confidence in dairy products will increase their utilization, serving to not only enhance sales of these products, but also to facilitate delivery of the highest quality product for a greater period of time. Indeed, delivery of the maximum product performance attainable as the sell-by-date is reached and surpassed is not possible without an understanding of the influence of contact surface properties on the interactions which bring about a deterioration in product quality. Finally, knowledge recently gained in this project with respect to interfacial chemistry should prove useful in selecting appropriate surface sterilizing systems, and should increase options available to dairy manufacturers by providing the information on which to base decisions for alternative packaging materials and sterilization strategies.

Publications (sponsored directly by the Dairy Center from 1 July 88 to 30 June 89):

Refereed Journal Articles

- McGuire, J. and S.A. Kirtley. Surface Characterization for Prediction of Food Particle Behavior at Interfaces: Theoretical Considerations and Limitations. *Journal of Food Engineering*. 8(4): 273. 1988.
- McGuire, J. and S.A. Kirtley. On Surface Characterization of Materials Targeted for Food Contact. *Journal of Food Science*. 54(1): 224. 1989.
- Kirtley, S.A. and J. McGuire. On Differences in Surface Constitution of Dairy Product Contact Materials. *Journal of Dairy Science*. In press. 1989.

Proceedings and Symposia

- McGuire, J. and S.A. Kirtley. Surface Characterization of Materials Targeted for Food Contact. Paper no. 43c, AIChE Summer National Meeting, Denver, CO. 1988.
- McGuire, J. Temperature Influences on Food Contact Surface Energetics. In *Proceedings* 5th International Congress on Engineering and Food, Cologne, Federal Republic of Germany. Elsevier Applied Science, New York and London, in press. 1989.
- Kirtley, S.A. and J. McGuire. Exploitation of Differences in Surface Constitution of Food Contact Materials. Paper no. 217, IFT Annual Meeting, Chicago, IL. 1989.
- Krisdhasima, V., E. Lee, R.D. Sproull and J. McGuire. Temperature Influences on Food Contact Surface Properties. Paper no. 218, IFT Annual Meeting, Chicago, IL. 1989.
- Krisdhasima, V., R.D. Sproull and J. McGuire. Application of Ellipsometry to the Study of Food Protein Behavior at Interfaces. Paper no. 702, IFT Annual Meeting, Chicago, IL. 1989.

Other

- Lee, E. Temperature Effects on Surface Energetic Parameters Evaluated at Solid-Liquid Interfaces. M.S. Thesis, Oregon State University, 1989.

Project Title:

Method for Identifying Batch of Origin in Semi-Continuous Cheese Making Processes

Personnel:

L. V. Ogden, Associate Professor, Food Science and Nutrition, Brigham Young University; Mike Dunn, Graduate Assistant, Food Science and Nutrition, Brigham Young University.

Funding Sources:

Western Dairy Foods Research Center, non-dairy board monies; Brigham Young University College of Biology and Agriculture and Department of Food Science and Nutrition.

Objectives:

This study will lay the ground work for on-line colorimetric batch identification by identification of markers that are maximally detectable to a Hunter Labscan II Colorimeter in either the L_a, or b dimension while being minimally detectable to consumers. It will establish how much marker will be necessary in alternate batches to facilitate confident identification of the "seam" between batches. Variable levels of annatto, and addition of beta carotene, tumeric, and canthaxanthan will be investigated as possible markers. Each marker will be evaluated and compared with respect to cost, ease of incorporation, level of detectibility, test reliability, and possible side effects.

Results:

Beta carotene, canthaxanthan (another carotenoid), turmeric, and annatto (at increased levels) were the colors selected for use as batch markers in the experimental system.

The experimental system consisted of three four-gallon vats contained in a heated water bath. Thirty pounds of milk was added to each vat. After inoculation with starter, all three vats were colored with 1.2 ml. of annatto. Two of the vats received additional coloration with a prepared marker solution, one at a lower level, and one at a higher level. Cheddar cheese manufacture was continued following a stirred curd make procedure. The result was three batches of cheese representing three different marker concentrations, one of which was a 0 level control (1.2 ml. annatto only).

Immediately after removing the cheese from the press, four readings were taken on each of the three batches using a Hunter Labscan II colorimeter. Hunter L, a, and b values as well as spectral data were collected. By repeating this entire process twice (a total of three times) for each of the four markers. A standard curve was plotted for each marker using either the L, a, b values or the spectral data. Ninety-five percent confidence limits were established for each curve. The minimum marker concentration needed to obtain a significant colorimetric difference was determined by setting the upper or lower limit of

the control (depending on the slope of the line) and solving for the actual marker value. The back to back confidence limits combine to keep the confidence at 95%.

By following the preceding approach, minimally detectable marker levels were determined for each of the four colors. The next step will be to carry our sensory panels on batches of cheese made at these levels to determine what effect marker addition will have on the overall sensory properties of the cheese.

Impact of Research

Inability to accurately identify the batch of origin of specific units of cheese for recall or downgrading is a problem in plants using cheddaring and/or block forming towers in cheese manufacturing. One plant manager estimated the unnecessary value reduction on his cheese to be \$250,000 per year assuming that 6.5 million pounds of cheese was unnecessarily downgraded and an average reduction in value of \$.04 per pound. Ability to identify batch of origin would prevent that loss.

Title: **OPTIMIZATION OF THE SENSORY CHARACTERISTICS
OF FLAVORED YOGURT**

Project No.: WDFRC 89-043

Personnel: Mina R. McDaniel, Associate Professor, Oregon State University
Floyd W. Bodyfelt, Professor, OSU
Nancy J. Micheals, Research Assistant, OSU
Steven J. Harper, Graduate Student, OSU
Debbie L. Barnes, Graduate Student, OSU

Funding Sources: Western Dairy Foods Research Center

Research Objectives:

A. Trained Panel Descriptive Analysis

1. To train a panel for and to rate sweetness, sourness and flavor attributes of a variety of Pacific Northwest commercial yogurts of different flavors using reference standards of sucrose, lactic acid, and flavoring systems.
2. To determine the sweetness/sourness ratios for the commercial yogurts.
3. To develop terminology to describe the body and texture of the samples, and complete a texture profile analysis of the samples to measure graininess, chalkiness, firmness, viscosity, gel character, etc.
4. To statistically correlate taste and texture descriptor ratings across all commercial products tested.

B. Analytical and Physical Testing

1. To analyze all samples for pH, titratable acidity, sugar, acid, and acetaldehyde using appropriate methods.
2. To correlate pH, titratable acidity and sugar analysis data with sensory descriptors.
3. To observe relationships between the sugar/acid ratios and the sweetness/sourness ratios.
4. To analyze for viscosity and other appropriate textural attributes.

C. Consumer Panel

1. To develop a ballot for consumer evaluation of the commercial yogurt samples.
2. To run replicate 50 member panels for evaluation of each set of samples for ascertaining if preferences exist among the samples, and to determine directional information for product improvement.
3. To evaluate the effective demographic factors such as age, sex, yogurt consumption patterns, etc.
4. To correlate consumer data with trained panel, analytical and physical measurements.
5. To study the acceptance of sundae-style yogurts with a separate consumer test.

D. Formula Optimization

1. To determine ranges of important flavor and textural qualities, and to prepare yogurt formulas incorporating these ranges.
2. To optimize the product formula using appropriate statistical techniques in a series of large consumer style sensory tests.

Results of Research (1989):

A pilot study was performed to gain information about and to pretest the methodology for conducting the above research. This study began with a preliminary interview survey to determine

he effectiveness of the survey format, and to gain preliminary demographic information.

A trained descriptive panel and consumers evaluated five different commercial brands of strawberry yogurt (3 sundae style, and 2 pre-stirred). From the trained panel, significant differences were found for all attributes tested with the exception of acetaldehyde. Especially large differences were found for color intensity, lumpiness and viscosity by appearance, amount of fruit pieces, overall flavor intensity, lumpiness and viscosity by mouthfeel, artificial strawberry flavor, sweetness and sourness. In comparison with the other yogurts:

Yogurt 1 was characterized by high color intensity, overall flavor intensity, and artificial strawberry flavor while being low in lumpiness by appearance and mouthfeel, and amount of fruit pieces;

Yogurt 2 showed a high level of size of fruit pieces, sweetness, and artificial strawberry flavor while being low in color intensity, viscosity and lumpiness by appearance and mouthfeel, amount of fruit pieces and sourness;

Yogurt 3 had a high level of chalkiness, fresh strawberry flavor and amount of fruit pieces, and a low level of sourness and artificial strawberry flavor;

Yogurt 4 was low in color intensity, but high in lumpiness by appearance, astringency, viscosity by appearance and mouthfeel, chalkiness, sourness, and fresh strawberry flavor;

Yogurt 5 was characterized by high levels of lumpiness by mouthfeel, amount of fruit pieces, sourness and fresh strawberry flavor, and low levels of overall flavor intensity, jammy and artificial strawberry flavor, size of fruit pieces and sweetness.

Overall, yogurts 1 and 2, as well as yogurts 4 and 5, were similar in many attributes. Principal component analysis or Discriminant analysis could be used to verify similarity of these yogurts.

The consumer test utilized six attributes, three analyzed on a 9-pt. hedonic scale and three on a just right scale for the yogurts. Yogurt 1 was rated highest in liking by consumers for overall impression, appearance and overall texture, and was rated near just right for sweetness, sourness and strawberry flavor. Yogurt 2 was similar to yogurt 1 except for having a slightly lower rating for sourness and strawberry flavor. Yogurt 3 also had a high degree of liking for appearance compared with 1 and 2, but was lower in overall impression, texture, and sweetness, and was just right for the sourness level. Yogurt 4 was rated like slightly for appearance, overall impression, and texture, and showed lower sweetness and higher sourness ratings. Yogurt 5 was lowest in all attributes except appearance, and had the highest degree of sourness. Looking at the descriptive and consumer data together allows us to characterize each yogurt.

This pilot study was a good starting point for examining the techniques and methods to be used for the actual descriptive and consumer studies in combination with simultaneous analytical and physical tests. Further research will concentrate on the objectives stated above using refined survey and consumer ballot techniques, and a more highly trained panel. Other flavors and more brands will be studied using sensory and analytical methods to determine optimal formulas for the sweetness/sourness ratios.

Impact of Research:

If accepted and utilized by the yogurt manufacturers this research will provide information regarding acceptance of the product by the consumers. An optimized formula could increase sales to both consumers and non-consumers of yogurt, consumption of fluid milk, and competition with national brands. If the demographic results indicate distinct populations of yogurt consumers, a better understanding of consumers desires will be noted, and advertising could be targeted to these groups to help increase consumption. In the United States, currently, yogurt consumption is low per capita compared to the European countries such as the Netherlands indicating the potential for increased acceptance and sales.

Project Title: Cogeneration of biogas and single cell protein from ultrafiltration permeate and whey.

Personnel: C. L. Hansen, Associate Professor and G. S. Choudhury, Research Associate, Nutrition and Food Sciences, Utah State University

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

Objectives:

1. Digest whole whey and whey milk permeate in a novel, computer controlled, anaerobic digester that coproduced biological protein and biogas.
2. Investigate the effect of supplemental micronutrient and macronutrient addition on overall reactor stability and performance.
3. Investigate quantity and quality of biological protein and methane that can be produced by determining mass balance relationships (kinetics) between organics removal, methane and biological protein production.

Results:

In about the past 10 years, anaerobic digester design has changed radically from previous complete mix designs to digesters which retain the slow growing anaerobic bacteria. Complete mix reactors require hydraulic retention times (HRT) of 10-30 days. The new designs pass waste water through the retained sludge where it is treated within 4 h to 5 d. Organic matter in the waste is taken up by the bacteria in the sludge and converted to methane, a small amount of carbon dioxide and more biological sludge (new cells). Waste water treatment in these new "cell retention" digesters is at least as good if not better than the old complete mix designs.

We selected the upflow anaerobic sludge blanket (UASB) reactor; a sludge retention design, for our research mainly because of the ease of harvesting biological sludge. The

biological sludge is a by-product of anaerobic treatment of whey permeate which can be sold as cattle or human food. Another by-product is biogas, which can be burned like natural gas for energy.

The UASB features a sludge bed which collects in the bottom half to two thirds of the reactor by gravity. Whey permeate is fed into the bottom of the reactor and passes through the sludge bed at a slow enough velocity that the sludge bed is not displaced.

Whey permeate digestion studies

It was more difficult than we expected to digest whey permeate in a UASB; our initial attempts were unsuccessful. We had to first complete the work of objective two to understand what the nutrient requirements were. We then redesigned our reactor with a greater length to facilitate better sludge retention. The present reactor is 81 cm (32") in length and 10 cm (4") in diameter. The top 13 cm (5") is expanded 50% to reduce velocity of treated water leaving the UASB thereby helping the bacteria to settle into the sludge bed.

Whey permeate was anaerobically digested in a USAB digester in separate experiments with different sub-objectives. The first sub-objective was to determine an upper limit on organic (COD) loading rate. The second was to determine an optimal removal efficiency. The upper loading limit was 15 mg COD/L-d with about 55-65% removal efficiency for COD. We were able to maintain better than 90% removal of COD by maintaining loading below 3 mg COD/L-d.

Micronutrient and buffer addition study

Batch study

It has become apparent from recent advances in the application of anaerobic systems to industrial wastewater treatment that anaerobic organisms and in particular, methane forming bacteria have unique nutritional requirements. Since whey permeate is not a nutrient rich material, it was necessary to determine if it contains sufficient micro and macro nutrients to supply needs of the acid and methane forming bacteria in order to efficiently convert the organic matter in permeate to mostly methane, carbon dioxide and biological protein. Conclusions of this part of the study were as follows:

- 1) Addition of trace metals; Co, Ni, Mo, Fe resulted in inhibition of the methanogens.
- 2) N & P in the permeate was not adequate and must be supplemented
- 3) pH control is the key to successful anaerobic digestion of whey permeate.

Quantity and quality of biological protein and methane produced

The amount of biogas produced depended on the loading rate as shown in Figure 1 and the amount of biological protein produced was most dependent on time since startup (Figure 2.). The quality of biogas was measured on the basis of percent methane, the higher the methane percentage the better. The biological protein quality is based on protein content.

Figure 1. shows change in gas production with increased loading. The total gas produced increases at a predictable rate as indicated in Figure 1., however the methane produced per g COD destroyed did not change appreciatively with loading rate. The methane produced per g COD destroyed was close to the theoretical maximum value of 0.35 L methane/g COD for the entire experiment. More total gas was produced at the higher loading rate, however the percent methane was reduced, making the gas less valuable for energy. At least 65% methane is recommended for use without pretreatment, in a boiler. At the higher loading rates the gas quality decreased to about 44% methane.

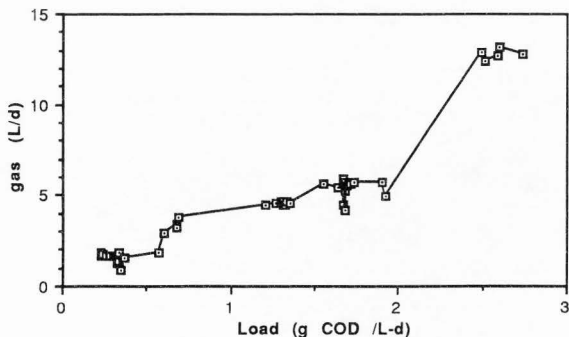


Figure 1. Gas production with increased organic loading

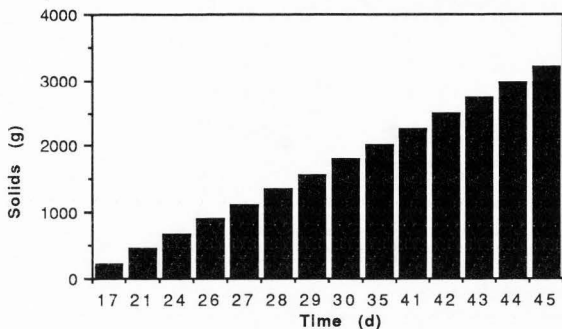


Figure 2. Solids production in the bioreactor

It is concluded that the UASB can produce about 15 g of solids/L-d. Unfortunately, most of these solids (69%) were not organic and therefore not useful for food or feed. The inorganic solids were probably due to precipitation of sodium bicarbonate in complex form. Large quantities (2.5 g/L permeate) of sodium bicarbonate had to be added to maintain a neutral pH in the digester.

Loading rate changed in a non linear fashion, but the rate of solids production was linear as shown in Figure 2. The rate of solids production did not respond to changes in loading rate. Solids production was dependent on time in a linear relationship. Apparently, the bacteria were reproducing at maximum possible rate at the lower (1 g COD/L-d) loading.

This project needs to be continued to learn how to control pH without adding expensive chemicals if this technology is to be economically viable. Our future work will investigate the addition of methanogens, loading rate, and increased sludge bed volume to control pH without chemicals.



Project Title:

High Yield, low moisture cheese from homogenized UF milk.

Personnel:

Donald J. McMahon, Assistant Professor, Department of Nutrition and Food Sciences, Utah State University;

Brian J. Orme, Graduate Student, Department of Nutrition and Food Sciences, Utah State University;

Funding Sources:

Western Dairy Foods Research Center.
Utah State University

Objectives:

1. Determine effects of homogenization treatment on fat losses from UF retentate curd.
2. Design a cheese making process so as to obtain cheese in the range of pH 5.0 - 5.4 and moisture content < 40%.
3. Determine effects of milk heat treatment on moisture, texture and body of cheese made from UF retentate.
4. Provide a manufacturing procedure for making acceptable low moisture cheese from pre-fermented UF retentate that could be adopted for a continuous cheese making process.

Results:

Objective 1:

Preliminary studies on the effects of homogenization on casein/fat complexing have been conducted. The laboratory homogenizer and heating systems used in these experiments, however, introduced too much variation into the results. This work is to be repeated using the newly installed Alfa Laval Sterilab UHT system to study the effect of temperature and homogenization pressure on the amount of casein/fat complexing that occurs during homogenization. The extent of this complexing will be determined by measuring the quantity of fat that sediments with the casein micelles during ultracentrifugation.

Objective 2.

Development of the correct curd structure is a vital part of cheese making. Cheese curd made from ultrafiltered milk retentate typically has poor textural properties. In order to duplicate the curd structure associated with cheese made from normal strength milk, we have conducted experiments to look at the relationships between milk concentration, pH, temperature, and enzyme concentration on the rate at which milk coagulates and forms a gel.

Enzymic coagulation time was found to be directly proportional to inverse of enzyme concentration at all retentate concentrations, pHs and temperatures studied but the proportionality constant varies widely with these parameters.

A series of charts included at the end of this report indicate that below pH 6.4, clotting time is not greatly affected by milk concentration, but at the natural pH of milk and concentrate (pH 6.7) concentration has a greater effect. The graphs also show that increasing temperature produces an approximately second order decrease in coagulation time at all concentrations and pHs studied.

Rennet curds of ultrafiltration concentrated milk firm very fast and can become tough and uncuttable if not cut within a narrow window. Formagraph curves of 5X retentate show that retentates with higher pH (pH 6.7) and lower temperature firm more slowly and produce firming curves more like those of unconcentrated milk. Further study will determine if using lower setting temperatures with higher rennet levels will produce a curd in a reasonable time with better texture and syneresis.

Impact of Research:

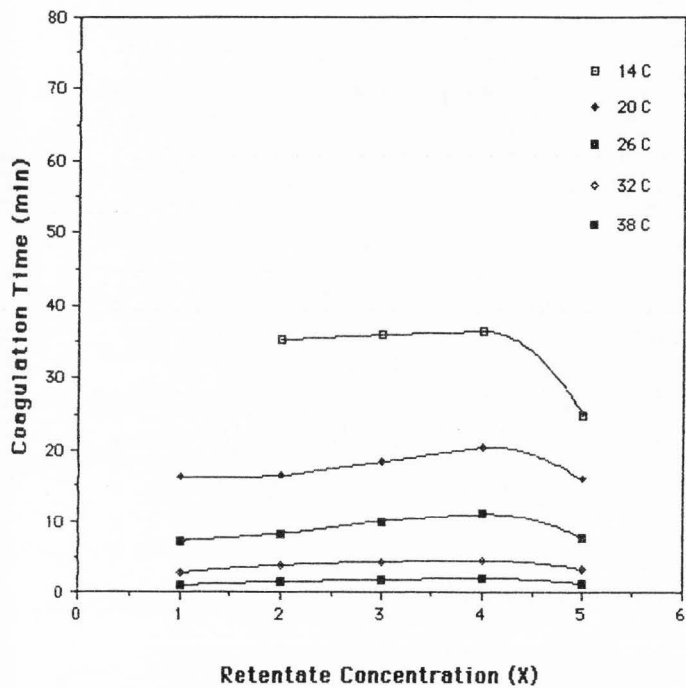
The technology of concentrating milk by ultrafiltration (UF) has progressed well over the past ten years. Much of this technology has been utilized in the development of new methodology for manufacture of cheese. The most successful applications have been in the production of high moisture cheeses by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



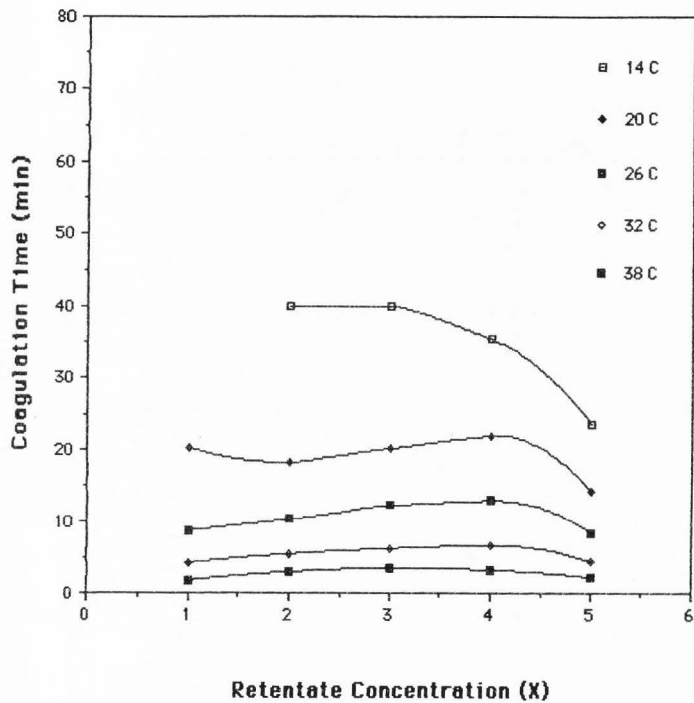
In making a low moisture cheese from UF retentate texture and body is usually unsatisfactory for use as a value-added 'natural' cheese. It is our intention that through the development of a new cheese making procedure, as described in this research proposal, it will be possible to make low moisture cheeses that will retain the high yield advantages of ultrafiltration. Cheeses in the moisture range of 35-45% are the most widely consumed cheeses in the U.S.A. today. Of the total 1987 cheese production of 6.3 billion pounds, 42% was American cheese (2.7 billion pounds) and 30% was Italian cheese (1.8 billion pounds). From this it would appear that the introduction of new varieties of cheese would have the greatest success if they were in these categories.

**Coagulation Time vs. Retentate Concentration for Various
Temperature and pH at Constant Enzyme Concentration**

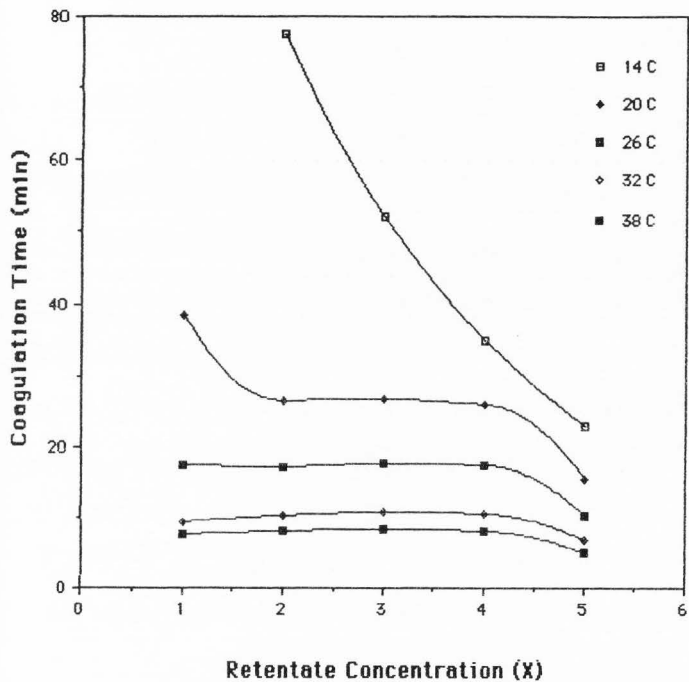
Coagulation Time vs. Milk Conc. @ pH 5.8



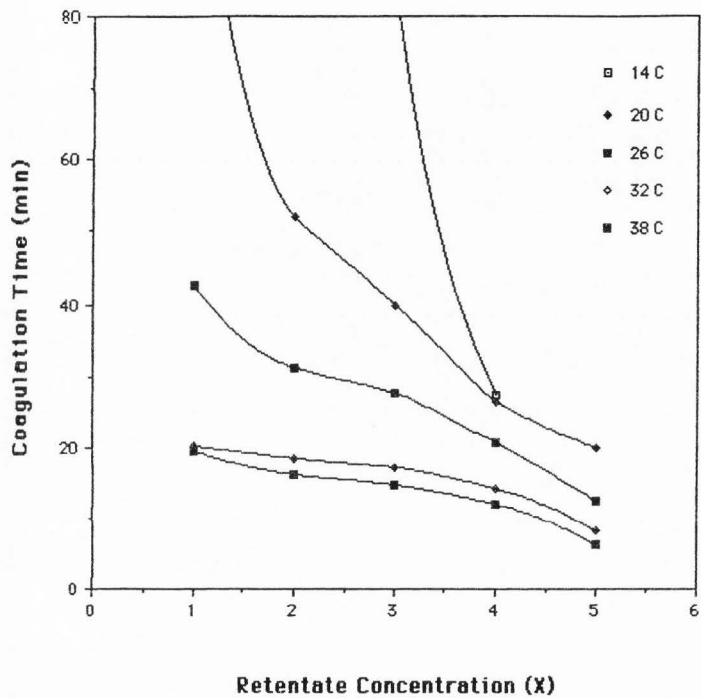
Coagulation Time vs. Milk Conc. @ pH 6.1



Coagulation Time vs. Milk Conc. @ pH 6.4



Coagulation Time vs. Milk Conc. @ pH 6.7



Project Title:

Continuous Production of Cottage Cheese from Ultrafiltered Skim Milk Retentate.

Personnel:

L. V. Ogden, Associate Professor, Food Science and Nutrition, Brigham Young University; Rick Lord, Graduate Research Assistant, Food Science and Nutrition, Brigham Young University.

Funding Sources:

Western Dairy Foods Research Center; College of Biology and Agriculture and Department of Food Science and Nutrition, Brigham Young University.

Objectives:

Learn to make cottage cheese using a continuous process from ultrafiltered skim milk retentate while studying the effect of retentate concentration and several processing variables on properties of curd

Results:

The effect of processing variables on curd characteristics were investigated. Retentate concentration, retentate preheat treatment, pH of cold acidification prior ultrafiltration, pH of cold acidification prior to curd formation, acidification agents, cooking time and cooking temperature were investigated.

Curd formation experiments to date have been conducted quiescently in tubes. Attempts to form curd in tubes with the retentate flowing have not been successful. It appears equipment will have to be designed that will allow a brief quiet period as the curd forms.

Cottage cheese curd samples from each of the curd forming experiments along with commercial curd samples were evaluated by a panel of descriptive judges. Samples were rated as to their degree of firmness, mealiness, matting and off flavor. Data from the taste panels indicate that curd firmness increases with higher cooking temperatures. Maximum curd firmness at a given cook temperature is achieved by cooking for about 60 minutes. Firmness decreases slightly at shorter and longer cook times. Curd firmness is also affected by acidifying agent used. HCl or citric acid produces firmer curd than phosphoric or lactic acid.

Mealiness was a problem with this curd. It appears to be almost entirely dependent on cook temperature. As cook temperature is increased the degree of mealiness also increases. The amount of mealiness can be reduced somewhat by not acidifying the skim milk prior to ultrafiltration. Even then the resulting curd is significantly more mealy than commercial cottage cheese curd.

To date cottage cheese curd samples have been made that are similar to commercial cottage cheese in firmness, degree of matting and flavor, but are more mealy than commercial curd. Firmness and mealiness seem to come together. Soft curd can be

made that lacks mealiness, but as firmness is increased mealiness also increases. More work must be done to reduce mealiness while retaining the desired degree of firmness.

Impact of Research:

Setting, cutting and cooking steps of cottage cheese manufacture by both the direct acid method and culturing methods have reached the limits of efficiency in conventional equipment. Vat sizes are as large as 40,000 to 50,000 pounds. Cross cutting of the curd is still a hand operation which limits the width and height of the vats. Large vats also result in long filling and draining times which add a non-productive hour or more to the production time. Larger vats would result in more non-productive time. Further efficiencies will require a continuous setting, cutting and cooking process and could be further improved by pre-concentration by ultrafiltration. A totally enclosed continuous system would also protect the quality and shelf stability of the product.

PROPERTIES OF LOW-FAT YOGURT MANUFACTURED FROM ULTRAFILTERED AND ULTRA-HIGH TEMPERATURE TREATED MILK

Personnel: Paul A.Savello, Assistant Professor, Utah State University
Richard Dargan, Graduate Assistant, Utah State University

JUSTIFICATION:

Yogurt production using ultrafiltration (UF) technology has only been researched and reported. Chapman et al. (2) produced yogurt of 21% total solids after UF. The product was comparable in flavor, body and texture to yogurt produced with milk fortified with nonfat dry milk solids. Abrahamsen and Holmen (1) ultrafiltered milk to produce yogurt that had a firmer coagulum and higher viscosity than a non-UF control. The acidity of the UF sample was more distinct.

Yogurt production using ultra-high temperature (UHT) treatments of the milk has also been reported. Schmidt et al. (8) and Labropoulos (5) reported that UHT treatment of yogurt milk produced a final product with lower firmness, lower viscosity and less syneresis. Labropoulos also reported that maximum whey protein denaturation (88%) followed a UHT treatment of 149° C for 3.3 sec compared to the same maximum whey protein denaturation with a vat treatment of 82° C for 5 min.

As UF concentration of yogurt milk gives a final product with firmer body and higher viscosity while UHT treatment of yogurt milk yields a product of lower firmness and reduced viscosity, the two technologies can prove beneficial to the production of yogurt with improved qualities. As UHT treatment of yogurt milk provides reduced syneresis in the final product, the combination of UF and UHT treatments can also yield a more consistent product with wider consumer appeal.

The advantages (including economic) of UF concentration and UHT treatment of lowfat milk for yogurt manufacture include:

- a. increase of total milk solids without addition of dried non-fat milk solids;
- b. control of yogurt milk quality by UF concentration prior to culture inoculation due to fewer dry ingredients being added to the yogurt milk;
- c. improved product quality with reduced fat levels;
- d. reduced heat input by UHT treatment compared to longer vat holding times to denature whey proteins;
- e. reduced inventory of added ingredients, including non-fat milk powder and stabilizers.

These advantages can prove beneficial if the yogurt produced is of acceptable quality. The product quality can be measured by flavor/texture, acidification time to geation, and minimum defects as syneresis, graininess, and flocculation.

UF technology can provide a means to control desired final product quality. UF concentration to different total milk solids levels together with different heat treatments prior to inoculation can indicate the relationships between milk solids/heat treatment to give different (yet acceptable) yogurt products.

PREVIOUS WORK/PRESENT OUTLOOK:

Chapman et al. (2) produced an acceptable yogurt product by concentrating milk to 18-20% total solids. The product was judged as acceptable with good flavor and texture. No objective measurements of viscosity, rheologic properties or gel strength were indicated in the report.

Yogurt milk that has been heat treated by vat, HTST, and UHT procedures with subsequent impacts on flavor and texture has been reported (4-8). Flavor profiles of the differently heated yogurt milks were not significantly different (4) whereas the textures of finished yogurts were different (6,8). The heat treatment of yogurt milk, therefore, plays a major role in the final body/texture of the product.

Combining UF concentration and different heat treatments to the yogurt milk can provide a profile to understand the effect(s) of each process as it relates to yogurt quality. By concentrating to the desired total milk solids level by UF without addition of external dried milk solids, more carefully controlled objective measurements can be made of body/texture.

OBJECTIVES:

The objectives of this research proposal are:

1. To measure the effects on the yogurt properties of viscosity, gel strength, syneresis, and water holding capacity by ultrafiltering yogurt milk to different total milk solids levels and applying different heat treatments;
2. To observe structural differences by scanning electron microscopy (SEM) and transmission electron microscopy (TEM) of the differently treated yogurt milks;
3. To measure the acceptability of yogurt flavor and body/texture by appropriate taste panel procedures;
4. To measure the effect on acidification time to desired gelation level by different heat treatments;
5. To measure the whey protein denaturation in the yogurt milk as a result of the different heat treatments.

PROCEDURES:

1. UF of yogurt milk. Milk will be ultrafiltered (batch-wise) to total milk solids of 14, 16 and 18%. The final yogurt milk will contain 0.5 to 2% milkfat.
2. Heat treatments of UF yogurt milk. The yogurt milk will be heat treated as follows:
 - a. Vat: 82 C for 20 min.
 - b. HTST: 95 C for 1.5 min.
 - c. UHT: 140 C for 6, 10, 20 sec.
3. Physical properties measurements.
 - a. Viscosity. Brookfield viscosity measurements will be conducted of the finished yogurt products;
 - b. Gel strength. Penetrometer depth measurements of appropriate time duration will indicate yogurt gel strength.
 - c. Syneresis. Syneresis measurements will be conducted according to Schmidt et al. (8). Surface whey is removed by aspiration after inclining yogurt samples 90 degrees.
 - d. Water holding capacity. Water holding capacity (WHC) of yogurt is determined according to Kalab et al. (3). The procedure involves centrifugation of the yogurt sample with subsequent weighing of the pellet. The WHC can be expressed as percent pellet weight relative to the yogurt sample weight.
 - e. Acidification time of the yogurt samples will be determined by monitoring acid development (i.e. pH) over the incubation time required to reach pH 4.9-5.0.
 - f. Flavor/Texture/Body. These properties of the finished yogurt samples will be evaluated by selected faculty members and graduate students. Proper descriptions of flavor qualities, texture and body qualities and defects will be prepared and given to panelists prior to taste panels.
 - g. Whey protein denaturation. Denatured whey protein will be determined by precipitation of casein at pH 4.6 followed by denatured whey protein determination by saturated salt precipitation.

Project Title:

Prediction of cooling rate of Cheddar cheese blocks:
Effect of cheese composition and cooling method

Personnel:

Oregon State University:

J.A. Torres, Assistant Professor; F.W. Bodyfelt, Professor; C. Grazier, Graduate Research Assistant; R. Simpson, Graduate Research Assistant; J. Bouzas, Graduate Research Assistant - Dept. of Food Science and Technology

Funding Sources:

Western Dairy Foods Research Center; Tillamook County Creamery Assoc. (Tillamook, OR); Oregon State University Agricultural Experiment Station; Utah Agricultural Experiment Station

Objectives:

The goal of this project is to examine the cooling of Cheddar cheese blocks before cheese aging which is believed to be one of the last processing steps requiring tighter control to achieve a more uniform and consistent cheese quality. This would increase consumer confidence in the product and increase Cheddar cheese consumption.

The specific objectives of this research project are:

1. To demonstrate that a more consistent quality can be achieved by controlling the cooling rate of the Cheddar cheese blocks to be aged.
2. To develop a computer program that calculates cooling rate as a function of cooling conditions and Cheddar cheese composition. This program will be made available to Cheddar cheese manufacturers.
3. To identify a starter bacteria to adventitious microorganisms ratio associated with a desirable Cheddar cheese quality as identified by sensory analysis and confirmed by chemical analysis.
4. To use a combined heat transfer and microbial activity model to determine the cooling rate that gives after cooling a desirable starter bacteria to adventitious microorganisms ratio.

Project Title:

Cooling rate of Cheddar cheese:
Comparison between 40 and 640 lb blocks
and uniform cooling of 640 lb blocks

Personnel:

Oregon State University:

J.A. Torres, Assistant Professor; F.W. Bodyfelt, Professor; C. Grazier, Graduate Research Assistant; R. Simpson, Graduate Research Assistant; J. Bouzas, Graduate Research Assistant - Dept. of Food Science and Technology

Utah State University:

Conly L. Hansen, Associate Professor, Dept. of Nutrition and Food Sciences

Funding Sources:

Western Dairy Foods Research Center; Tillamook County Creamery Assoc. (Tillamook, OR); Oregon State University Agricultural Experiment Station; Utah Agricultural Experiment Station

Objectives:

The goal of this research project is to suggest cooling process changes to achieve a better temperature control and temperature uniformity during the cooling of 640 lb Cheddar cheese blocks.

The specific objectives of this research project are:

1. To quantify the effect of heterogeneous temperature distributions on the residual starter culture activity and the growth of adventitious microorganisms during aging of 640 lb cheese blocks.
2. To quantify the effect of heterogeneous temperature distributions and microbial activity on the chemical and sensory characteristics of 640 lb cheese blocks.
3. To implement a heat transfer model for the cooling of 640 lb cheese blocks.
4. To implement a combined model for heat transfer and microbial activity of residual starter and typical adventitious microorganisms.
5. To use a mathematical optimization method to determine cooling conditions that reduce sensory differences between the various locations in a 640 lb cheese blocks.

Results:

The experimental procedures used in this research were developed primarily to accommodate the large experimental error associated with sensory analysis. The design includes four batches with two replications at each of four temperature treatments sampled throughout the ripening process. Samples were drawn from commercial production after the pressing step in the process used at Tillamook County Creamery Assoc. (Tillamook, OR). Blocks were cut and vacuum packaged under sanitary conditions. Batch collection was timed for early and late lactation to take advantage of maximum variability among batches. Samples were stored under constant temperature conditions (5°C, 15°C, 25°C and 35°C). Sample size varied according to analysis need (microbiology, chemistry and sensory). In all cases, samples were small enough to reach storage temperature within 1-2 hours.

Heat Transfer Modelling

A computer program for the heat transfer model described in the proposal has been implemented for the cooling of single blocks (any size and shape). The model takes into account the effect of the thermal properties of the cheese and the packing materials (one or more materials: wood, metal or plastic) and has been validated using data obtained at Tillamook County Creamery Assoc. and that published by Reinbold and Ernstrom (1). Further tests will be conducted at OSU and USU to make it available to dairy processors.

Microbiological Analysis

Several elements of the manufacturing process affect the types and quantities of bacteria present in the cheese as it goes into cooling and ripening: (a) pH of the curd; (b) the amount and timing of the salting; (c) the

composition and inoculum level of the starter culture; (d) sanitary conditions of the equipment and the raw materials as well as the extent of the heat treatment of the milk before use. At Tillamook County Creamery Assoc. milk used for Cheddar cheese is flash heated and not pasteurized.

Initially, the starter culture can be found in the aging cheese microflora in the greatest numbers (Fig.1). In Cheddar, this is likely to be *Lactococcus cremoris*. Within the first few days, a slight amount of growth will be observed. This is followed by a rapid die-off of cells primarily due to a continued drop in the pH of the cheese. Enzymes of both viable and dead starter cells are involved in flavor development. Starter bacteria which reach too high a level or survive too long have been associated with production of bitter flavor defects.

In cheese produced under good manufacturing conditions, the initial counts of non-starter organisms are low (Fig.1). Of the adventitious organisms, the lactobacilli are well represented. Characteristic Cheddar flavor can be attained without the presence of the adventitious organisms, but is achieved more rapidly when they are present.

Enumeration of the non-starter fractions of bacteria present in Cheddar cheese is straightforward using selective or indicative media. The starter bacteria is a different problem because they require the use of non-selective medium and isolation of individual colonies for further characterization (morphological and biochemical). This procedure is time-consuming, expensive and most of the times inconclusive. Development of the API system has facilitated this identification but remains an expensive procedure.

Samples collected at Tillamook were analyzed for total plate counts, coliforms, lactobacilli and non-starter lactobacilli. Our analysis of

microbiological data has concentrated on the non-starter lactobacilli counts as a function of time and storage temperature. At 5°C, there is essentially no change in microbial counts for up to 80 days (Fig.2). It is interesting to note that this sample could be used as a reference sample where the lack of microbial growth should be reflected in specific chemical and sensory properties. At 15°C there is significant growth up to day 40 (Fig.3). At 25°C there is significant growth up to day 16 (Fig.4), and at 35°C the growth phase seem to end at day 6, after which significant cell count reduction occur (Fig.5). A statistical analysis of the growth data suggested the need to modify our sampling plan to include 6-10 cheese samples during the exponential growth phase.

Two theoretical models can be used to analyze the exponential growth phase: the first order and the Verhulst equation (eqs. 1 and 2, respectively).

$$dN = \mu N dt \quad (1)$$

$$dN = \mu [(b - N)/b] N dt \quad (2)$$

where N = number of microbial counts, μ = specific growth rate, t = time, and b = maximum microbial counts. The effect of temperature on the specific growth rate, μ , can be analyzed with either one of the following three models:

$$\mu = \mu_0 \exp [-E_a/RT] \quad (3)$$

$$\mu = \mu_0 (1 + cT) \quad (4)$$

$$\mu^{\frac{1}{b}} = b (T - T_0) \quad (5)$$

where μ_0 , c , b are constants, E_a = energy of activation for growth, R = universal gas constant, T = absolute temperature and T_0 = theoretical minimum growth temperature. A preliminary analysis of the effect of temperature on the specific growth rate gave an estimate for the energy of activation of about 16 Kcal/g-mole. Additional experiments at other storage temperature conditions (12°C and 20°C) are needed to confirm this activation energy value and the

suitability of the Arrhenius model as compared to the other available models (eqs. 4 and 5). However, this low activation energy value would suggest that microbial growth of non-starter lactobacilli could be controlled by the diffusion of nutrients in Cheddar cheese (see Table 1).

Enumeration of the starter culture has presented a few experimental problems. New batches will be enumerated using a three step approach: (1) enumeration on a semi-selective medium (betaglycerophosphate); (2) pure culture isolation and API characterization at 4 representative time points for each temperature treatment; and (3) extrapolation of the semi-selective medium-API test results to the results obtained at other times.

Chemical Analysis

Chemical analysis requirements for the completion of this project include: carbohydrate and organic acid analysis by HPLC, D-L lactic acid determination by an enzymatic method (5,6), degree of proteolysis by a trinitrobenzenesulphonic acid spectrophotometric method (7), pH, and titratable acidity. Salt concentration, fat, moisture and total protein content were thought to be needed only at time 0. However, preliminary experiments show significant syneresis during storage, suggesting the need for more close monitoring of these values.

We chose the following methodology for the carbohydrate and organic acid analysis of Cheddar cheese (2,3,4). Samples are extracted using an acetonitrile:water (ratio needs to be optimized), the mixture is centrifuged at 1600 g and the supernatant filtered through a regenerated cellulose filter. Ten μ l of the filtered solution will be injected into a Bio-Rad Aminex HPX-87H column with a Cation- H^+ Microguard column. The mobile phase to be used is 0.0090N H_2SO_4 (flow rate of 0.8 ml/min). Variable wavelength UV and refractive

index detectors, installed in tandem, will be used for the quantification of organic acids and carbohydrates, respectively. Preliminary runs using pure carbohydrate and organic acid solutions gave good separations of major components (Fig.6).

Sensory Analysis

A descriptive panel has been trained and an appropriate set of aroma and flavor-by-mouth descriptor have been identified. Sensory testing has been conducted on samples from two batches pulled from manufacturing dates in January. Sensory testing was done at 90 and 120 days into the ripening process for both batches. At 90 days, the cheese ripened at 35°C was unpalatable and was not presented to the panel. The 5°C, 15°C, and 25°C cheeses were scored using 20 different aroma and flavor descriptors (Fig.7) and a 15 point intensity scale (Fig.8). No significant differences were observed between batches. At 90 days, significant differences between temperature treatments were as follows (Tables 2 and 3):

- 1) Overall intensity of both flavor and aroma, pungent acidic and pungent sulfur aroma, and goaty flavor were greater for cheeses ripened at higher ripening temperatures.
- 2) Buttery aroma and flavor were significantly lower in the 25°C cheese compared to the 5°C and 15°C cheeses.
- 3) Fruity, goaty, dirty, and yeasty aroma and sour, salty, bitter, fruity, dirty, and sulfur flavors were significantly greater in cheese ripened at 25°C.

At 120 days similar trends were observed with the following exceptions:

- 1) Goaty and dirty aroma, and dirty and sulfur flavor were greater at higher ripening temperatures.
- 2) Sour and salty flavors were significantly lower in the 5°C cheese compared to the 15°C and 25°C.
- 3) The 25°C cheese was significantly sweeter than the 5°C cheeses.

In comparing 90 and 120 day cheese within each temperature treatment, differences were found only in cheese ripened at 25°C.

- 1) Overall intensity of flavor and aroma increased significantly.
- 2) Nutty aroma decreased.
- 3) Dirty aroma, and dirty and sulfur flavors increased.

In general, panelist by treatment interaction was minimal where significant differences in descriptor scores were detected. This allowed us to be confident in the ability of the panelists to describe the cheese characteristics consistently. The only exception to this was in the scoring of sour flavor where one panelist scored markedly different from the others. Review sessions emphasizing the scoring of the sour flavor will be conducted before further testing.

Considering these findings, we have determined that more and earlier analysis is indicated for future batches. Later batches will be tested at 7, 30, and 60 days in addition to 90 and 120 days. Consent of the Human Subjects Board has been obtained to test before the legal minimum of 60 days for cheese made with unpasteurized milk.

Impact of Research:

American is the major type of cheese produced in the United States (2.8 billion pounds in 1986). The vast majority of this production is Cheddar

REFERENCES

1. Reinbold, R.S. and Ernstrom, C.A. 1988. Effect of non-uniform cooling on moisture, salt, and pH distribution in 290-kg blocks of stirred-curd Cheddar cheese. *J. Dairy Sci.* 71:1499.
2. Marsili, R.T., Ostapenko, H., Simmons, R.E. and Green, D.E. 1981. High performance liquid chromatography determination of organic acid in dairy products. *J. Food Sci.* 46:52.
3. Pirisino, J.F. 1983. High performance liquid chromatographic determination of lactose, glucose, galactose in lactose-reduced milk. *J. Food Sci.* 48:742.
4. Huffman, L.M. and Kristoffersen, T. 1984. Role of lactose in Cheddar cheese manufacturing and ripening. *N.Z.J. Dairy Sci. Technol.* 19:151.
5. Gutmann, I. and Wahlefeld, A.W. 1974. L-(+)-Lactate determination with lactate dehydrogenase and NAD. In *Methods in Enzymatic Analysis*, Bergmeyer, H.U. (ed.), 2nd edition, Vol.3, pp.1464. Academic Press, NY.
6. Gawehn, K. and Bergmeyer, H.U. 1974. D-(-)-Lactate determination with lactate dehydrogenase and NAD. In *Methods in Enzymatic Analysis*, Bergmeyer, H.U. (ed.), 2nd edition, Vol.3, pp.1492. Academic Press, NY.
7. Polychroniadou, A. 1988. A simple procedure using trinitrobenzenesulphonic acid for monitoring proteolysis in cheese. *J. Dairy Research* 55:585.

cheese. Unfortunately, there are wide fluctuations in the sensory quality of individual lots of Cheddar cheese. Conservatively, experienced cheese graders frequently categorize 30-40% of all American Cheddar cheese as being "high acid (sour)" or "bitter" in off-flavor.

One of the processing steps believed to be responsible for the lack of uniformity in Cheddar cheese quality is cooling of the cheese block before aging. This research project examines the microbiological, chemical and sensory consequences of lack of temperature control during this processing step and will apply engineering principles to suggest cooling conditions leading to more uniform and consistent quality.

We believe that Cheddar cheese sales are not increasing in great part because of the high variability in product quality. The economical impact is enormous because: (a) much Cheddar cheese has to be marketed at a younger age than is perhaps optimal for best price; (b) the frequency of the sourness/bitterness defect reduces its quality image and hence reduced its market value (price/unit) and also limits its overall consumer acceptance (sales volume).

TABLE 1

Typical E_a values, Kcal/mol

Diffusion	0-15
Microbial growth	20-60
Enzyme reactions	10-15
Hydrolysis	15
Lipid oxidation	10-25
Nutrient losses	20-30
Non-enzymatic browning	25-50
Spore destruction	60-80
Vegetative cell destruction	50-150
Protein denaturation	80-120

TABLE 1: DESCRIPTIVE ANALYSIS OF CHEDDAR CHEESE SAMPLES: POOLED DATA FOR BATCHES 1 AND 2

AROMA SENSORY SCORES

TEMP TIME	Overall Intensity	Buttery	Nutty	Fruity	Pungent Acidic	Pungent Sulfur	Goaty	Dirty	Yeasty

5°C									
90 days	6.8	4.5	3.1	1.7	2.0	1.3	1.9	1.2	0.4
120 days	6.8	4.9	3.2	1.3	2.0	1.2	1.9	1.1	0.3
15°C									
90 days	8.0	5.2	3.8	2.0	3.1	2.3	2.8	1.3	0.6
120 days	8.6	5.6	4.0	2.0	3.2	2.5	3.3	2.3	0.8
25°C									
90 days	10.0	3.7	3.4	3.5	4.9	3.9	4.8	3.9	2.0
120 days	10.9	3.6	2.6	4.2	5.5	3.7	5.6	4.8	2.7

TABLE 3: DESCRIPTIVE ANALYSIS OF CHEDDAR CHEESE SAMPLES: POOLED DATA FOR BATCHES 1 AND 2

FLAVOR-BY-MOUTH SENSORY SCORES

TEMP TIME	O.I.	Sour	Salty	Bitter	Sweet	Buttery	Nutty	Fruity	Goaty	Dirty	Sulfur
<hr/>											
5°C											
90 days	6.9	4.9	4.9	3.5	1.7	4.1	2.4	1.1	1.4	1.1	0.6
120 days	6.9	4.8	4.8	3.3	1.5	4.1	2.4	1.2	1.4	1.3	0.6
15°C											
90 days	8.3	5.6	4.9	3.4	1.5	4.0	2.7	1.5	2.9	1.5	1.4
120 days	8.2	6.0	5.4	4.0	1.8	3.9	3.1	1.5	2.7	2.3	1.5
25°C											
90 days	10.9	6.9	6.0	6.2	2.4	2.4	2.5	2.6	5.8	4.5	3.4
120 days	11.6	6.7	5.9	7.0	3.5	2.0	2.4	3.1	5.8	6.0	4.3
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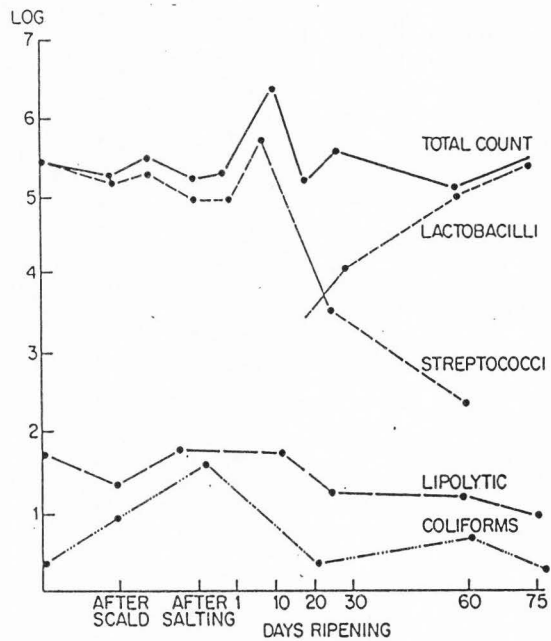


FIGURE 1. Typical Microbial Activity During Processing and Aging of Cheddar Cheese

FU

E5

E4

E3

E2

E1

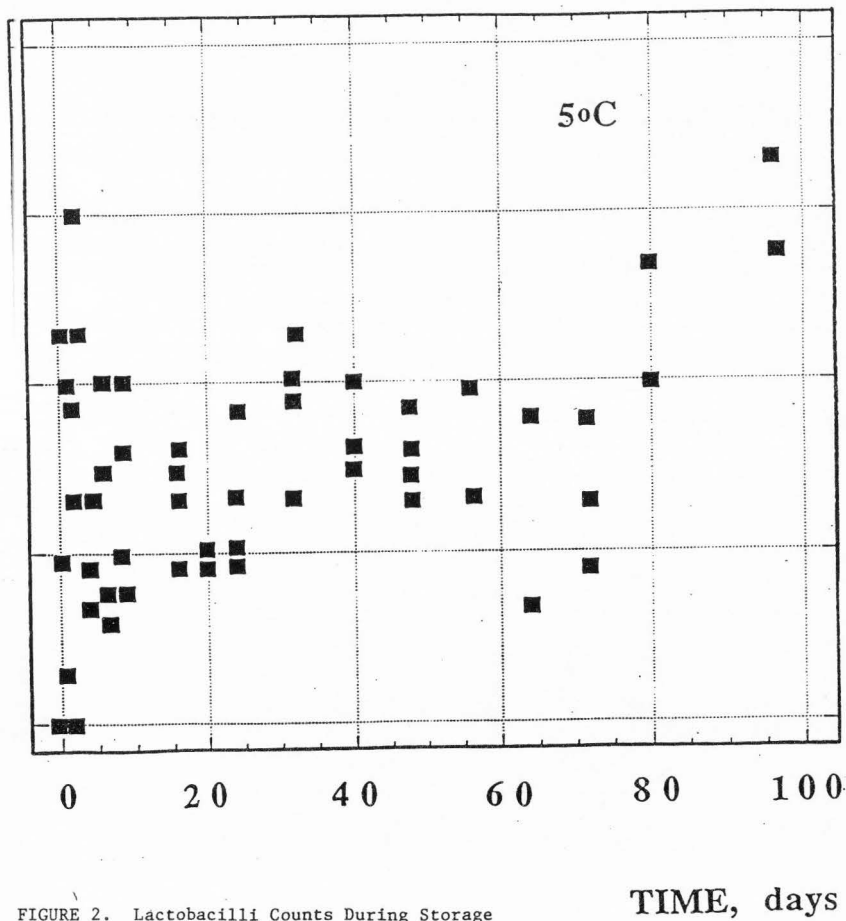
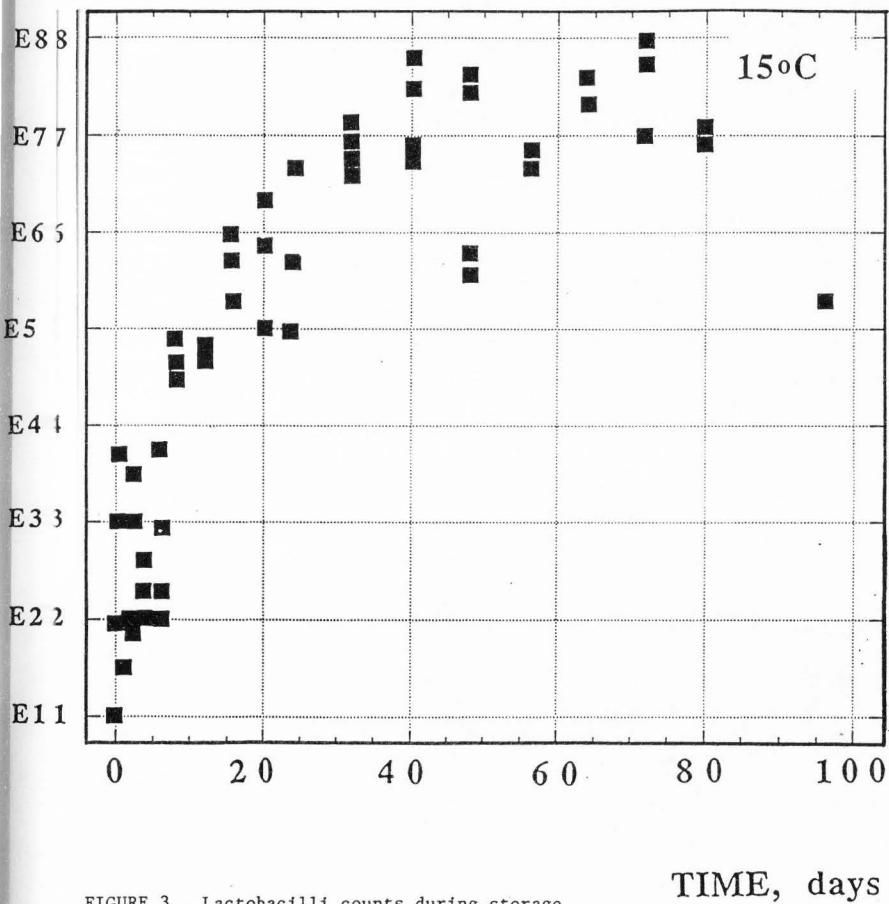


FIGURE 2. Lactobacilli Counts During Storage at Constant Temperature

TIME, days

FU



CFU

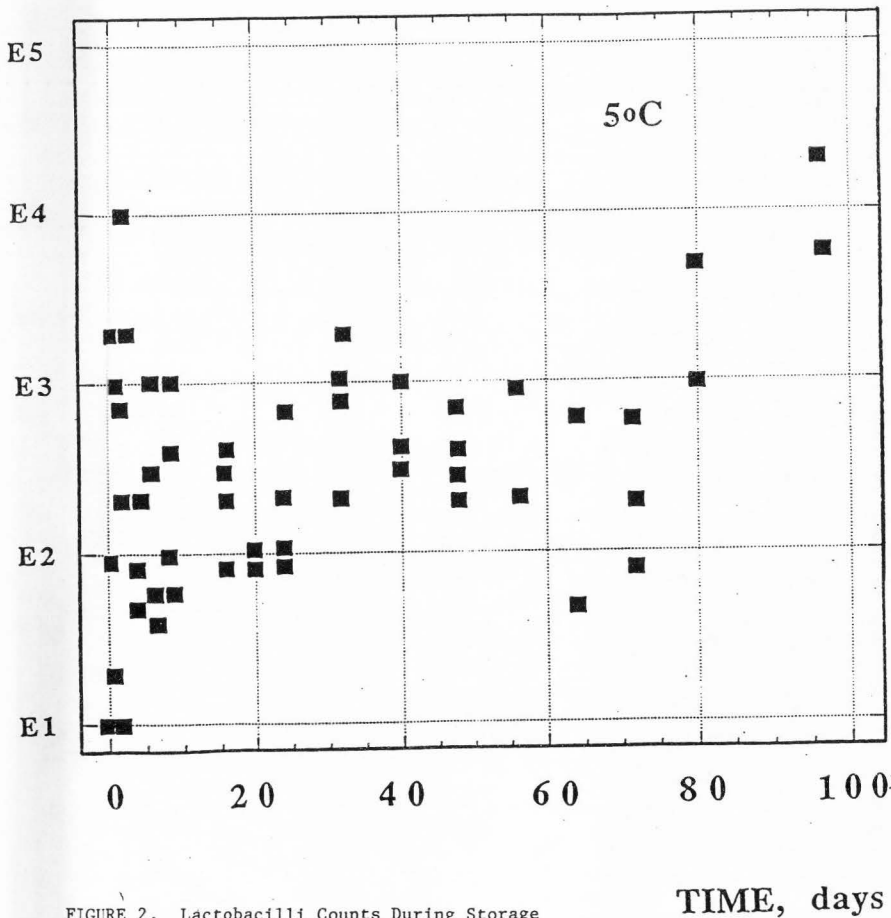


FIGURE 2. Lactobacilli Counts During Storage at Constant Temperature

TIME, days

CFU

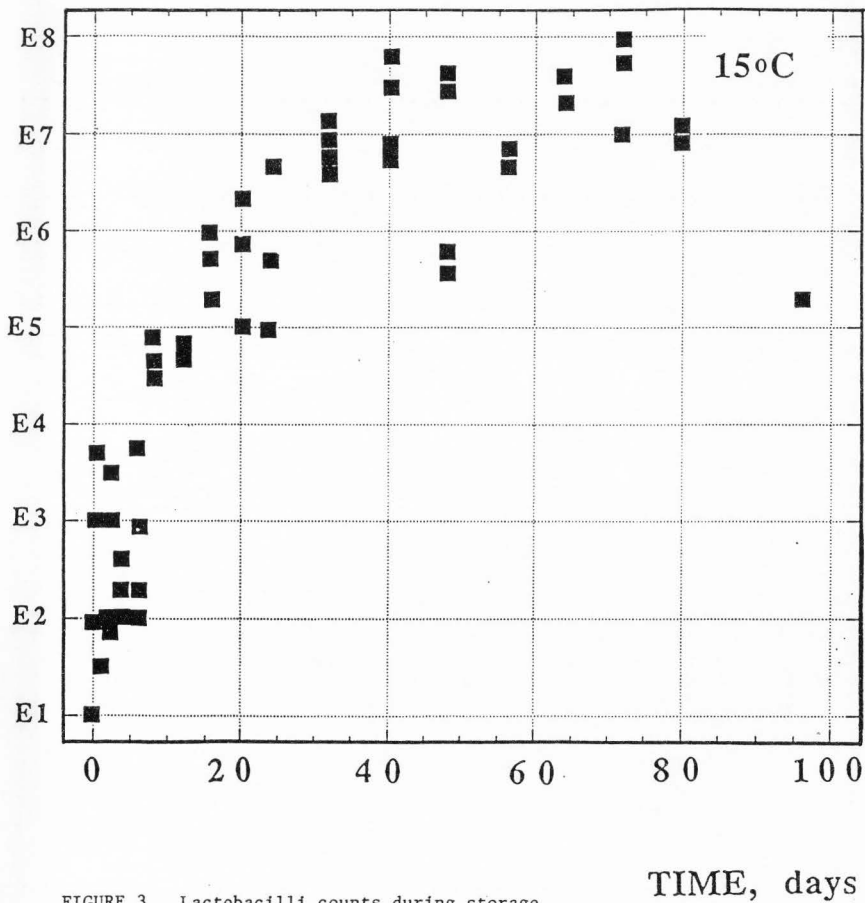


FIGURE 3. Lactobacilli counts during storage at constant temperature

CFU

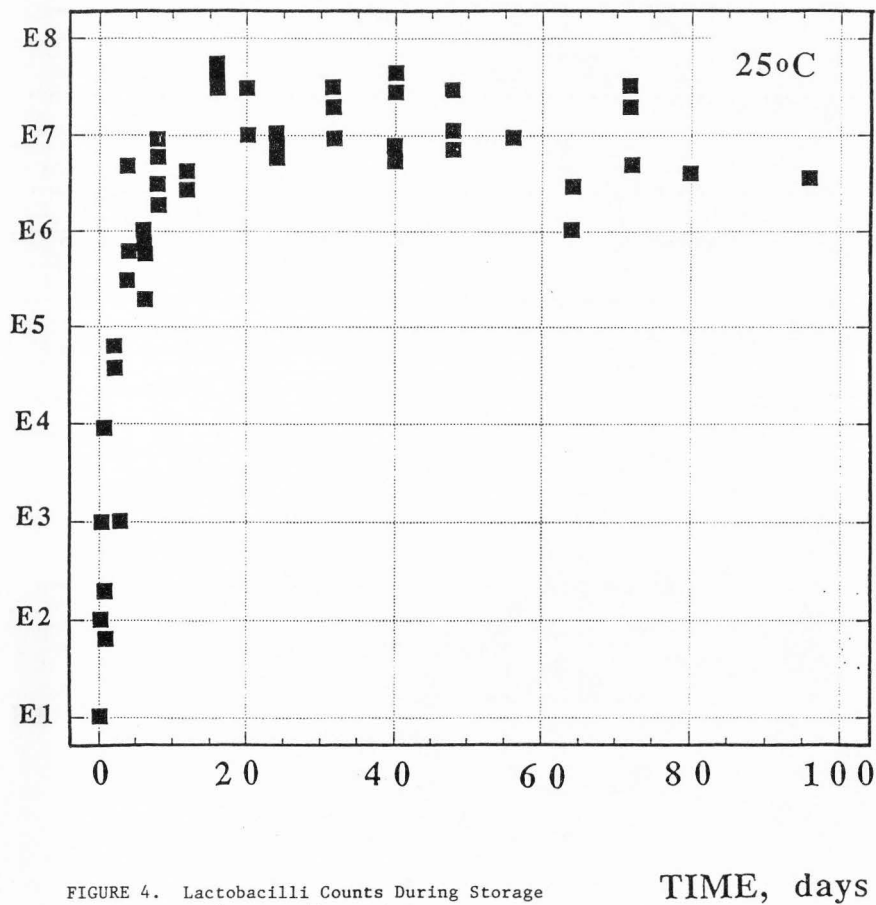


FIGURE 4. Lactobacilli Counts During Storage at Constant Temperature

TIME, days

CFU

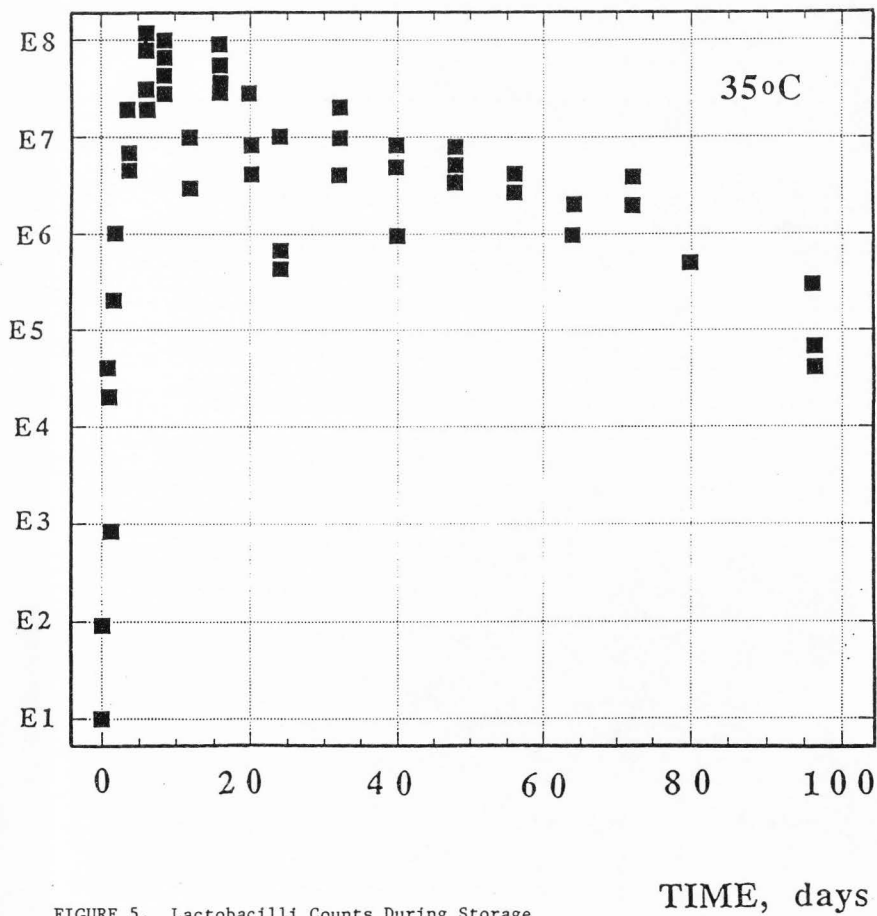


FIGURE 5. Lactobacilli Counts During Storage at Constant Temperature

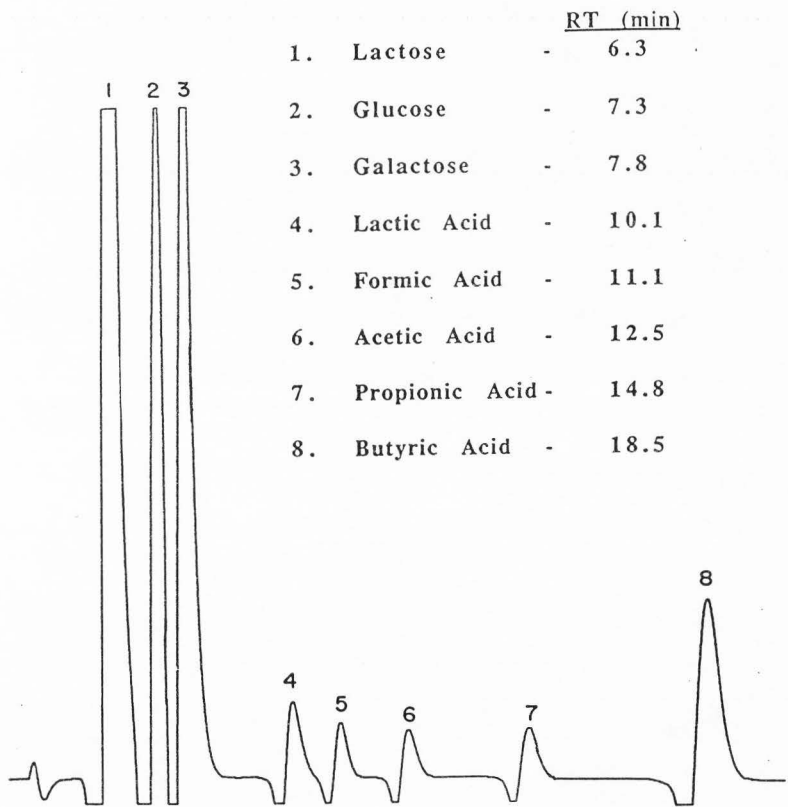


FIGURE 6. HPLC run of an aqueous solution of organic acids and carbohydrates

Assay conditions: Bio-Rad Aminex HPX-87H column w/ Cation-H⁺ Microguard.
 Mobile phase, 0.0090N H₂SO₄ (0.8 ml/min). Refractive Index Detector.
 Butyric acid (50 mM), other organic acids (15 mM), carbohydrates (25 mM).

	<u>Sample #</u>	<u>Sample#</u>
AROMA		
Overall Intensity	_____	_____
Nutty	_____	_____
Fruity	_____	_____
Pungent (acidic-formic)	_____	_____
Pungent (sulfur)	_____	_____
Goaty (butyric)	_____	_____
Dirty (isovaleric)	_____	_____
Yeasty	_____	_____
Other (please list)	_____	_____
FLAVOR		
Overall Intensity	_____	_____
Sour	_____	_____
Salty	_____	_____
Bitter	_____	_____
Sweet	_____	_____
Buttery	_____	_____
Nutty	_____	_____
Fruity	_____	_____
Goaty (butyric)	_____	_____
Dirty (isovaleric)	_____	_____
Sulfur	_____	_____
Other (please list)	_____	_____

FIGURE 7. Aroma and Flavor-by-mouth Descriptors used by the Trained Panel

DESCRIPTIVE CHEDDAR CHEESE PANEL

Scoring Procedure

0 = None	8
1 = Just detectable	9 = Moderate to large
2	10
3 = Slight (oil)	11 = Large ("grape" drink)
4	12
5 = Slight to moderate	13 = Large to extreme
6 = Sour, bitter, salt solutions	14
7 = Moderate ("orange" drink)	15 = Extreme ("Cinnamon" Gum)

FIGURE 8. Scoring Performance Used by a Trained Panel

TITLE:

A new method for measuring syneresis of renneted gels applied to development of cheese.

PERSONNEL:

Conly L. Hansen, Professor, Nutrition and Food Sciences, Utah State University.

OBJECTIVES:

The primary objective is to develop a rapid, inexpensive, accurate, real time method of measuring syneresis.

Specific Objectives

- 1) Determine the necessary optical components and the relative optimal spacing.
- 2) Design and calibrate the sampling vial for the desired optical characteristics.
- 3) Create a program that will use the measured amount of light scattered to compute the particle size.
- 4) Determine the optimal angle for collection of scattering data.
- 5) Make some measurements of syneresis with this method.

SIGNIFICANCE OF PROPOSED PROJECT:

Syneresis is important in many dairy products and especially in the manufacture of cheese. It is the underlying physical mechanism that produces cheese from milk. Over the years there have been many projects devoted to understanding syneresis. The secrets of syneresis still elude researchers (Walstra et al., 1985). Today the factors that affect syneresis are understood better than ever

before but syneresis itself is still some what of a mystery. The major reason for this is the inability to make accurate measurements (Fox , P. F., 1987). This factor permeates all research on syneresis and the factors that affect it. If the syneresis of curd particles were measured accurately we would be able to understand syneresis better and make inroads into controlling it. By controlling it we could better control the quality and optimize the quantity of cheese produced, we could improve shelf life of dairy products and insure the product was more appealing at the time of sale, we could create new products by altering the process during manufacture.

To control syneresis you must understand it first. Knowing the rate of syneresis is inherent to understanding it. Over 400 methods have been tried to accurately measure the rate of syneresis and there are inaccuracies inherent in each (Stoll, W.F., 1966). The properties of the surrounding media can affect the rate of syneresis (Walstra et al., 1985).

Still another family of methods depends upon a dilution of a substance in the whey. After the curd is formed this tracer is added to the whey. At time intervals samples are drawn and the concentration of the tracer is measured. But this too has inherent inaccuracies as invariably some of the tracer is absorbed into or bound onto the curd itself (Zviedraus, P. and Graham, E.R.B., 1981). In fact for cheese made from goats milk this is so bad as to yield almost unusable results (Nilsen, K.O. and Abrahamson, R.K., 1985).

A recent approach proposed by Pearse et al. (1985) utilizes the optical properties of the whey as a measure of syneresis. Samples of the whey drawn at intervals shows that the optical characteristics change with time. This is what would be expected as the concentration of whey solids is diluted. But their method is not free of errors (Pearse, et al., 1984).

And with all of the above methods time is a major factor. A lot of time is required to run an analysis on a batch and hence makes an exhaustive analysis of even one factor difficult (Ernstrom, C.A., 1988). But the method we propose will be much faster and more accurate.

This method will use the light scattering characteristics of the curd itself to analyze the curd size. This method will not have the drawback of being absorbed into the curd as in the tracer method, or the inconvenience of having to separate the curd from the whey for measuring, both of which are fraught with error. By making more measurements in a run the size of the curd vs. time may be plotted and a rate of shrinkage can be calculated. Since the curd is not removed from the

whey, and since the environment can remain intact the effects of the measurement on the curd will be minimal. This method will give us a much more accurate picture of the rate of syneresis.

This technology is new to the dairy industry and may prove valuable in many areas. It may eventually provide a method for measuring the size of proteins and fat structures in milk. And related technologies may help in analysis of moisture content, fat and solids not fat in a quicker fashion.

TITLE:

Evaluation of Milk Protein and Microbial Polysaccharide Interaction

PERSONNEL:

R. Olsen
C. Hansen
G. Choudhury

FUNDING SOURCES:

Western Dairy Foods Research Center

OBJECTIVES:

- 1) To determine effects of exopolysaccharide on renneted milk gel syneresis
- 2) To determine effects of exopolysaccharide on cheese quality and nutrient recovery.

RESULTS:

1. Ropy strains of Lactococcus Lactis decreased the rate of whey syneresis in renneted milk gels. Various methods were investigated including drainage of cut curd in a cheese-cloth lined funnel, UV absorption of diluted whey, centrifugation and periodic drainage of coagulated curd. Most of the results reported were obtained using the periodic drainage method. Comparisons were made to commercially available polysaccharides commonly used in dairy products (see graph 1). Effects of pH, heat, culture level, total solids and fat were examined. Ropy cultures generally reduced syneresis. The effect was more pronounced at higher pH values (see graphs 2-5).
2. Lab scale trials were performed in 4 1 stainless steel containers with temperature controlled water baths. Fresh cultures were prepared from ropy and non-ropy strains of streptococcus thermophilus.

Fourteen trials were completed and the final cheese were analyzed for total solids, protein, fat and ash. Neither the unadjusted nor the adjusted yields were significantly different. The nutrient recovery data also shows no significant difference.

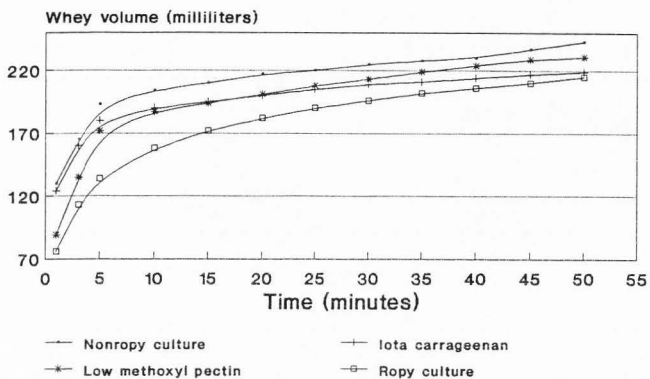
Although the adjusted yields and nutrient recoveries were not significantly different, it is possible that interactions were too subtle to be observed. One fact may have been the amount of polysaccharide present. The amount of starter culture used was based upon common usage levels. Since polysaccharide yield in the culture was approximately 5g polysaccharide/1000g culture, the amount of polysaccharide was diluted to 0.005% in the cheese-milk.

In order to increase the amount of polysaccharide added to the cheese-milk without increasing the amount of streptococcus thermophilus and to avoid the errors associated with small scale batches, a large pilot scale trial was carried out. Ropy and non-ropy Lactococcus Lactis cultures were used to increase the amount of polysaccharide. High cooking temperatures controlled subsequent acid production from the more temperature sensitive L. Lactis culture. Yields, again, were not significantly different (see charts 1 and 2). The ropy vat did show improved protein recovery, but not enough to cause a significant increase in yield.

ECONOMIC EFFECTS:

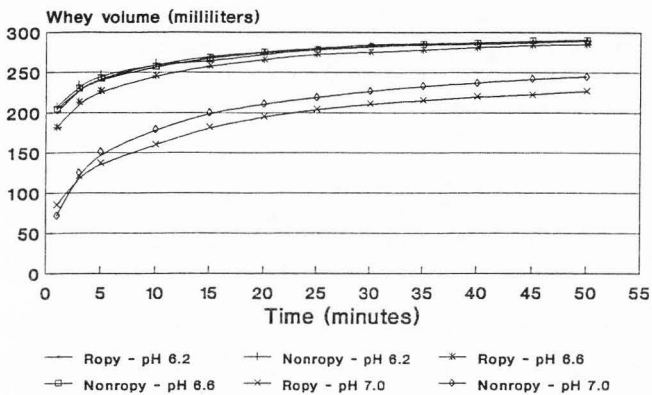
1. Potential method for moisture control in milk gels which have experienced various treatments affecting water holding capacity.
2. Incorporation of polysaccharide material into the cheese curd may improve body and texture. At this point, it is not apparent that polysaccharide incorporation has been accomplished. Body and texture effects are being evaluated.

Figure 1. Effects of polysaccharide materials on syneresis with ropy and nonropy cultures



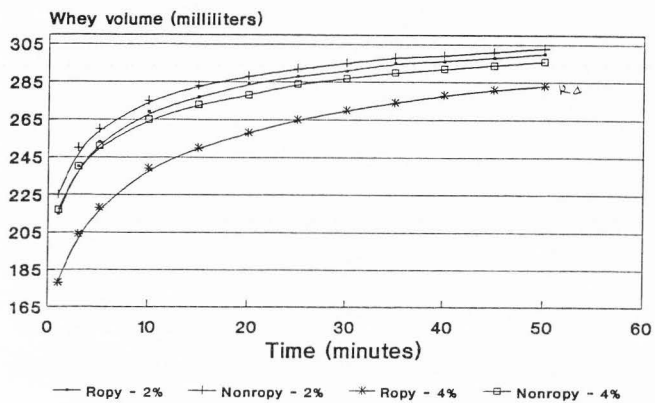
Graph 1

Effects of pH on syneresis using ropy and nonropy cultures



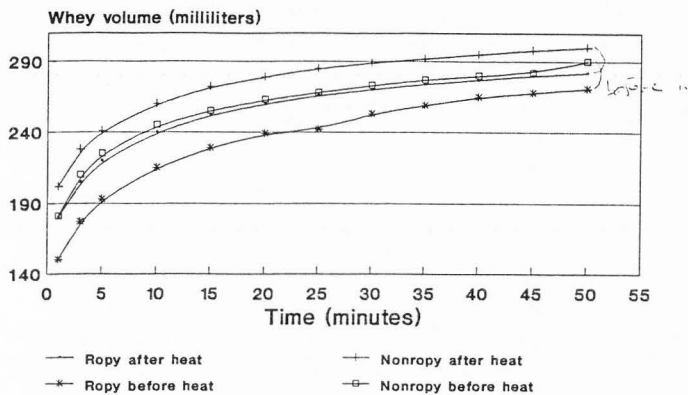
Graph 2

Effects of culture level on syneresis using ropy and nonropy cultures



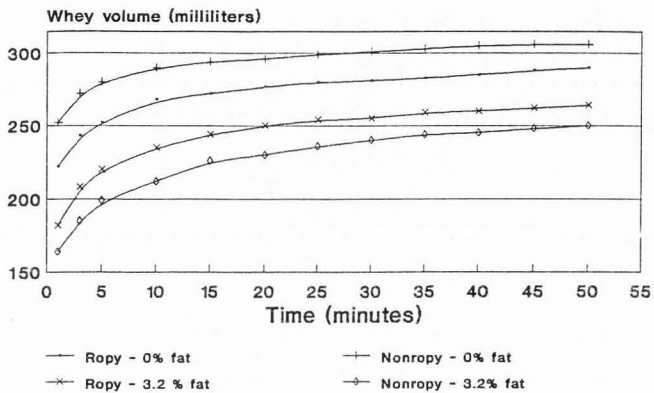
Graph 3

Effects of heat on syneresis using ropy and nonropy cultures



Graph 4

Effects of fat on syneresis using ropy and nonropy cultures



Graph 5

CHEESE YIELD DATA (LARGE TRIAL)

	<u>ROPY</u>	<u>NON-ROPY</u>
Unadjusted Yield	7.7	7.7
Adjusted Yield (45% H ₂ O)	8.3	8.3

Chart 1

NUTRIENT RECOVERY (LARGE TRIAL)

	<u>ROPY</u>	<u>NON-ROPY</u>
Protein	71.3	70.5
Fat	88.1	88.3

Chart 2

PROJECT TITLE:

Improvement of Mozzarella cheese yield and physical properties through proteinase modification of starter cultures

PERSONNEL:

Dr. G. H. Richardson, Professor, NFS Dept., USU
Dr. C. J. Oberg, Research Associate, NFS Dept., USU
Lynn V. Moyes, Research Technician, NFS Dept., USU
Amos Wang, Graduate Assistant, NFS Dept., USU

FUNDING SOURCES:

Western Dairy Foods Research Center

OBJECTIVES:

1. Find proteinase negative (Prt⁻) thermolactic cultures that can be used to manufacture Mozzarella cheese and improve the physical properties of curd without causing adverse flavor reactions.
2. Screen the available proteinase negative strains of S. cremoris for acid production and survival at the Mozzarella cooking temperatures.
3. Isolate and test proteinase negative strains of S. thermophilous and L. bulgaricus for casein proteolysis and acid production. Trial production studies will follow using direct acid Mozzarella curd as the control.
4. Obtain current physical testing procedures from Pizza Hut and other Mozzarella cheese buyers, select representative methods, and quantitate the changes caused by the proteinase systems involved. The physical properties of the cheese to be measured will include stretchability, cook color, and meltability.

RESULTS:

1. A number of Prt^- strains of Streptococcus thermophilous and Lactobacillus bulgaricus were found and evaluated in Mozzarella cheese production.
2. Activity tests were run at 37, 42, 44, and 46°C on the following proteinase negative strains of Streptococcus cremoris; UC 320 Prt^+ , UC 320 Prt^- , UC 85 Prt^- , UC 45 Prt^- , and UC 310 Prt^- . Results showed noticeable activity up to 42°C for several strains. High temperature isolation of S. cremoris UC Prt^- strains was also done. Results showed only UC 85 Prt^- grew at all the temperatures tested, and that 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) using these organisms. High temperature incubation of S. cremoris Prt^- cultures at 42°C was done to select for cultures that could adapt to elevated temperatures. Only UC 85 Prt^- exhibited growth at this temperature and no new isolates were found.
3. A large number of strains of both thermolactic organisms were screened for proteolytic ability with the OPA test. These cultures were also analyzed by amino acid analysis which was found to provide a much more detailed profile of their proteolytic characteristics. A number of Prt^- strains of both S. thermophilous and L. bulgaricus were characterized for

both proteolysis and acid production. Mozzarella cheese was manufactured using these cultures, their Prt⁺ parents, and the direct acid method. The physical properties of these cheeses were then compared over a 28 d period.

4. The curd was evaluated using methods to measure color changes and meltability during heat treatment. A Minolta reflectance color meter was used and the b* values were used to measure the darkening that occurred with heat treatment. A tube method was used to evaluate the meltability of the curd upon heating. Differences were found that proved to be strain dependent. (Figure 1 and Figure 2) A low viscosity Brookfield viscometer was combined with a Helipath stand to allow objective measurements of the rheology, specifically stretchability, of the curd at 60°.

(Figure 1 and Figure 2)

PUBLICATIONS:

1. Oberg, C.J., A. Wang, L.V. Moyes, and G.H. Richardson. 1989. Effect of proteolytic activity of thermolactic cultures on physical properties of Mozzarella cheese. J. Dairy Sci. (Abstract accepted for Annual meeting) (Manuscript in preparation)
2. Weimer, B.C., C.J. Oberg, L.V. Moyes, R.J. Brown, and G.H. Richardson. 1989. Comparison amino acid analysis and o-phthalaldehyde methods to characterize proteolysis by Lactobacillus bulgaricus. J. Dairy Sci. (submitted).
3. Oberg, C.J., B.C. Weimer, L.V. Moyes, R.J. Brown, and G.H. Richardson. 1989. Proteolytic characterization of Lactobacillus bulgaricus strains by amino acid analysis. J. Dairy Sci. (In preparation).

4. Oberg, C.J., L.V. Moyes, and G.H. Richardson. 1989. Acid production of Streptococcus cremoris in curd made at elevated temperatures. J. Dairy Sci. (In preparation)

IMPACT OF RESEARCH:

The second leading cheese produced in the U. S. is Mozzarella cheese. Proteinase negative starter cultures used in the production of Cheddar cheese, cottage cheese, and acid casein have been shown to increase yield, along with providing a number of other advantages. If proteinase negative cultures could be used in the production of Mozzarella and other high temperature Italian cheeses, an increase in yield may be possible. Additionally, if the effects of proteinase activity can be measured, there may be a possibility of improvement in the stretching, along with other physical properties. We need to learn more about the effect proteinase activity of the thermolactic cultures have on the physical qualities of these cheese types.

The major purchasers of pizza cheese are concerned that the physical qualities of Mozzarella curd begin to deteriorate at about ten days of age. The proteolytic activity of the starter culture is thought to be the cause of reduction of stretch. By incorporation of less proteolytic thermolactic starter cultures, we desire to extend these superior qualities. The better and more consistent the physical properties, the more sales for the product.

FIGURE 1

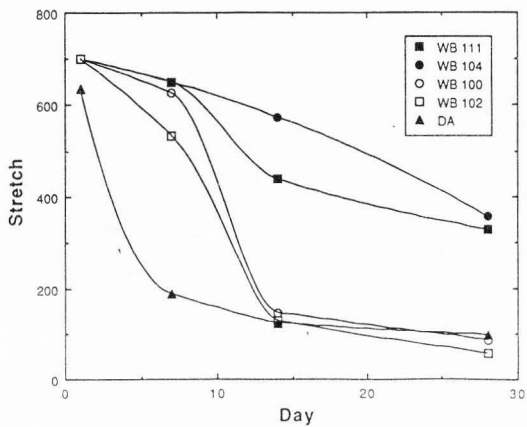
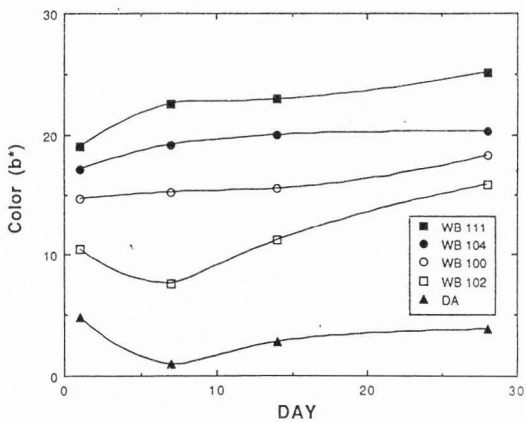
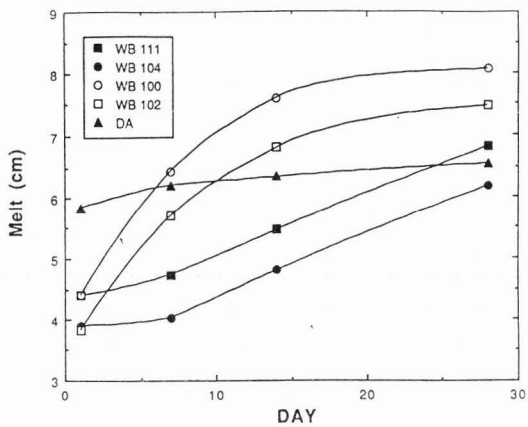
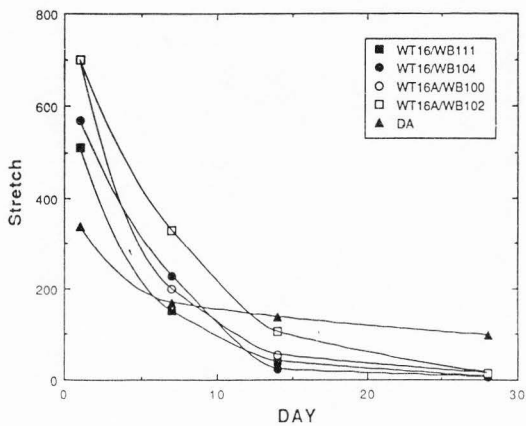
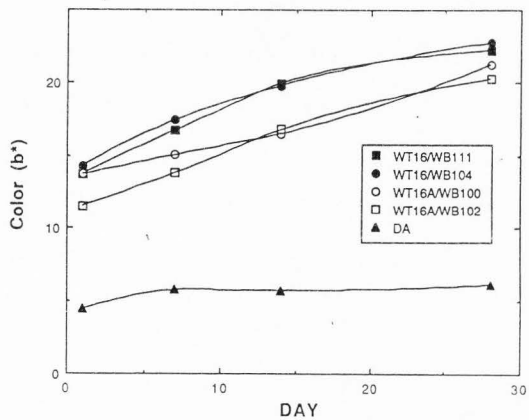
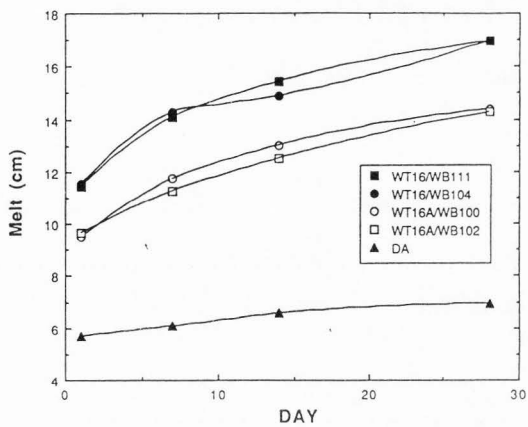


FIGURE 2



Project Title:

Improved Control of Cheese Manufacture Through Vat Monitoring.

Personnel:

G. H. Richardson, Professor, Nutrition and Food Sciences, Utah State University; Don McMahan, Professor, Nutrition and Food Sciences, Utah State University; Snow Brand Milk Products Co. Ltd., Japan; Stoelting, Inc., Kiel, Wisconsin; Remy Grappin, INRA, France; Stoelting Co. Inc., WI; Golden Cheese Co., Corona, CA; Western General Dairies, Twin Falls, ID; GHR Corp., Logan; Michael J. LeFevre, Research Assistant, Nutrition and Food Sciences, Utah State University.

Funding Sources:

Western Dairy Foods Research Center and Utah Agricultural Experiment Station.

Objectives:

1. Evaluate the control of curd strength during cottage cheese manufacture. Establish software that would be most helpful for the cheese industry.
2. Use the same system to monitor the coagulation of milk for Cheddar cheese manufacture. Determine the limits of curd strength that would cause significant product loss or prevent sufficient

moisture removal. These data would then be applied to expert systems.

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

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.

Results: A hot wire coagulation probe was obtained from Snow Brand Milk Products Co., Ltd.. Auxiliary equipment including data acquisition material, power supplies, and pH and temperature sensors were also acquired. Control and measurement of the hot wire was adapted for use with IBM PC/XT/AT computer systems. Software was written to collect data during Cheddar cheese and cottage cheese manufacture. We have shown that the hot wire system will sense agitation and curd healing as well as coagulation of milk in the vat. Tests comparing the coagulation points of milk using a broad range of instrument types are in progress and will help to prepare correlation values. Information obtained from these other instruments may also be incorporated into the software parameters.

Impact of Research:

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

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III. Media Appeal (check if you consider paper to be of interest to news media):

- Yes

Abstract and Abstract Heading (see instruction sheet for example)

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Gel formation in acid coagulated milk. D.J. McMahon, Utah State University, Logan.

Skim milk was slowly acidified to pH 4.6 by addition of glucono- δ -lactone. Coagulation was monitored using a Formagraph to measure gel modulus and a spectrophotometer to record changes in turbidity. Gel formation occurred when the pH reached 5.0 and gel modulus increased as pH decreased. The resultant gel was weaker than that formed after hydrolysis of κ -casein by chymosin. When milk is both acidified and renneted (as for manufacture of cottage cheese) a cumulative effect was observed. A gel typical of enzymic hydrolysis is formed first, then when the pH reaches 5.0 a further increase in gel modulus occurs. This cumulative effect is not observed if the enzyme concentration is low enough that pH 5.0 is reached before enzymic coagulation occurs.

key words: acid, coagulation, milk

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Effect of concentration on coagulation of ultrafiltered milk. B.J. Orme* and D.J. McMahon, Utah State University, Logan.

Pasteurized whole milk was ultrafiltered to 15.5% protein and diluted, with permeate from the same milk, to protein levels of 2-15.5%. The diluted retentates were treated with antibiotics and held at 22°C throughout the experiment. Calf rennet was added to the samples and coagulation time measured using the Formagraph method with modification to prevent surface drying of concentrates. Enzymic coagulation time of milk decreased with increased protein concentration and leveled to a plateau at 8-11% protein. Increasing protein concentration above 11% further decreased coagulation time. Coagulation time was directly related to the inverse of enzyme activity at all protein levels studied ($R^2 = .99$), but the proportionality constant decreased with increasing protein concentration.

key words:: ultrafiltration, coagulation, concentration

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Abstract and Abstract Heading (see instruction sheet for example)

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Ultra-high temperature processing of milk ultrafiltered to 3× concentration and effects of phosphate addition on gelation. P.S. Savello*, D.J. McMahon and R.J. Brown, Utah State University, Logan.

Whole milk and skim milk were ultrafiltered to 3× concentration (volume reduction). Calcium sequestering agents (0.1% of disodium phosphate, sodium tripolyphosphate or sodium hexametaphosphate) were added. The milk was homogenized then ultra-high temperature (UHT) treated. An indirect UHT processor was designed and assembled for laboratory-scale treatment of milk at a flow rate of 200 ml/min. Milk was preheated to 70°C then heated to 135°C with an 8 s holding time. Samples of the UHT milk were stored at room temperature. All samples containing added phosphate salts sedimented extensively after 60 d. Control samples containing no added salts were stable after 120 d. Effects of UHT treatment and phosphate salts on rennet coagulation of milk were measured using a Formagraph. Before UHT, rennet coagulation time was shortened upon addition of phosphate salts but after UHT treatment the milk containing the phosphate salts did not coagulate.

key words: UHT, ultrafiltration, age gelation

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Purification of microbial milk-clotting enzymes by immunoaffinity chromatography. C.I. Osuala* and R.J. Brown, Utah State University, Logan.

Milk-clotting enzymes were purified on a column of porous glass beads derivatized with antibodies against commercially-available microbial milk-clotting preparations. Antibodies diluted in 1 mM pH 7.0 sodium acetate buffer were coupled to the matrix by mixing for 16 h at 5°C. Unreacted sites on the column were blocked by recycling 0.2 M pH 7.0 buffered glycine through the column. Enzyme preparations were diluted in 1 mM pH 5.0 sodium acetate, then adsorbed by recycling through the column for 12 h at 5°C. The column was washed with coupling buffer until absorbance at 280 nm returned to base line. Adsorbed enzyme was eluted from the column with 0.2 M pH 4.0 sodium acetate. Purification by immunoaffinity chromatography yielded enzymes with high clotting activity.

key words: microbial, coagulation, purification

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Relationship between κ -casein hydrolysis and milk coagulation.

F. He* and R.J. Brown, Utah State University, Logan.

Extent of κ -casein hydrolysis was determined throughout enzymic coagulation of milk. (Coagulation of undiluted milk was followed by measuring turbidity at 600 nm with a temperature controlled spectrophotometer. Trichloroacetic acid (TCA) was added to stop κ -casein hydrolysis at different stages of coagulation. Amino acid analysis of TCA soluble peptides was used to measure extent of κ -casein hydrolysis. Casein micelles started aggregating immediately after enzyme addition. Coagulation did not require complete conversion of κ -casein to para- κ -casein. The ratio between κ -casein hydrolysis and extent of coagulation was affected by calcium concentration. Calcium also affected rate of enzymic hydrolysis of κ -casein.

Key words: coagulation, κ -casein, hydrolysis

Shammet
Last name of first author

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Proteolytic activity of proteinases on macropeptide purified from κ -casein. K.M. Shammet* and R.J. Brown, Utah State University, Logan.

Proteolytic activity of some proteinases (chymosin, pepsin, *Mucor meihie* rennet, *Endothia parasitica* rennet, trypsin and chymotrypsin) on pure macropeptide isolated from κ -casein was measured. Macropeptide solutions (10 mg macropeptide in 1 ml .05 M pH 6.6 phosphate buffer) were incubated with the enzymes at 37 °C for 1, 5, 15, 30 and 60 min. Reactions were stopped by addition of 8 M urea containing .025 ml pepstatin solution (11 mg pepstatin in 1 ml methanol). Reaction mixtures were separated into fraction on an R1P-HPLC C3 column. Macropeptide was resistant to the milk-clotting enzymes, but was degraded by trypsin and chymotrypsin.

key words: κ -casein, macropeptide, proteolysis

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Corresponding author C. A. ErnstromAddress Department of Nutrition & Food SciencesUtah State UniversityLogan, UT 84322-8700Phone (801) 750-2102**II. Selection Preference (check 1st choice and indicate**

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Abstract and Abstract Heading (see instruction sheet for example)

Enzymic milk coagulation: Jersey-Holstein comparison. E.D. Bastian*, R.J. Brown, and C.A. Ernstrom, Utah State University, Logan.

Individual milk samples from 21 Jersey and 21 Holstein cows were collected monthly during one lactation period. Coagulation time and curd firmness were estimated using Formagraph tracings. Chymosin activity was held constant through the experiment. Monthly average clotting times ranged from 5.5-7.7 min for Jerseys and 6.9-11.6 min for Holsteins. Curd firmness was 35.5-43.2 mm for Holsteins and 48.8-58.7 mm for Jerseys. Holstein coagulation time increased toward the end of lactation. Jersey curd firmness increased as lactation progressed while Holstein curd firmness decreased.

key words: coagulation, Jersey, Holstein

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Abstract and Abstract Heading (see instruction sheet for example)

Effect of proteolytic activity of thermolactic cultures on physical properties of Mozzarella cheese. C.J. Oberg*, A. Wang, L.V. Moyes, and G.H. Richardson, Utah State University, Logan and Weber State College, Ogden, Utah.

A collection of commercial strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were analyzed for proteolytic activity by the O-phthaldialdehyde test. Some proteinase deficient strains were generated using standard mutation techniques for both genera. Slight differences were found for the proteinase deficient strains of *S. thermophilus*, but large differences were noted among the *L. bulgaricus* variants. Cheese was manufactured using single rod or cocci strains and rod/cocci pairs either proteolytic or deficient in proteolysis. These were compared to cheese manufactured by the direct acid method. Resulting cheese was analyzed for cook color with reflectance colorimetry, for melting with the tube test, and for stretch with a helical viscometer. Cheese was analyzed at 1, 7, 14, and 28 d of age. No differences were observed in cheese made with proteinase deficient strains of *S. thermophilus*. Cheese made with proteinase deficient strains of *L. bulgaricus* browned less, melted better, and stretched less. Cheese made with proteinase deficient paired cultures had less browning. All cultured cheeses had better stretch, better melt and more browning than direct acid cheese.

Key words: Mozzarella, proteolysis, culture

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Abstract and Abstract Heading (see instruction sheet for example)

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Effect of exopolysaccharides from *Streptococcus thermophilus* on Mozzarella cheese yield. R.L. Olsen, Utah State University, Logan.

Strains of exopolysaccharide-producing lactic acid bacteria were isolated from commercial sources. Production of exopolysaccharide was monitored by appearance of ropiness in grown cultures. High incubation temperatures were used to induce non-ropy control cultures. Cheesemaking trials were performed in 4 l stainless steel lab-scale vats. Milk was standardized to 2.0% fat. Control and experimental cheeses were made simultaneously and replicated eight times. Fresh cultures were used with each replication. Levels of 1.0% *Streptococcus thermophilus* and 0.5% non-ropy *Lactobacillus bulgaricus* were used. Average yield, moisture, fat and protein were not significantly different in cheese made with ropy cultures.

Key words: cheese, yield, polysaccharide

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Abstract and Abstract Heading (see instruction sheet for example)

Influence of Processing Parameters on Nutrient Recovery During Ultrafiltration of Milk. S.K. Collinge* and C.A. Ernstrom, Utah State University, Logan.

Nutrient recovery, water usage, and time expenditure were compared while varying the beginning point of diafiltration. Milk was pasteurized at 72°C for 16 s and acidified to pH 5.8. Three batches of milk were ultrafiltered to 60, 65, or 70% volume reduction before diafiltration, and concentrated to 5× (80% volume reduction). Amount of diafiltration water was adjusted to control the buffer capacity/lactose ratio so the final pH of the fermented retentate was 5.1 ± .1. Starting diafiltration at 70% volume reduction took less time and water without affecting nutrient recovery. The effect of preheating and acidifying milk on nutrient recovery was studied. Whole milk was heated to 60, 72, and 82°C for 16 s. Milk representing each heat treatment was divided into three batches, one unacidified (pH 6.6), the others acidified to pH 6.2 and 5.8. The milk was ultrafiltered, diafiltered, and concentrated to 5×. Recovery of calcium, phosphorus, and riboflavin were significantly reduced following acidification of milk. Riboflavin recovery was higher when milk was preheated to 60°C as opposed to 72 or 82°C.

key words: ultrafiltration, nutrient, retention

1989 JOINT ANNUAL MEETING

AMERICAN DAIRY SCIENCE ASSOCIATION AND AMERICAN SOCIETY OF ANIMAL SCIENCE

Triple Crown Convention Connection, Lexington, Kentucky

July 31 - August 4, 1989

J.M. Elliot, Chairman, Program Committee

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Abstract and Abstract Heading (see instruction sheet for example)

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Attenuation of proteinase activity results from cointegrate formation in *Lactococcus lactis* ssp. *lactis* C₂O. J.K. Kondo* and K. Gillies, Utah State University, Logan.

Loss of proteinase activity (Prt) is observed in lactose positive (Lac⁺) transconjugants obtained from conjugal matings between *L. lactis* ssp. *lactis* C₂O and LM2301 and LM2306. Both C₂O x LM2301 and C₂O x LM2306 matings yielded Lac⁺Prt⁺ and Lac⁺Prt^d (proteinase deficient) transconjugants. Plasmid profiles of Lac⁺Prt^d transconjugants from C₂O x LM2306 matings were identical to C₂O indicating that drug resistance had transferred from LM2306 to C₂O. Similarly, plasmid profiles of Lac⁺Prt⁺ transconjugants from C₂O x LM2301 matings were identical to C₂O. The majority of other transconjugants contained 100 kb cointegrate plasmids resulting from the fusion of the 55 kb LacPrt plasmid, pJK550, and the 45 kb transfer-factor plasmid, pJK448. Restriction endonuclease mapping indicated that pJK550 and pJK448 are identical to pSK08 and pRS01 in *L. lactis* ssp. *lactis* ML3. Unlike cointegrates from a ML3 x LM2301 mating, transconjugants from C₂O x LM2301 and C₂O x LM2306 matings contained cointegrates which employ only insertion sequence ISS1T on pJK550 for insertion into pJK448. Similar to the ML3 system, a number of target sequences can be used as insertion sites on pJK448. However, fusion events in a 6.6 kb *Bam*HI-*Kpn*I fragment of pJK448 resulted in a substantial reduction in proteinase activity in C₂O x LM2301 Lac⁺Prt^d transconjugants. These results suggest that cointegrate formation may be a possible mechanism for the instability of proteinase activity of lactococci.

Key words: cointegrate, proteinase, plasmid

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Abstract and Abstract Heading (see instruction sheet for example):

Electro-transformation of *Lactococcus lactis* ssp. *lactis* with 100 kb conjugative, cointegrate plasmid, pJK206 and 55 kb non-conjugative plasmid, pJK225. K. Gillies* and J.K. Kondo, Utah State University, Logan

Plasmid-cured *Lactococcus lactis* ssp. *lactis* LM0230 and LM2306 (both Lac⁻Prt⁻) were transformed via electroporation with pJK206 (Lac⁺Prt⁺, 100 kb cointegrate plasmid isolated from a *L. lactis* ssp. *lactis* C₂O x LM2306 mating) and pJK225 (Lac⁺Prt⁺, 55 kb plasmid isolated from a C₂O x LM2306 mating). Under optimal conditions, recipients were transformed at a frequency of 20 per µg of supercoiled pJK225 and 1 per µg of relaxed, open circular pJK225, while no transformants were obtained using linear pJK225. Electro-transformation with pJK206 occurred at a frequency of 1 per µg of DNA. Transformants contained plasmid DNA that had not undergone rearrangements or deletions during the transfer process and each exhibited the Lac⁺Prt⁺ phenotype. Host strain background may have an influence on Prt expression since one transfer of a LM0230//pJK206 transformant resulted in the loss of Prt in 20% of the cells. We demonstrate with these experiments, that large, native plasmid DNA can be transferred via electroporation. In addition, the 55 kb plasmid in *L. lactis* ssp. *lactis* C₂O encodes both lactose utilization and proteinase functions.

key words: electro-transformation, plasmid, *Lactococcus lactis*