CDR

Center for Dairy Research

Annual Report 1987-1988

College of Agricultural and Life Sciences
University of Wisconsin - Madison

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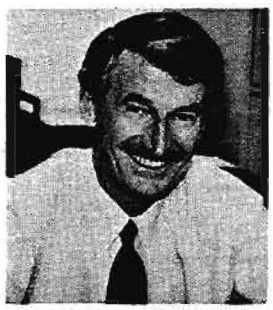
From the Director



The Center for Dairy Research has grown substantially since its formation in 1986. It has administrative oversight of 34 research projects or programs funded by the Wisconsin Milk Marketing Board, National Dairy Promotion and Research Board, State of Wisconsin, University of Wisconsin, and private industry. Research areas encompass (1) Safety and quality of milk products (2) Cheese research, (3) Milk components and their uses. In the latter, milkfat modification and utilization receives greatest emphasis. The original concept of fostering multi-disciplinary research is being realized with projects underway in six academic departments including production, processing, and economics of milk and milk products.

As we strive, step by step, to be a high caliber research center, we are frequently reminded of our dependence on feedback from the dairy producers and dairy foods processors which help us design effective applied and basic research projects. As we see it, to succeed in our goal of increasing the utilization of milk and milk products, the food industry and researchers must team up to produce products of appeal to the evermore demanding consumer. A properous dairy economy will depend upon the dialogue between researchers, marketers, and business people involved in this industry.

A unique and rapidly evolving project within the Center is the World-wide Information and Technology Exchange Program. Designed to bring together dairy research efforts and information, WITEP coordinates an annual conference, a seminar series, special conferences and workshops, a scientist exchange program, and the preparation of various published materials.

We hope you find this report informative and a source of enthusiasm. We invite all segments of the dairy research effort -- marketing and promotion boards, universities, individual researchers, and private industry -- to help us move forward and join us in building the resources of scientific skills, communication networks, and innovative ideas in dairy research. This unified effort, I believe, is critical for the solid and dynamic growth of dairy research.

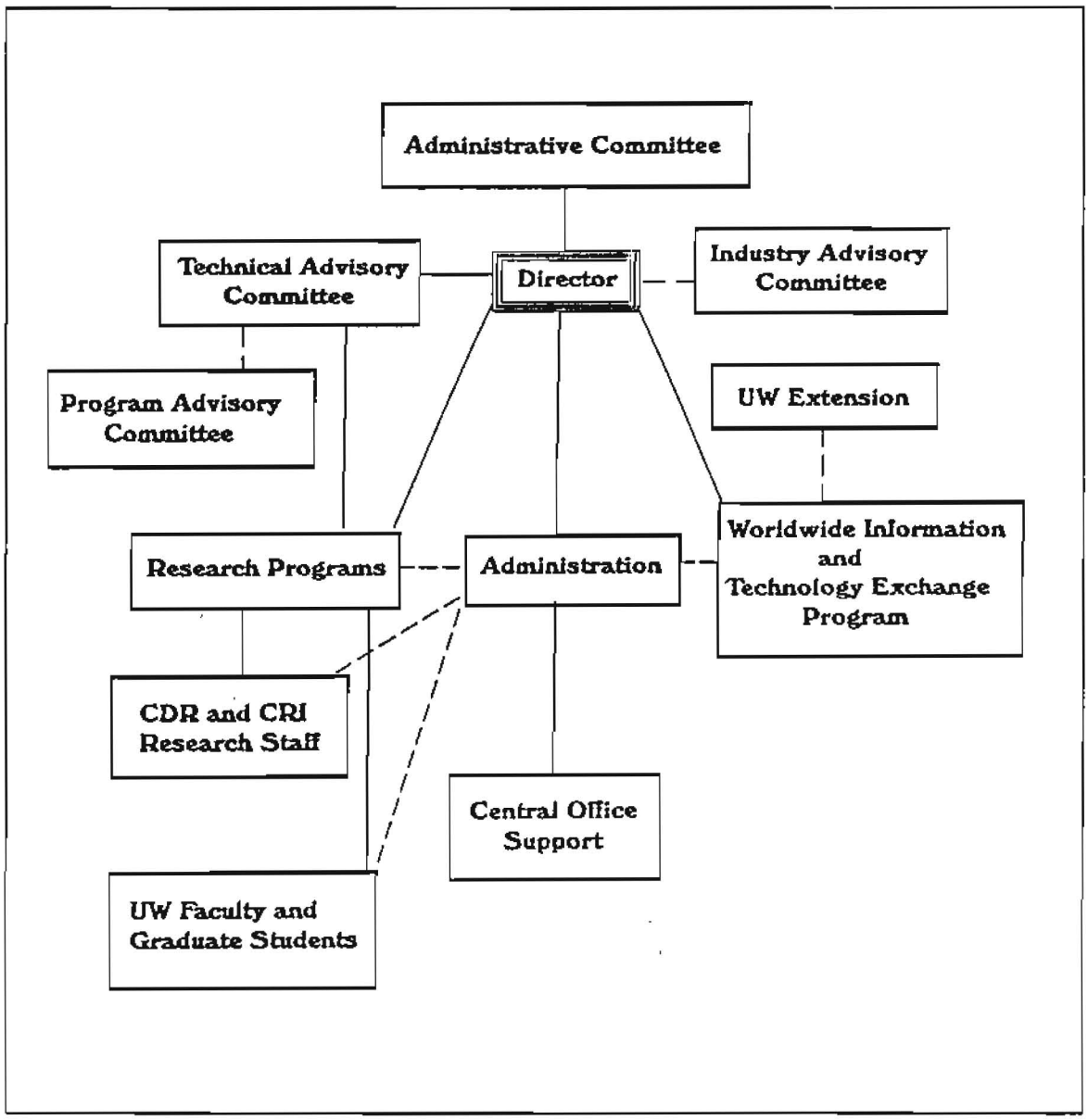
Sincerely,

Norman F. Olson

Norman F. Olson

Chapter 1 The Center for Dairy Research

Organizational Structure of CDR



CDR Staff and Associates

CDR Staff

Director Norman F. Olson

Administration

Melinda Adams, administrative program coordinator

Margaret Jackson, administrative office assistant

Shawna McGee, administrative office support

Mary Ann Murray, fiscal clerk

Jeanne Wagner, administrative office support

Research

Carol Chen, dairy chemist

J.P. Chen, PhD, food engineer

Brian Gould, PhD, dairy economist

Mark Johnson, PhD, cheese microbiologist

Brian Riesterer, cheese technologist

Bill Tricomi, MS, laboratory manager

Worldwide Information and Technology Exchange Program (WITEP)

Sarah Quinones, program coordinator

Wayne Wenzel, assisant to the WITEP coordinator

University of Wisconsin graduate students affiliated with CDR

The following are MS and PhD graduate students pursuing research projects sponsored by CDR.

James Beyer
Tarun Bhowmik
Kay Craig
Misun Kim
Janice Johnson
Lisa Pannell
Teresa White
Sirirat Rengpipat
Peggy Spangler

Visiting Scientists funded by CDR

The following scientists were sponsored to do short-term research projects by CDR's World-wide Information and Technology Exchange Program. These individuals worked in collaboration with CDR-affiliated researchers.

Betty de Haan, The Netherlands
Boamin Liang, China
Morsi El Soda, Egypt
Moustaffa El-Shenawy, Egypt
Demetrios Papageorgiou, Greece

Administrative Advisory Committee

This committee establishes the overall policies and program goals of the Center.

Robert Bock, Dean, Graduate School
Harvey Grosskopf, Wisconsin Milk Marketing Board
Charles Hunt, Vice President, Dairy Foods Research Centers, National Dairy Promotion and Research Board
Neal Jorgenson (ex officio), Associate Dean, College of Agricultural and Life Sciences
Leslie Lamb (ex officio), Wisconsin Milk Marketing Board
Norman Olson, Director, Center for Dairy Research
Leo Walsh, Dean, College of Agricultural and Life Sciences, UW-Madison

Technical Advisory Committee

This committee assists in planning the research program of the Center.

Robert Bremel, Dept. of Dairy Science
Michael Doyle, Dept. of Food Microbiology and Toxicology (Food Research Institute)
Janet Greger, Dept. of Nutritional Sciences
Charles Hill, Dept. of Chemical Engineering
Charles Hunt (ex officio), National Dairy Promotion and Research Board
Edward Jesse, Dept. of Agricultural Economics
Eric Johnson, Dept. of Food Microbiology and Toxicology (Food Research Institute)
Mark Johnson (ex officio), Center for Dairy Research
Neal Jorgenson (ex officio), Associate Dean, College of Agricultural and Life Sciences
Kenneth Lee, committee chair, Dept. of Food Science
Robert Lindsay, Dept. of Food Science
Joe O'Donnell (ex officio), Wisconsin Milk Marketing Board
Norman Olson, Director, Center for Dairy Research, Dept. of Food Science

Historical developments leading to the formation of the Center for Dairy Research

1976 Walter V. Price Cheese Research Institute (WVPCRI) formed.

1979 State of Wisconsin allocated funds for WVPCRI adding two full-time researchers and funding for five research assistantships. Staff gradually increased to 3.5 FTE's.

1979-80 New facility for the U.S. Dairy Forage Research Center built on Madison campus. Current research emphasizes forage utilization, production efficiency, control of milk composition, and animal health.

1983 Wisconsin Milk Marketing Board (WMMB) is formed by Wisconsin dairy farmers through mandatory milk marketing order.

1984 WVPCRI sponsored First Annual Cheese Research & Technology Conference.

1985 Five graduate students and six visiting scientists supported by WVPCRI program.

WMMB staff and UW faculty discussed creation of a dairy research center.

1986 Center for Dairy Research (CDR) formed with the operating budget provided by a 10-year irrevocable trust fund set up by WMMB.

CDR submitted proposals to WMMB, National Dairy Promotion and Research Board, and the State of Wisconsin for additional funding.

1987 CDR holds its First Annual Meeting on January 21.

CDR co-sponsors Fourth Annual Cheese Research and Technology Conference on March 25 - 26.

1988 First research conference sponsored by CDR titled Milkfat: trends and utilization, April 20 - 21.

National Dairy Promotion and Research Board recognizes CDR as one of six United States dairy foods research centers and provides funding commit ment, May 16.

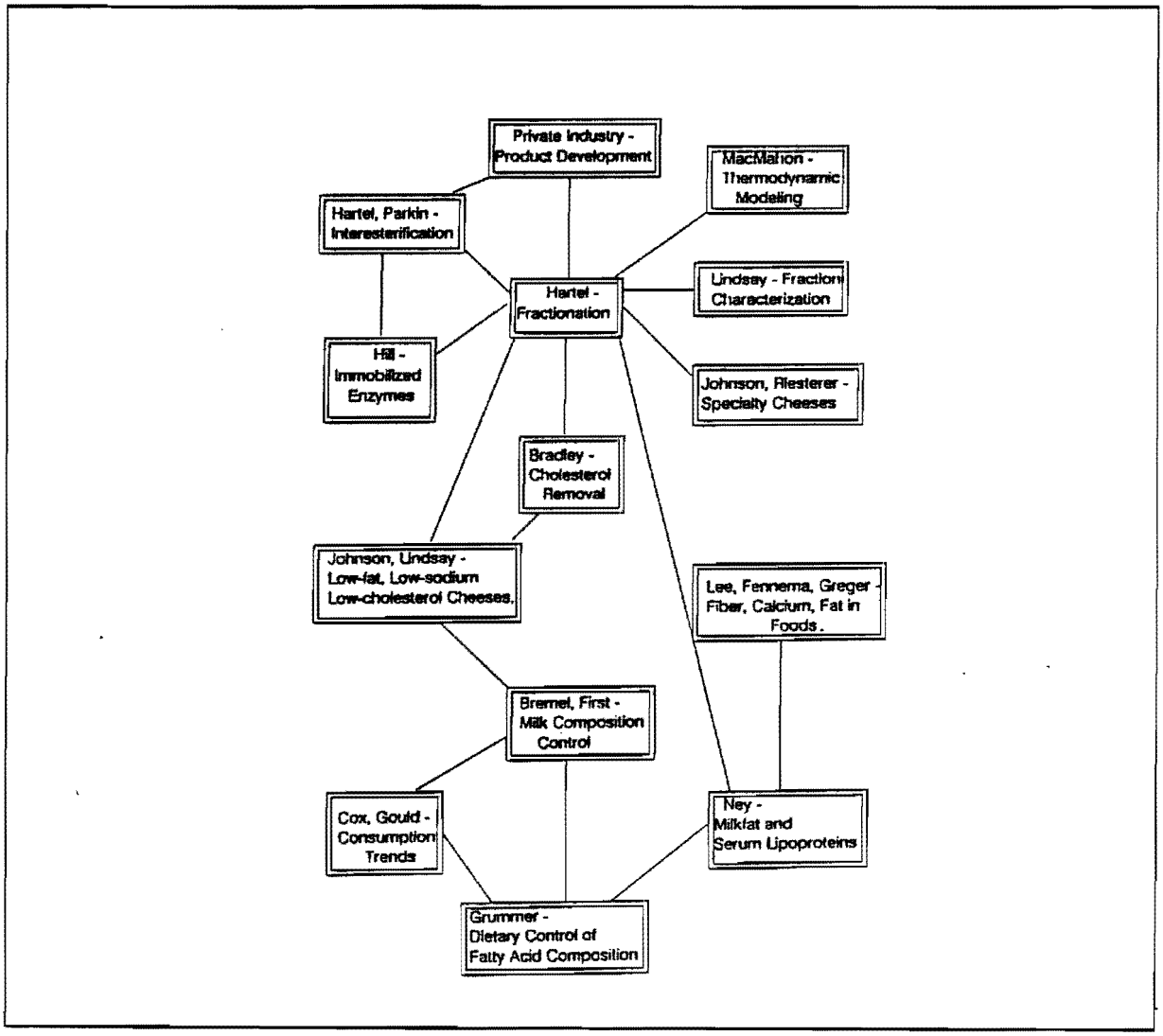
Dedication of new office complex over the Deck, May 16.

1989 -91 Babcock Hall additions and remodeling will increase staff offices and laboratory space for CDR.

Chapter 2 CDR Research Program

Introduction

Based upon the technological needs of the dairy industry and the continued concern of finding means to maintain a vital dairy economy, CDR has identified the following five areas as the primary areas of research focus: cheese technology, dairy foods quality and safety, whey utilization, product and process development, and new uses for milkfat. CDR promotes research projects that take an interdisciplinary approach which utilize the tremendous resources of the University of Wisconsin. The diagram below illustrates various faculty from food science, chemical engineering, nutritional sciences, dairy science, and CDR involved in our milkfat research program. This chapter lists the projects underway in 1987-1988 and lists research reports on some of those projects.



CDR Research Projects Listing

These projects receive financial support from one or several of the following organizations: Wisconsin Milk Marketing Board, National Dairy Promotion and Research Board, private industry, and the University of Wisconsin.

Safety of Milk and Milk Products

SAFETY

Differentiating virulent from avirulent *Listeria monocytogenes*; Dr. Michael Doyle, Food Research Institute.

Procedures for detection of *Escherichia coli* 0157:H7 in dairy products; Dr. Michael Doyle, Food Research Institute.

Prevention of survival and growth of pathogens in milk and cheese by enhancement of the activity of lactoferrin/lysozyme; Dr. Eric Johnson, Food Research Institute.

Fate of Listeria during pasteurization and cheesemaking; Dr. Elmer Marth, Food Science Department, and Dr. Michael Doyle, Food Research Institute.

Safety of milk and milk products; Dr. Elmer Marth, Food Science Department.

Behavior of *Listeria monocytogenes* during the preparation of lactic starter cultures and in raw milk inoculated with lactic acid bacteria; Dr. Elmer Marth, Food Science Department.

Effects of heat treatments and cheesemaking variables on pathogen survival and growth; Dr. Eric Johnson and Dr. John Nelson, Food Research Institute.

Fate of *Listeria monocytogenes* in whey and skim milk with 6% or 12% salt and held at 4° and 22°C; Dr. Elmer Marth, Food Science Department, and Dr. Demetrios Papageorgiou, CDR visiting scientist.

Fate of *Listeria monocytogenes* during the manufacture and ripening of Blue cheese and Feta cheese; Dr. Elmer Marth, Food Science Department, and Dr. Demetrios Papageorgiou, CDR visiting scientist.

Effect of common preservatives on *Listeria monocytogenes*; Dr. Elmer Marth, Food Science Department, and Dr. Moustafa El-Shenawy, CDR visiting scientist.

NUTRITION

Anticarcinogenic effects of milk components; Dr. Michael Pariza, Food Research Institute.

Sodium, Potassium, Calcium, Magnesium, and Chloride metabolism of human subjects fed milk and calcium supplement; Dr. Janet Greger, Nutritional Sciences Department.

Milk: A point of entry into the human diet for mevalonate-suppressive plant secondary metabolites; Dr. Charles Elson, Nutritional Sciences Department.

MILKFAT

Effect of butterfat versus long-chain saturated fats on cholesterol absorption and plasma lipoprotein composition in rats; Dr. Denise Ney, Nutritional Sciences Department.

Cheese Research

CHEESE RIPENING

Generation of and roles of proline in providing flavor and pathogen protection in cheese; Dr. Eric Johnson, Food Research Institute.

Texture development of cheese made from ultrafiltered milk; Dr. Damo Srinivasan, Food Science Department.

Enhancing flavor characteristics of Cheddar and selected semi-hard and semi-soft cheeses made from ultrafiltered milk; Dr. Norman Olson, Food Science Department.

Microencapsulation of cheese ripening systems; Dr. Norman Olson, Food Science Department, and Dr. Morsi El-Soda, CDR visiting scientist.

Acceleration of cheese ripening through the use of selected protease-peptidase preparations, freeze-shocked lactobacilli and microencapsulated enzymes and microorganisms; Dr. Norman Olson, Food Science Department.

Characterization of the protease, peptidase, and lipase of selected bacteria that may be used to enchance cheese flavor; Dr. Elmer Marth, Food Science Department.

SPECIALTY CHEESES

Development of basic technology for improving the flavor and consumer acceptability of reduced-sodium Cheddar cheese; Dr. Robert Lindsay, Food Science Department.

Development of the process technology and flavor enhancement of reduced-fat cheese; Dr. Mark Johnson, CDR.

Development of a technology base for specialty cheeses; Dr. Mark Johnson, CDR.

CHEESE TECHNOLOGY

Manufacture of cheese from milk pasteurized at high temperatures; Dr. Mark Johnson, CDR.
Use of acidulants in manufacturing Mozzarella cheese from ultrafiltered milk; Dr. Norman Olson, Food Science Department.

Effects of concentrations of cation, especially calcium, on the primary and secondary phases of enzymatic milk clotting; Dr. Richard Hartel, Food Science Department.

Application of linear programming for the development and formulation of processed cheese products; Dr. John Norback, Food Science Department.

Characterization of lactobacilli that are involved in white mineral crystal formation on cheese surfaces; Dr. Eric Johnson, Food Research Institute.

Development of cheese manufacturing and packaging processes to minimize milk salt crystallization on cheese during storage and distribution; Dr. Mark Johnson, CDR.

Milk Components and Their Uses

MILKFAT

Enzymic modification of butterfat in supercritical $C0_2$; Dr. Richard Hartel and Dr. Kirk Parkin, Food Science Department.

Use of immobilized enzymes in the treatment of milkfat; Dr. Charles Hill, Chemical Engineering Department.

Extraction of cholesterol from milkfat; Dr. Robert Bradley, Food Science Department.

Thermodynamic modeling of lipids in supercritical CO_2; Dr. Patrick McMahon, Chemical Engineering Department.

Milkfat fractionation/investigation of the properties of cold spreadable butter; Dr. Robert Lindsay, Food Science Department.

PROTEIN

Incorporation and expression of bovine casein in transgenic animals; Dr. Robert Bremel, Dairy Science Department.

LACTOSE AND WHEY

Exo-Polysaccharide production from whey and whey permeate; Dr. Douglas Cameron, Chemical Engineering Department, and Dr. Eric Johnson, Food Research Institute.

Food Processing and New Product Development

Freeze concentration of fluid dairy products; Dr. Richard Hartel, Food Science Department.

New dairy foods from added fiber and added calcium; Dr. Ken Lee, Food Science Department, Dr. Janet Greger, Nutritional Sciences Department, and Dr. Owen Fennema, Food Science Department.

Research Reports

Project Title:

Fate of *Listeria monocytogenes* in whey and skim milk with 6% or 12% salt and held at 4 and 22°C.

Personnel:

E.H. Marth, Professor, Food Science Department, and D. Papageorgiou, CDR visiting scientist.

Funding:

Center for Dairy Research (WITEP), the Aristotelian University of Thessaloniki, Greece, and Kraft, Inc., Glenview, IL.

Objectives:

To determine the behavior of *Listeria monocytogenes* in salted whey and skim milk during storage at 4 and 22°C.

Results and Procedures:

Autoclaved samples of skim milk and deproteinated whey were fortified with 6 or 12% salt, inoculated to contain ca, 1.0×10^3 c.f.u./ml (in the products with 6% salt) or ca, 5.0×10^3 c.f.u./ml (in the products with 12% salt) *L. monocytogenes*, strain Scott A or California, and incubated at 4 and 22°C. The pH of the 6% salted whey was 5.65, the 6% salted skim milk 6.20, the 12% salted whey 5.50, and the 12% salted skim milk 6.00. These pH values remained relatively constant during the entire time of the experiment.

Growth curves were derived, and generation times and maximum populations calculated for the combination of the two strains, 6% salt concentration, and the two incubation temperatures. The loss of viability by the two strains during incubation of the 12% salted whey and skim milk at both temperatures also was studied. *Listeria* counts were obtained by surface-plating samples on Tryptose Agar and, when necessary, cold-enrichment in Tryptose broth was done. All results are averages of duplicate trials.

The growth rate of the two strains of *L. monocytogenes* was about the same in the two 6% salted products at a given incubation temperature, and generation times were at least 10 times shorter at 22°C than at 4°C. A lag phase of 6 to 12 hours was noted at 22°C, and generation times were 3.73 hours and 3.56 hours in 6% salted whey for strain Scott A and California, respectively, and 4.24 hours and 4.12 hours in 6% salted skim milk, for the two strains, respectively. A lag phase of 5 to 10 days was noted at 4°C, and generation times were 45.29 hours and 37.33 hours in 6% salted whey for strains Scott A and California, respectively, and 44.05 hours and 47.06 hours in 6% salted skim milk for the two strains, respectively. Maximum populations reached after 108 to 120 hours of incubation at 22°C in the 6% salted products for the two strains were in the range of 7.70 to 8.10 Log_{10} c.f.u./ml (Fig. 1). At 4°C, maximum populations reached in the 6% salted products for the two strains, were in the range of 7.58 to 8.04 Log_{10} c.f.u./ml after 50 to 55 days of incubation (Fig. 2).

In 12% salted whey and skim milk incubated at 22°C, *Listeria* decreased in numbers (Fig. 3). Strain California in 12% salted whey, decreased in numbers from an initial level of 5.4×10^3 c.f.u./ml to less than 1 c.f.u./2 ml after 105 days of incubation (using direct plating on Tryptose Agar (TA), cold-enrichment continues). The same strain (California) in 12% salted skim milk decreased in numbers from an initial inoculum 5.5×10^3 c.f.u./ml to less than 1 c.f.u./2ml after 75 days at 22°C, using direct plating on TA, but cold enrichment gave positive results after 75 and 80 days. In contrast to strain California, strain Scott A proved to be more salt tolerant. Populations of strain Scott A in 12% salted whey decreased from an initial 3.91 Log_{10} c.f.u./ml to 2.79 Log_{10} c.f.u./ml after 130 days at 22°C. In 12% salted skim milk, numbers of strain Scott A decreased from an initial inoculum 3.88 Log_{10} c.f.u./ml to 2.58 Log_{10} c.f.u./ml after 130 days of incubation at 22°C (Fig. 3). The loss of viability of both strains in 12% salted whey and skim milk after 130 days of storage at 4°C was very low and in the range of less than 0.7 order of magnitude (Fig. 4).

Impact of Research:

These results indicate both the ability of *L. monocytogenes* to grow and survive in the presence of relatively large amounts of salt. This information in important when brines of certain salt concentrations are used with cheese.

Figure 1. Growth of *L. monocytogenes* in 6% salted whey and skim milk at 22°C.

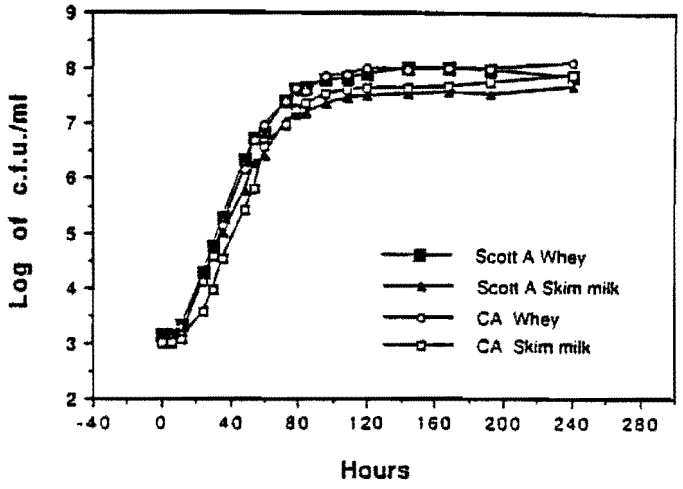


Figure 2. Growth of *L. monocytogenes* in 6% salted whey and skim milk at 4°C.

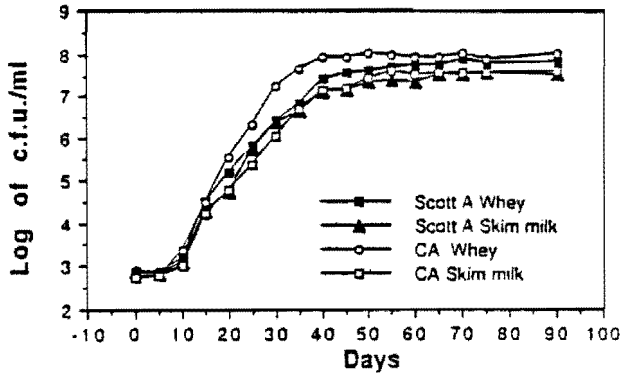


Figure 3. Survival of *L. monocytogenes* in 12% salted whey and skim milk at 22°C.

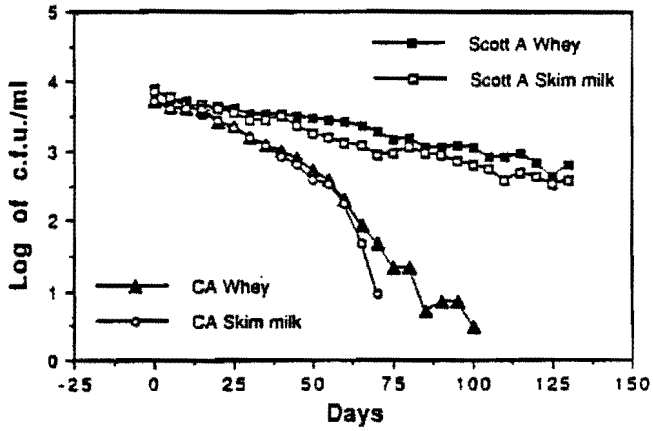
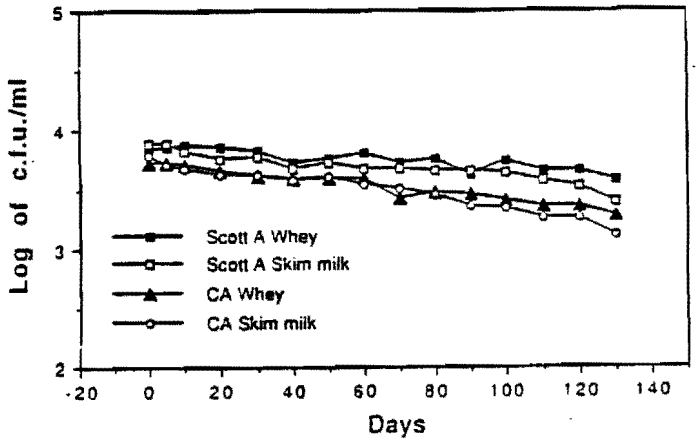


Figure 4. Survival of *L. monocytogenes* in 12% salted whey and skim milk at 4°C.



Project Title:

Fate of *Listeria monocytogenes* during the manufacture and ripening of Blue cheese.

Personnel:

E.H. Marth, Professor, Food Science Department, and D. Papageorgiou, CDR visiting scientist.

Funding:

Center for Dairy Research (WITEP), the Aristotelian University of Thessaloniki, Greece, and Kraft, Inc., Glenview, IL.

Objectives:

To determine the behavior of *Listeria monocytogenes* during the manufacture and ripening of Blue cheese.

Results and Procedures:

Pasteurized skim milk was standardized to a milkfat content of 3.7% by the addition of pasteurized homogenized cream (35% milkfat); was inoculated to contain ca. 1.0-2.0 x 10^3 *L. monocytogenes* (strain Scott A or California) c.f.u./ml; and was made into Blue cheese. A combination of *Streptococcus lactis* and *Streptococcus cremoris* was used as the starter culture (2% v/v). *Streptococcus lactis*, subsp. *diacetilactis*, also was used (0.15% of the starter) to help create open-structured cheese. *Penicillium roqueforti* spore powder (2.5 g) was added (dusted using salt shaker) during hooping of the curd from 67 pounds of milk.

Blue cheese was ripened at the relative humidity 90-98%, at 9-12°C, for 84 days, and then the cheese was stored at 4°C. Milk, curd, whey, and cheese were tested for pH and for numbers of *Listeria*. Duplicate samples were used to enumerate *L. monocytogenes* by surface plating of appropriate dilutions (made in Tryptose broth with 2% sodium citrate) on McBride Listeria Agar. When necessary, cold-enrichment of the first dilutions was done.

The pH of the 1-day-old cheese, before salting, was in the range of 4.65 to 4.95 (six at pH 5.3 because of unsatisfactory mold development). Pieces of cheese were wrapped after 65 days of ripening.

Results of this work indicate that:

1. *L. monocytogenes* was entrapped in curd during cheesemaking, with the population in curd being ca. 0.62 Log_{10} c.f.u./g greater than in the inoculated milk. The whey contained less than 85 c.f.u. of the pathogen/ml.

2. *L. monocytogenes* strain Scott A in cheese increased in numbers by ca. 1.2 Log_{10} c.f.u./g during the first 24 hours until the pH values of cheese dropped to 4.7-4.9. The same strain increased in numbers by only 0.3 Log_{10} c.f.u./g and growth of the pathogen ceased as the pH of the curd dropped to 4.85, 4 hours after hooping in one lot with rapid acid production (Lot 1).

3. *L. monocytogenes* strain California in cheese increased in numbers by ca. 0.72 Log_{10} c.f.u./g during the first 24 hours until the pH values dropped to 4.95-4.90. The same strain, in a lot with rapid acid production (lot 4), increased in number only by 0.12 $Log_{10/g}$, and growth of the pathogen ceased as the pH of the curd dropped to 5.0, 4 hours after hooping.

4. The population of the pathogen decreased continuously during the first 40 to 50 days of ripening of the cheese. The population of strain Scott A dropped by an average of 2.65 Log_{10}c.f.u./g compared to the population of the 1-day-old cheese before salting. The population of strain California dropped by an average of 2.73 Log_{10} c.f.u./g compared to the population of the 1-day-old cheese before salting.

5. Satisfactory mold growth occurred in 30 to 80 days and the pH of the 80-day-old cheese was in the range of 5.70 to 6.20 (except for one lot which was at pH 5.3 because of unsatisfactory mold development). No growth of *L. monocytogenes* was observed during this period even with the favorable pH in cheese. This probably was the result of the high salt content in the range of 4.26 to 4.85%. This salt content,

and a moisture content in the cheese of 37.68 to 40.02%, with 11% as "bound water" giving about 33.54 to 35.62% free moisture results in an effective brine concentration of 10.68 to 12.32% (Table 1). This amount of salt in solution is too high to permit growth of *L. monocytogenes*.

6. After 12 weeks of ripening, Blue cheese was stored at 4°C. The 90-day-old cheese had a very low population of the pathogen, less than 2.4 Log_{10} c.f.u./g, but the pathogen was still detectable by direct plating in McBride Listeria Agar. This work has not yet been completed.

Impact of the Research:

These results emphasize the tenacity of *L. monocytogenes* in the presence of salt. Consequently, one cannot depend on the environment of Blue cheese to promptly inactivate the pathogen during the cheese ripening process. Thus, to insure *Listeria*-free Blue cheese, the pathogen must be kept out of cheese during cheesemaking and subsequent handling of the product. Use pasteurized milk and adequate hygienic practices to accomplish this goal.

Table 1.

Lot	Moisture(%)	FDM(%)	NaCl(%)	Salt in waterphase
1	39.31	55.20	4.56	11.52
2	39.54	52.10	4.56	11.47
3	40.02	54.18	4.26	10.68
4	38.80	50.65	4.85	12.32
5	38.04	50.83	4.56	11.87
6	37.68	52.15	4.26	11.27

Project Title:

Fate of *Listeria monocytogenes* during the manufacture, ripening, and storage of Feta cheese.

Personnel:

E.H. Marth, Food Science Department, and D. Papageorgiou, CDR visiting scientist.

Funding:

Center for Dairy Research (WITEP), the Aristotelian University of Thessaloniki, Greece, and Kraft, Inc., Glenview, IL.

Objectives:

To determine the behavior of *Listeria monocytogenes* during the manufacture, ripening, and storage of Feta cheese.

Procedures:

Pasteurized whole cow's milk was inoculated to contain ca. 5.0×10^3 *L. monocytogenes* (strain Scott A or California) c.f.u./ml and made into Feta cheese according to standard procedures. *Lactobacillus bulgaricus* and *Streptococcus* thermophillus (1:1, v/v) were used as starter culture (1%, v/v).

Cheese in brine was held at 22°C for 5 days (24 hours in 12% salt brine followed by 4 days in 6% salt brine) followed by storage at 4°C. Duplicate samples were used to enumerate *L. monocytogenes* by surface plating of appropriate dilutions (made in Tryptose Broth with 2% sodium citrate) on McBride Listeria Agar. Selected *Listeria* colonies from many samples were isolated and were kept on slants of Tryptose agar until they were confirmed biochemically.

Results indicate:

1. *L. monocytogenes* was entrapped in curd during cheese making, with the population in curd being 0.92 Log_{10} c.f.u./g greater than in the inoculated milk. The whey contained less than 3.2×10^2 c.f.u. of the pathogen/ml, which is further evidence that *Listeria* was entrapped in curd.

2. *L. monocytogenes* in cheese increased in numbers by ca. 1.5 Log_{10} c.f.u./g during the first 2 days of ripening at 22°C. Because of the concentration of the pathogen in the curd and growth of *L. monocytogenes* during the first 2 days of ripening, the population was 2.4 Log_{10} c.f.u./g greater in cheese than in the inoculated milk, with a maximum number of 1.5×10^6 c.f.u./g (Fig. 1 and Fig. 2).

3. No difference was observed between the two strains, Scott A and California, in their behavior in cheese during the first 5 days. Growth of *Listeria* during the first 2 days, mainly the first 24 hours, resulted from a combination of favorable conditions such as: a high temperature (up to 22°C), a high moisture content (up to 55%) unsalted curd at first or a salt content of less than 4.87% in the water phase, and the favorable pH. Populations of *L. monocytogenes* increased during the time that the pH dropped from an initial value of 6.6 to 5.0, and growth of the pathogen ceased as the pH value of 2-day-old cheese dropped to 4.6. (Fig. 1 and Fig. 2).

4. *L. monocytogenes* survived during storage of Feta cheese at 4°C for more than 90 days (Fig. 3 and Fig. 4). The pH of the cheese during the entire storage time at 4°C was approximately 4.3. Strain Scott A survived in greater numbers than did strain California. After 90 days of storage at 4°C, the population of strain Scott A dropped by an average of 1.28 Log_{10} c.f.u./g compared to the population of the 2-day-old cheese (Fig. 3). In contrast with strain Scott A, strain California proved to be less tolerant of conditions in the cheese. After 90 days of storage at 4°C, the average decrease in population was 3.07 Log_{10} c.f.u./g compared to the population of the 2-day-old cheese (Fig. 4).

5. After 24 hours, the 12% salt brine had a *L. monocytogenes* population approximately 2.68 Log_{10} c.f.u./ml. This resulted from the migration of *Listeria* from the cheese into the brine. Although nutrients and some small pieces of cheese scraped off during handling were present in the brine, other experiments have shown that *Listeria* doesn't grow in 12% brine. During the ripening of cheese in 6% salt brine

the same phenomenon of leaching of nutrients and small pieces of cheese into the brine, also occurred. However 6% salt brine allowed growth of *L. monocytogenes*, and after 5 days of cheese ripening at 22°C, the population of *L. monocytogenes* was slightly higher in the brine than in the cheese. After this initial growth in 6% brine, *L. monocytogenes* survived for more than 90 days during storage at 4°C. (Fig. 5 and Fig. 6). In most samples (64/84), the brine had a slightly higher population than in the cheese.

Impact of Research:

Results of this work indicate that if present in milk and thus in Feta cheese, *L. monocytogenes* can grow in the cheese during the initial stage of cheese ripening. Populations of *L. monocytogenes* reached clearly would be hazardous to the health of susceptible consumers. This again emphasizes the importance of using properly pasteurized milk and adequate hygienic practices in cheesemaking. Furthermore, *L. monocytogenes* survived more than 90 days in Feta cheese undergoing ripening at 4°C. This again demonstrates that ripening cheese made from raw or heat-treated milk at or above 1.7°C for 60 days does not insure that cheese will be free of pathogens.

Figure 1. Fate of *L. monocytogenes* strain Scott A in Feta cheese during days 0-5 at 22°C.

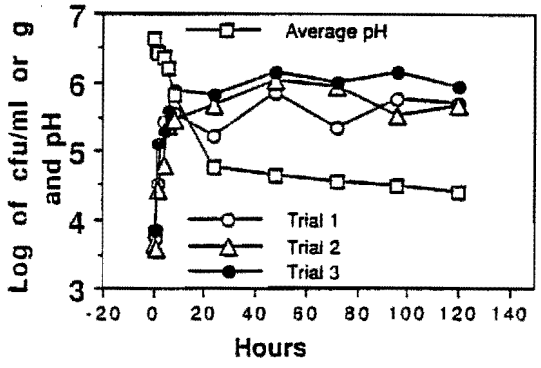


Figure 2. Fate of *L. monocytogenes* strain California in Feta cheese during days 0-5 at 22°C.

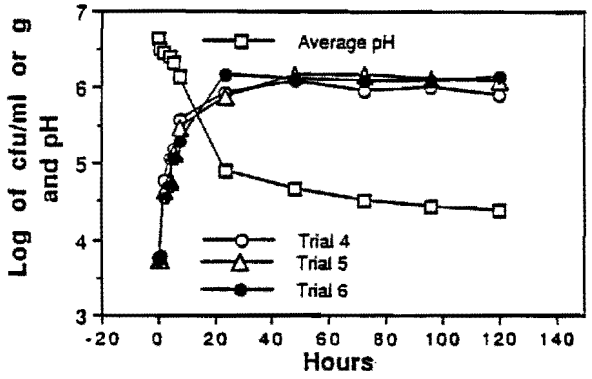


Figure 3. Survival of L. *moncytogenes* strain Scott A during storage of Feta cheese at 4°C.

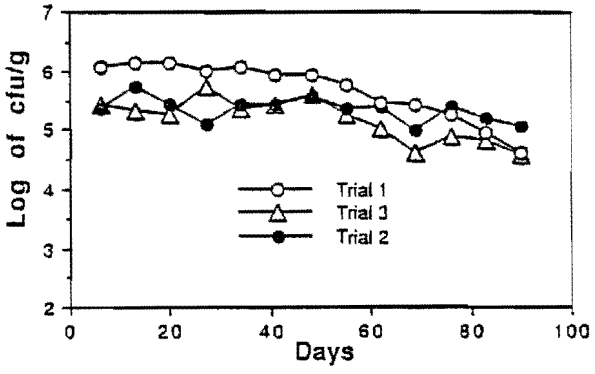


Figure 4. Survival of *L. monocytogenes* strain California during storage of Feta cheese at 4°C.

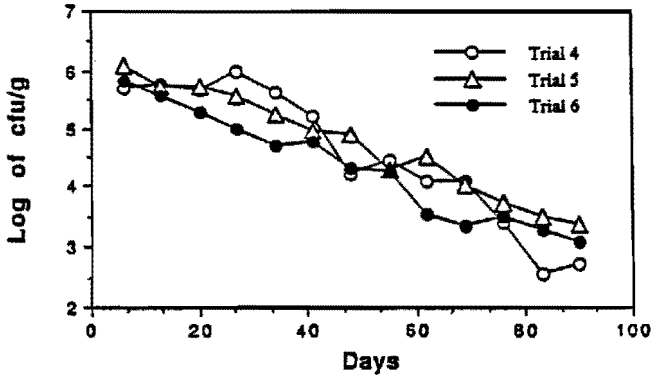


Figure 5. Survival of *L. monocytogenes* strain Scott A in brine during storage of Feta cheese at 4°C.

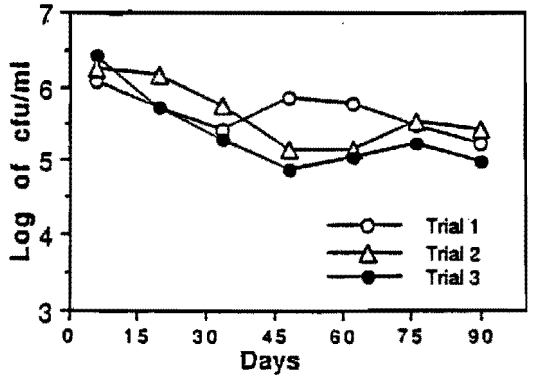
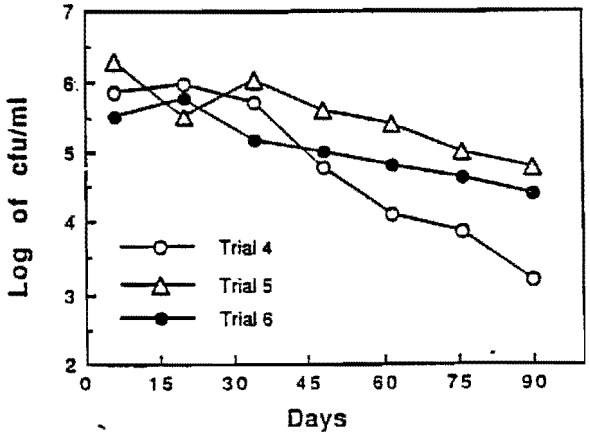


Figure 6. Survival of *L. monocytogenes* strain California in brine during storage of Feta cheese at 4°C.



Project Title:

Esterolytic activity of *Streptococcus lactis*, *Streptococcus cremoris*, and their mutants.

Personnel:

E.H. Marth, Professor, Food Science Department; K. Kamaly, graduate student, Food Science Department; and M. El Soda, CDR visiting scientist.

Funding:

Center for Dairy Research (WITEP), the Cultural and Educational Bureau of the Arab Republic of Egypt.

Objectives:

Determine esterolytic activity of some lactic acid bacteria.

A comparative study on the esterase system of Lac$^+$ and Lac$^-$ strains of *Streptococcus lactis* and *S. cremoris* was carried out. The obtained results indicate that the specific activities associated with hydrolysis of a series of para-or ortho-nitrophenyl derivatives of fatty acids by cell-free extracts of *S. lactis* and *S. cremoris* and their mutants are as listed in Table 1. The lactic streptococci had an active esterase system. In general, the rate of hydrolysis of p-nitrophenyl esters of fatty acids by the cell-free extracts was higher than that of the o-nitrophenyls. With cell-free extracts, hydrolysis of o- and p-nitrophenyl esters of capric (C-10) was slow, suggesting slow esterase activity. The specific activity for hydrolysis of p-nitrophenyl caprate (C-10) by *S. lactis* (C2) was 61, whereas a specific activity of only 1 could be measured for hydrolysis of the ortho compound. A similar trend also was observed for hydrolysis of the other substrates by the various strains (Table 1). The preferential hydrolysis of the p-nitrophenyl derivatives is probably because the straight configuration of these molecules is more accessible to the active site of the enzyme. The general trend for hydrolysis of p-nitrophenyl derivatives of fatty acids by lactic streptococci was:

acetate<butyrate<caproate>caprylate>caprate.

From data in Table 1 it also was evident that although the parent and mutant strains of *S. lactis* showed rather similar specific activities for most substrates tested, significant differences were observed between *S. cremoris* (KH) and the mutant strain, *S. cremoris* (KHA2). For example, the specific activity of the mutant strain, *S. cremoris* (KHA2), for p-nitrophenyl was about 70% higher than that of the parent strain. A similar trend was observed for the rate of hydrolysis of p-nitrophenyl esters of butyrate, caproate, caprylate, and caprate. It is apparent from the data in Table 1 that the relative rate of hydrolysis of the C-6 and C-8 fatty acid esters of p-nitrophenyl by *S. cremoris* (KHA 2) was clearly about 50% higher than that of *S. lactis* (25Sp). Data in Table 1 also illustrates that *S. lactis* (C2) showed almost 70% more esterase activity than did *S. cremoris* (KH).

Parent and mutant strains of *S. lactis* and *S. cremoris* possessed active esterases, and well-defined bands of specific activity were detected after electrophoresis of cell-free extracts (Tables 2 and 3). Based on electrophoretic mobility (R_f values), the parent *S. lactis* (C2) and its mutant, *S. lactis* (25Sp), showed colored zones of marked esterase activity located between R_f 0.35 and R_f 0.56. Three activity bands were detected on α -naphthyl acetate for both strains, the major band had R_f 0.50 while the two minor bands were located at R_f 0.56 and 0.43.

The number of bands exhibited by *S. lactis* (C2) and *S. lactis* (25Sp) were similar for α-naphthyl acetate, β-naphthyl acetate, and β-naphthyl butyrate (Table 2). None of the *S. lactis* strains showed activity against caproate and caprylate derivatives of β-naphthyl. It is noteworthy that the parent strain, *S. lactis* (C2), exhibited two esterase bands with R_f values of 0.50 and 0.52 for α -naphthyl butyrate (Table 2) and 0.45 and 0.52 for α-naphthyl caprylate, whereas the mutant strain, *S. lactis* (25Sp), had only one activity band located at R_f 0.50 for α -naphthyl butyrate (Table 2) and 0.56 for α-naphthyl caprylate.

Differences in the number of esterase bands also were observed for *S. cremoris* (Table 3). When β-naphthyl acetate was the substrate, only one

activity band (R_f 0.35) was detected in the cell-free extract from the parent strain. The mutant strain, *S. cremoris* (KHA2), exhibited two bands. The opposite was true for α -naphthyl butyrate; four activity bands appeared in the cell-free extract from the parent strain whereas only two were present in the extract from *S. cremoris* (KHA2) (Table 3). It also is noteworthy that *S. cremoris* (KHA2) could be distinguished from the parent strain, *S. cremoris* (KH), by its ability to form an activity band with both β-naphthyl butyrate and β-naphthyl caprylate. The parent strain appeared to lack this enzyme system (Table 3).

Results presented here indicate the quantitative difference in activity of esterases as noted with different o- and p-nitrophenyl derivatives of fatty acids. Also, use of polyacrylamide gel electrophoresis to determine the number and substrate specificity of esterases may provide a method of characterizing strains of streptococci according to esterase patterns. The considerable differences in the activity levels and number of esterase bands between parent strains and their mutants are difficult to explain with the presently available genetic information; more work in this area is being carried out.

Impact of Research:

It seems important for future selection of mutants to be used in making cheese and other dairy products to examine activities other than lactose hydrolysis and general proteolysis, since differences in the peptidase level of these strains and their mutants also were observed by Kamaly and Marth.

(Tables 1, 2, and 3 follow.)

Table 1. Specific activity[a,b] of the esterases from *Streptococcus lactis* (C2), *Streptococcus cremoris*, (KH) and their mutants.

Strain	C2		C4		C6		C8		C10	
	P	O	P	O	P	O	P	O	P	O
S. cremoris (KH)	33	2	41	2	51	6	30	7	18	0.5
S. cremoris (KHA2)	109	6	114	4	324	21	221	8	105	1
S. lactis (C2)	85	9	120	7	155	22	116	10	61	1
S. lactis (25Sp)	92	8	120	11	147	22	103	40	55	1

[a]These results represent the mean of three experiments.

[b]Specific activity is defined as the number of units per mg of protein. A unit of enzyme activity was defined as the variation of 0.01 unit of absorbance at 410 nm in 1 min.

Abbreviations:
P: para-nitrophenyl, O: ortho-nitrophenyl, C2: acetate, C4: butyrate, C6: caproate, C8: caprylate, and C10: caprate.

Table 2. Electrophoretic mobility (R_f[a]) of different esterases in cell-free extract from *Streptococcus lactis* in polyacrylamide gels.

Strain	Substrate	R_f values					
		0.35	0.43	0.45	0.50	0.52	0.56
S. lactis(C2)	α-naphthyl acetate		+		+		+
	β-naphthyl acetate				+		+
	α-naphthyl butyrate				+	+	
	β-naphthyl butyrate					+	
	α-naphthyl caproate						+
	β-naphthyl caproate						
	α-naphthyl caprylate			+		+	
	β-naphthyl caprylate						
S. lactis(25Sp)	α-naphthyl acetate		+		+		+
	β-naphthyl acetate	+	+				
	α-naphthyl butyrate				+		
	β-naphthyl butyrate						+
	α-naphthyl caproate						+
	β-naphthyl caproate						
	α-naphthyl caprylate						+
	β-naphthyl caprylate						

[a] R_f expressed as the ratio of the distance traveled by the band of activity to that of bromophenol blue as the tracking dye.

Table 3. Electrophoretic mobility (R_f[a]) of different esterases in cell-free extract from *Streptococcus cremoris* in polyacrylamide gels.

Strain	Substrate	R_f values							
		0.27	0.35	0.40	0.45	0.50	0.52	0.56	0.61
S. Cremoris (KH)	α-naphthyl acetate		+	+					
	β-naphthyl acetate		+						
	α-naphthyl butyrate	+			+			+	+
	β-naphthyl butyrate								
	α-naphthyl caproate	+							
	β-naphthyl caproate								
	α-naphthyl caprylate				+			+	
	β-naphthyl caprylate								
S. cremoris (KHA2)	α-naphthyl acetate		+	+					
	β-naphthyl acetate		+	+					
	α-naphthyl butyrate							+	+
	β-naphthyl butyrate						+		
	α-naphthyl caproate							+	
	β-naphthyl caproate						+		
	α-naphthyl caprylate					+		+	
	β-naphthyl caprylate								

[a] R_f expressed as the ratio of the distance traveled by the band of activity to that of bromophenol blue

Project Title:

Effect of preservatives on *Listeria monocytogenes*.

Personnel:

E. H. Marth, Professor, Food Science Department and M. El-Shenawy, CDR visiting scientist.

Funding:

Center for Dairy Research (WITEP); Kraft, Inc., Glenview, IL; and Chr. Hansen's Laboratory, Milwaukee, WI.

Objectives:

Listeria monocytogenes is a gram-positive, nonsporeforming, aerobic to facultatively anaerobic, rod-shaped bacterium. It is a pathogen that causes listeriosis, a disease that primarily affects pregnant women, infants, and adults with a compromised immune system. In food-related outbreaks of listeriosis, the mortality rate is about 30%.

Little is known about its behavior, including control, in food. One possible approach to control of *L. monocytogenes* is to employ acceptable food preservatives (e.g., sodium benzoate, potassium sorbate, sodium propionate, etc.) when this is appropriate. According to our results from earlier work, it is possible that antilisterial effects of some preservatives (e.g., benzoic, sorbic, and propionic acids) can be enhanced by the presence of other food-type organic acids (acetic, tartaric, lactic, and citric acid).

Our current research involves determining the ability of *L. monocytogenes* to grow/survive at 13 or 35°C in Tryptose Broth (TB) adjusted to pH 5.6 or 5.0 with acetic, tartaric, lactic, or citric acid and supplemented with 0.05, 0.15, or 0.3% sodium benzoate, potassium sorbate, or sodium propionate. Results of the experiments at 13°C were not complete when this was written. The following discussion applies to behavior of *L. monocytogenes* at 35°C. Controls also were done using unadjusted TB and TB adjusted to pH 5.6 or 5.0 without added preservatives.

Results:

Trials with Sodium Benzoate

Behavior of *L. monocytogenes* using acetic acid:

Using acetic acid to adjust the pH of TB to 5.6 (control) resulted in growth of *L. monocytogenes* after approximately 4 hours at 35°C. The maximum population, ca. 10^8/ml, was attained after 32 hours. When the pH was reduced to 5.0, growth occurred after a lag phase of about 16 hours. The maximum population, after 96-120 hours, was about the same as at pH 5.6.

Addition of 0.05 and 0.15% sodium benzoate at pH 5.6 permitted growth of the pathogen. Presence of 0.3% completely inhibited growth of *L. monocytogenes*. At pH 5.0, 0.05% sodium benzoate allowed slight growth after 48 hours of incubation. It was clear that as the concentration of the preservative increased and the pH decreased the lag phase and generation time were increased and maximum populations attained were decreased. The other two concentrations of benzoate, 0.15 or 0.31, caused complete inactivation of the pathogen after 96 and 40 hours, respectively.

Behavior of *L. monocytogenes* using tartaric acid:

Tartaric acid was used to adjust the pH of the medium to 5.6; growth occurred when the medium contained 0.05 or 0.15% benzoate. The lag phase and generation time were prolonged as the concentration of the preservative was increased and the maximum population attained was decreased. Presence of 0.3% benzoate at pH 5 did not allow any growth of the pathogen. Sodium benzoate at 0.05% permitted slight growth of *L. monocytogenes* after 48 hours of incubation. Addition of 0.15 or 0.3% benzoate caused complete inactivation after 88 and 20 hours of incubation, respectively.

Behavior of *L. monocytogenes* using citric acid:

When citric acid was used to adjust the pH of the medium to 5.6, growth of the pathogen occurred in the presence of 0.05 or 0.15%, but not 0.3% sodium benzoate. When the pH of the medium was reduced to 5.0 using citric acid, growth of the pathogen occurred at concentrations of 0.05 or 0.15% sodium benzoate. Complete inactivation of the pathogen was noted after 40 hours of incubation at pH 5 when the medium contained 0.3% benzoate.

Behavior of *L. monocytogenes* using lactic acid:

When the pH of TB was adjusted to 5.6 using lactic acid, growth of the pathogen occurred in the presence of 0.05 or 0.15% sodium benzoate. A concentration of 0.3% sodium benzoate in the medium did not allow growth until the end of the experiment. With the pH at 5.0, the pathogen grew when the medium contained either 0.05 or 0.15% sodium benzoate. Complete inactivation of the pathogen occurred after 48 hours of incubation when the medium contained 0.3% sodium benzoate.

Trials with Potassium Sorbate:

Behavior of *L. monocytogenes* using acetic acid:

When the pH of the medium was adjusted to 5.6 using acetic acid, growth of *L. monocytogenes* occurred with concentrations of 0.05 or 0.15% potassium sorbate. Addition of 0.3% sorbate was accompanied by slight growth after a lag phase of about 32 hours.

Reducing the pH to 5 resulted in growth of the pathogen when the medium contained 0.05% sorbate. Addition of 0.15% sorbate to the medium resulted in a slight decrease in numbers but 0.3% completely inactivated the pathogen after 48 hours.

Behavior of *L. monocytogenes* using tartaric acid:

Tartaric acid markedly enhanced the effect of sorbate. Although there was substantial growth at pH 5.6 when 0.05 or 0.15% of the preservative was present, a concentration of 0.3% potassium sorbate virtually prevented growth of the pathogen. Decreasing the pH to 5.0 was accompanied by growth of *L. monocytogenes* after 60 hours when the medium contained 0.05% potassium sorbate. Concentrations of 0.15 or 0.3% of the preservative resulted in complete inactivation of the pathogen after 32 or 56 hours of incubation, respectively.

Behavior of *L. monocytogenes* using citric acid:

Adjusting the pH to 5.6 permitted growth at all concentrations of the preservative. Lag phases and generation times were extended and maximum populations achieved decreased as the concentration of the preservative was increased. The same was true when the pH was reduced to 5.0, except that 0.3% sorbate caused complete inactivation of the pathogen after 56 hours.

Behavior of *L. monocytogenes* using lactic acid:

At pH 5.6 the pathogen grew in all concentrations of the preservative that were tested, but when the pH was reduced to 5.0, growth occurred only at concentrations of 0.05 or 0.15% potassium sorbate. Addition of 0.3% of the preservative to the medium caused inactivation of the pathogen after 56 hours of incubation.

Trials with Sodium Propionate:

Behavior of *L. monocytogenes* using acetic acid:

The pathogen grew in all concentrations of sodium propionate either at pH 5.6 or 5 when acetic acid was used, except for 0.3% of propionate that caused a gradual decrease in numbers of *L. monocytogenes*. Lag phases and generation times were prolonged as the concentrations of the preservative were increased and as the pH of the medium was decreased.

Behavior of *L. monocytogenes* using tartaric acid:

Tartaric acid enhanced the antimicrobial activity of sodium propionate more than did acetic acid. The pathogen grew in all concentrations of propionate used in this investigation except at pH 5.0 where concentrations of 0.15 and 0.3% sodium propionate caused complete inactivation after 72 or 112 hours of incubation, respectively.

Behavior of *L. monocytogenes* using citric acid:

Adjusting the pH of the medium to 5.6 or 5.0 resulted in growth of the pathogen in all concentrations of propionate tested, except that 0.3% sodium propionate inhibited growth of the pathogen at pH 5.0.

Behavior of *L. monocytogenes* using lactic acid:

When lactic acid was used the pathogen grew at all concentrations of propionate and pH values except 0.3% at pH 5.0 and that completely inhibited growth of *L. monocytogenes.*

Impact of Research:

Our results provide information on circumstances under which common preservatives might be useful in the control of *L. monocytogenes.*

Project Title:

Texture development in cheese made from ultrafiltered milk.

Personnel:

D. Srinivasan, Assistant Professor, Food Science Department, N.F. Olson, Professor, Food Science Department, M.E. Johnson, Associate Scientist, CDR, and S. H. Kim, graduate student, Food Science Department.

Funding:

Wisconsin Milk Marketing Board.

Objectives:

1. To elucidate fundamental causes for poor texture development in cheese made from ultrafiltered milk. This will include:

   a.) the extent and mode of retention of whey proteins and their effect on cheese curd structure and texture development during ripening of Cheddar cheese,

   b.) and the role of casein micelle concentration on cheese curd structure under various coagulation temperatures and rennet concentration.

2. To develop practical methods to improve texture development of hard cheeses (Cheddar cheese) made from ultrafiltered milk.

Impact of Research:

The outcome of the proposed research will greatly enhance our understanding of the factors which affect curd structure formation and subsequent texture development in cheese make from ultrafiltered milk. Successful development of methods to improve the acceptability of cheese from concentrated milk will facilitate implementation of ultrafiltration techniques in cheese manufacture. Such a development will decrease the cost of cheese production, increase the sale of dairy products, and enhance the competitiveness of the cheese industry.

Research Summary:

I. Ultrafiltration of milk at 50°C for an extended period of time might cause, apart from changing the absolute concentrations of milk constituents, alterations in the state of equilibrium of various components between the serum and micellar phases. Such changes may affect the aggregation properties of the casein micelles. In order to elucidate whether there is a change in the equilibrium state of casein micelles during ultrafiltration and what effect such changes have on their aggregation properties, the following studies were conducted:

Pasteurized skim milk was ultrafiltered to 5-fold concentration using E-500 membrane (Desalination Systems, Inc., CA) in a laboratory scale ultrafiltration unit (Tri-Clover, Kenosha, WI). Ultrafiltration was carried out at various temperatures, viz., 25°, 40°, 50°, and 55°C. After ultrafiltration, the 5-fold concentrated sample was diluted to 50-fold (i.e., one-tenth of single strength) with the permeate. The temperature of the sample was immediately lowered to 30°C and incubated in a waterbath at that temperature. Aliquots (3 ml) of the sample were taken at regular intervals of time and the kinetics of rennet-induced aggregation of the casein micelles was studied using an aggregometer. The changes in the rate of aggregation (obtained from the initial slope of turbidity versus time curve) as a function of incubation time was determined.

In a control experiment, pasteurized milk was stored at 4°C. After 24 hours, the milk was diluted 10-fold (one-tenth of single strength milk) using the permeate from the ultrafiltration experiment. The temperature was immediately raised to 30°C and incubated at that temperature. Aliquots (3 ml) were withdrawn at regular intervals of time and the rennet induced aggregation of the casein micelles was studied. As before, the change in the rate of aggregation as a function of incubation time was determined.

The rationale of these experimental designs are as follows: If ultrafiltration of milk causes unusual changes in the state of equilibrium of the components of the milk system, one should be able to observe these changes from the time dependent relaxation behavior in the equilibrium state upon reconstitution to the original conditions.

The major results of these experiments are shown in Figure 1. It should be noted that in the case of the control (i.e., the sample whose temperature was increased from 4°C to 30°C and incubated at that temperature), the rate of aggregation of casein micelles increased with incubation time. This indicates that upon changing the temperature from 4°C to 30°C, the milk system undergoes a slow change in the distribution of components between the serum and micellar phases and reaches a new equilibrium state after about 3 to 4 hours. During this slow equilibration process, the changes that occur in the physico-chemical properties of casein micelles promote their enzymatic aggregation/coagulation properties. It is known that pre-cooling of milk before renneting increases the coagulation time. However, incubation or aging of pre-cooled milk at renneting temperature (i.e., 30°C) has been shown to restore the coagulation properties to that of uncooled milk. The results shown here in fact support those observations and provide a kinetic date for those observations.

The time-dependent aggregation behavior of casein micelles in ultrafiltered milks are also shown in Figure 1. It should be noted that in contrast to the control, the rate of aggregation of the micelles of ultrafiltered milk decreased with incubation time at renneting temperature; this indicates that the casein micelles of the ultrafiltered milk also undergo a slow equilibration process. However, this equilibration process is completely different from that of the behavior of the control. In other words, ultrafiltration of milk at higher temperatures alters the chemical equilibrium of the milk system, but the changes in the distribution of components during ultrafiltration is different from that of the distribution at low temperatures, i.e., in the opposite direction. The resultant casein micelles possess greater rate of enzymatic coagulation/aggregation properties than that of the regular milk. However, when aged at the

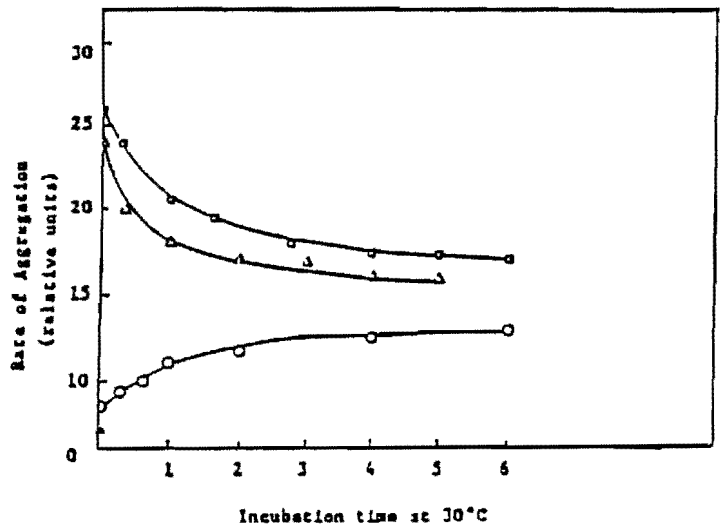
renneting temperature, i.e., at 30°C, the casein micelles of ultrafiltered milk undergo a re-equilibration process which decreases their renneting properties; after 3 hours the renneting properties of the ultrafiltered milk seem to approach that of the regular milk. Further studies are in progress to understand the time dependent physico-chemical changes in the casein micelles of ultrafiltered milk that are responsible for the observed behavior.

The significance of these studies: Since the rate of aggregation and coagulation of casein micelles has a pronounced effect on the properties of curd structure and subsequent texture development, understanding the factors that affect the coagulation/aggregation rate of casein micelles of ultrafiltered milk would provide ways to manipulate the rate and thus the texture of cheese.

II. It has been shown by other researchers that substantial changes in the composition of milk occur during ultrafiltration. These include changes in the levels of calcium, phosphate, and citrate. In order to examine whether addition of citrate or calcium affect the rate of aggregation/coagulation of micelles, we first studied the effects of these ions on the rennet-induced coagulation of k-casein in a model system. The results of these studies are shown in Figure 2. At lower concentrations, citrate decreased the clotting-time; however, above 7 mM (I= 0.035), the rate of rennet induced coagulation of k-casein decreased, and the clotting time increased dramatically. When 10 mM calcium chloride was included in the reaction medium, the rate of coagulation decreased and the

(Continued on the next page.)

Figure 1. Effect of aging at 30°C on the aggregation properties of casein micelles of ultrafiltered milk.

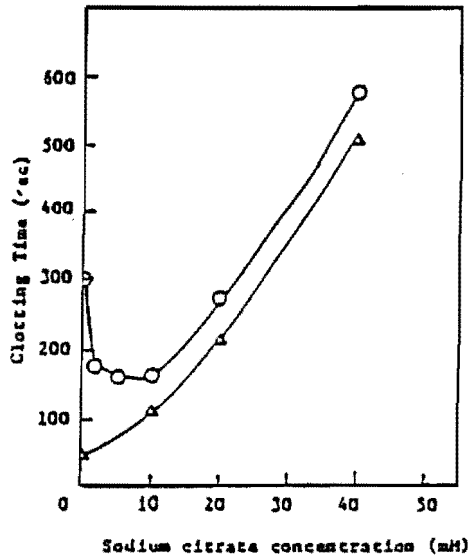


- Milk was stored at 4°C and then incubated at 30°C (Control).
- Milk was ultrafiltered at 50°C and then incubated at 30°C.
- Milk was ultrafiltered at 55°C and then incubated at 30°C.

clotting time increased with citrate concentration. It should be pointed out that, in both cases, addition of 40 mM sodium citrate increased the clotting time by about a 2- (in the absence of $CaCl_2$) to 10-fold (in the presence of 10mM $CaCl_2$). Although these results were obtained with pure k-casein, the data suggest that it is possible to manipulate the aggregation/coagulation properties of casein micelles of ultrafiltered milk by adding sodium citrate. These investigations are currently in progress.

III. We are in the process of developing a reliable, non-destructive method to measure the kinetics of coagulation of ultrafiltered milk prepared under various experimental conditions. Our initial approach was to use the Instron Universal Machine. Although the preliminary experiments were highly successful, the baseline instability was unacceptable. This was because of the large capacity of the load cell that we used. We are in the process of locating a load cell with 500g or 2 kg capacity. This method will be highly useful to study the kinetics of coagulation of ultrafiltered milk under various experimental conditions. This investigation would provide information on the optimum conditions at which the rate of curd setting of ultrafiltered milk is similar to that of regular milk. The curd prepared under these conditions will be analyzed for texture development.

Figure 2. Effect of citrate on the rennet-induced clotting of k-casein in the presence and absence of 10mM $CaCl_2$.



o—o In the absence of $CaCl_2$
Δ—Δ In the presence of $CaCl_2$

Project Title:

Development of cheese manufacturing and packaging processes to minimize milk salt crystallization on cheese during storage and distribution.

Personnel:

N.F. Olson, Professor, Food Science Department, M.E. Johnson, Associate Scientist, CDR, and B.A. Riesterer, Associate Researcher, CDR.

Funding:

Wisconsin Milk Marketing Board.

Objectives:

During distribution and handling of Cheddar cheese, a white crystalline material sometimes appears on the surface of the cheese. Although the material is harmless, it adversely affects the consumer acceptance of the cheese. Preliminary studies have indicated that the white substance is calcium lactate. The cause of crystallization of calcium lactate on the surface of cheese is unknown. This project is studying several factors that may influence calcium lactate crystal development. The factors involved are manufacturing conditions, pH at drain, pH at mill, and salt levels in the finished product. The amount of calcium, lactic acid, and residual lactose in the curd at mill probably influences the amount of calcium lactate in the cheese. Salt and pH can affect the solubility of calcium and calcium lactate. Preliminary investigations have also shown that packaging and handling conditions may affect crystal formation and are also being studied.

Results of Research July 1987 - July 1988:

Cheddar cheese was made at Lake-to-lake Dairy in Kiel, Wisconsin on November 10, 1987. Four experimental vats were made according to parameters designed to prevent crystal formation. Four vats were made according to the plant's normal processing schedule and were monitored during manufacture as control vats.

Five 40 pound blocks from each of the eight vats were stored at 40°F for five months. On April 12, 1988 four blocks from each vat were cut into approximately 9 ounce pieces. Half of the pieces from each block were packaged and vacuum sealed. The remaining pieces were packaged using the plant's normal routine (Co_2 flush, no vacuum). The remaining uncut blocks from each vat was used to evaluate flavor, texture, and body. Half of the pieces from each packaging method was stored at 40°F while the other half was stored at 50°F. Each of the 9 ounce pieces are currently being examined monthly for crystal growth.

Examinations on May 12, June 13, July 11, and August 10 revealed that there was no crystal development in vacuum packaged cheeses. Very few normal packaged cheeses ($C0_2$ flush, no vacuum) showed any sign of crystal growth. If there were crystals, the arbitrary grading scale rated the crystal development as 1. A score of 10 would mean crystals covered the cheese. A score of 1 means a single, very small spot or crystal was observed.

Observations will continue through November. Also, additional observations will be made by taking several pieces of cheese from each vat and packaging method and placing them under other storage conditions that are thought to induce crystal formation. These conditions include storage under fluorescent light, and warming and cooling cycles. The cheese will also undergo chemical analysis to determine if packaging conditions prevented crystal development.

Impact of Research:

It was estimated by representatives of the cheese industry that 4% of the Cheddar and similar varieties of cheese develop this white haze at the retail level. Consumers reject the cheese since they assume that the haze is white mold. If defective cheese were considered waste, this would produce a $57 million annual loss ($1.30/pd) to the Wisconsin cheese manufacturing industry. This research will lead to manufacturing and handling procedures necessary to avoid or minimize calcium lactate crystallization.

Project Title:

Accelerated ripening of cheese using liposome-entrapped enzymes.

Personnel:

M. El Soda, CDR visiting scientist, M.E. Johnson, Associate Scientist, CDR, P. L. Spangler, graduate student; L. Jensen, Associate Researcher, CDR; and N.F. Olson, Professor, Food Science Department.

Funding:

Wisconsin Milk Marketing Board (WITEP), Center for Dairy Research.

Objectives:

1. To study temperature-sensitive liposomes as a controlled release system for the acceleration of cheese ripening.

2. To compare liposome-entrapped enzymes to other accelerated ripening systems in conventional and UF Gouda.

Objective 1

Liposomes technology, which was recently proposed as an effective tool for the acceleration of cheese ripening, offers the possibility of preparing a wide array of vesicles varying in size, net charge, stability, and sensitivity to pH and/or temperature. Phospholipid vesicles differing in size, net charge, or stability were previously evaluated for the acceleration of cheese ripening. The aim of the present investigation was to use liposomes that would break apart or disrupt at a well-defined temperature (temperature sensitive liposomes).

Results:

Domiati cheese, a feta-type curd, was manufactured. Renneting was accomplished at a low temperature (28°C) for three hours in order not to destabilize the temperature-sensitive liposomes. Encapsulated corolase PN, a neutral proteinase from *Aspergillus* sp. (Rohm Tech, West Germany) was added to the cheesemilk of the first batch while encapsulated calcein (a fluorescent dye) was added to the second batch; empty capsuless were added to the third batch and was considered a control. All the cheese-making steps were performed at temperatures not exceeding 30°C in order not to destabilize the vesicles. Cheese from each vat was treated in one of three ways:

1. Ripened at 12°C and was not subjected to further treatments,

2. Heated to 40°C/two hours in order to destabilize the vesicles and then ripened at 12°C,

3. Samples were kept at 12°C for 48 hours, heated to 40°C for two hours, and then returned to 12°C.

PTA and TCA soluble nitrogen were determined in the different cheeses. Samples were also subjected to fluorescence measurement.

Table 1 shows that the initial fluorescences values for all cheeses are similar. The readings are probably due to the fluorescence of riboflavin in the cheese. After heating the cheese for two hours at 40°C a significant increase in fluorescence could be measured in the calcein-entrapped liposome treated cheese. This indicates that heating the cheese will lead to liposome leakage and to the release of the dye. Calcein was also released in cheese held for two days at 12°C. This is probably a result of vesicle disruption due to the joint action of salt, pH (3), and phospholipase. However, heating the samples increased dye release. After seven days of ripening most of the calcein was released from the vesicles indicating total disruption of the liposomes and that heat treatment is not necessary for release of calcein from the vesicles after only one week of ripening.

The rate of protein breakdown during a four-week ripening period is given in Table 2. There are very little differences in the TCA soluable N and PTA soluable N values between the control cheese and various treatments. This is probably due to the denaturation of the Corolase PN enzyme during liposomes preparation (two hours at 50°C).

Impact of Research:

Although temperature-sensitive liposomes might be a good candidate for targetable drug delivery, they do not seem to be the appropriate controlled release system for accelerated cheese ripening due to the fact that relatively high temperatures are to be used during vesicle formation which can lead to partial or total denaturation of the enzyme to be entrapped. The obligation to use a low coagulation and manufacturing temperature during the cheese-making process is also another limitation for the application of temperature-sensitive liposomes for the acceleration of cheese ripening. On the other hand, it seems that liposomes engineered to have a phase transition at a pH that is reasonably well defined (pH 5.5-5.0) will probably be more appropriate for enzyme delivery under cheesemaking conditions, and work in this area is currently in progress.

Objective 2

Experiments where liposome-entrapped Corolase PN and free Corolase PN were added to Gouda cheese made using either the conventional manufacturing procedure or from ultrafiltered milk were also carried out. The rate of ripening in the different cheeses was followed by measuring PTA and TCA soluble nitrogen.

The results (Fig. 1 and 2) indicated a higher rate of ripening in the conventional Gouda when compared to the cheese made from UF milk. Ripening in both cheeses was significantly increased in the cheese with free proteinase-added, but a pronounced, bitter flavor developed very rapidly in the cheese samples. On the other hand, liposome-entrapped enzyme lead to a lower rate of protein breakdown in the cheeses but, the resultant cheese showed less bitterness.

The rate of ripening in UF cheese, which is known to be more resistant, can be enhanced without significant impairment in cheese flavor using liposome-entrapped proteinase. Experiments where exopeptidases obtained from starter organisms are combined with the entrapped gross proteolytic agent should be carried out in order to obtain a rapidly ripened UF cheese showing enhanced organoleptic properties.

Table 1. Calcein release from temperature-sensitive liposomes in Domiati cheese.

	Fluorescence Values		
	Liposome + Calcein	Liposomes + Proteinase	Control
Cheese after manufacture	24135	23137	21847
Cheese heated 40° C/2hr.	75644	22005	20732
Cheese after 2 days/12C	45743	25876	25000
Cheese 2 days/12C then heated 40° C/2hr.	72814	24300	23282
Cheese after 7 days/12° C	69333	n.d.	24899

n.d.= not determined

Table 2. Effect of temperature-sensitive liposome-entrapped proteinase on the rate of protein breakdown in cheese as measured by PTA and TCA soluble nitrogen.

Age (weeks)		PTA Soluble nitrogen Treatment			TCA soluble nitrogen Treatment		
		1	2	3	1	2	3
Liposomes + calcein	0	11.9			15.6		
	1	9.6	8	7.3	21.8	19.9	20.3
	2	11.0	10.4	10.6	30.8	27.4	28.8
	4	19.9	17.5	15.9	44.5	47.1	44.6
Liposomes + proteinase	0	7.4			12.3		
	1	8.2	8.8	7.7	20.5	21.2	26.7
	2	9.2	11.0	11.2	31.2	30.8	33.7
	4	11.9	20.3	12.4	37.3	41.0	35.2
Control	0	9.8			12.2		
	1	10.0	9.4	7.9	19.7	17.3	16.7
	2	9.1	10.6	13.9	28.2	29.7	32.9
	4	15.4	20.2	14.7	46.7	44.7	44.8

Treatment 1= Cheese ripened at 12°C and not subjected to further treatments.

Treatment 2= Cheese heated to 40°C/2hr. and then ripened at 12°C.

Treatment 3= Cheese ripened at 12°C/2 days, heated to 40°C/2hr. and then returned to 12°C.

Figure 1. PTA soluble N of Gouda cheese versus time.

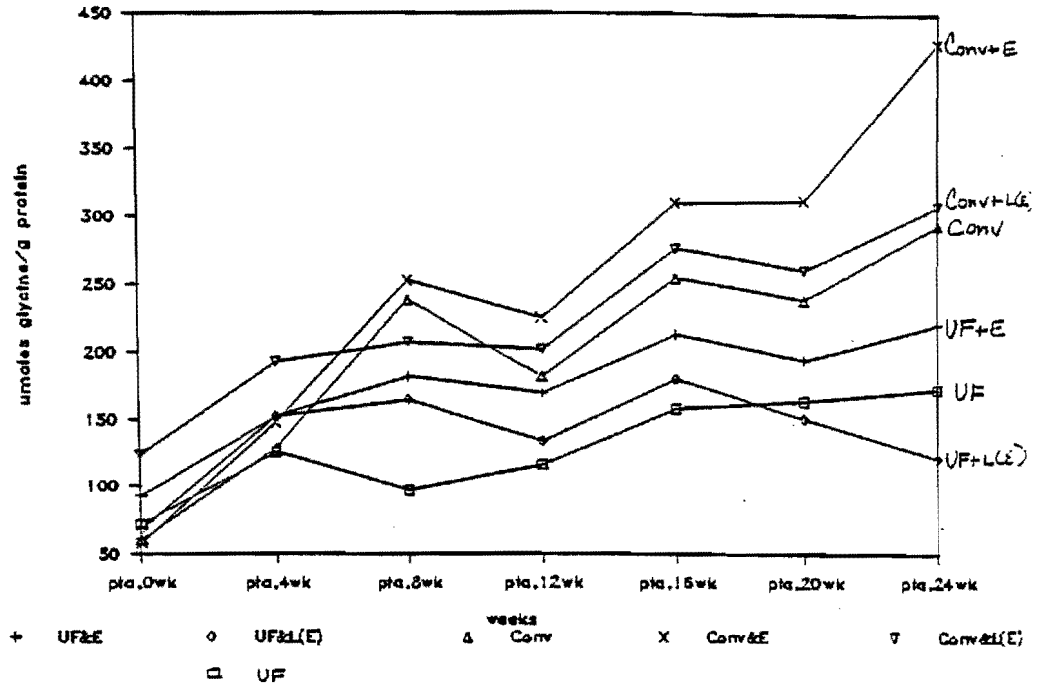
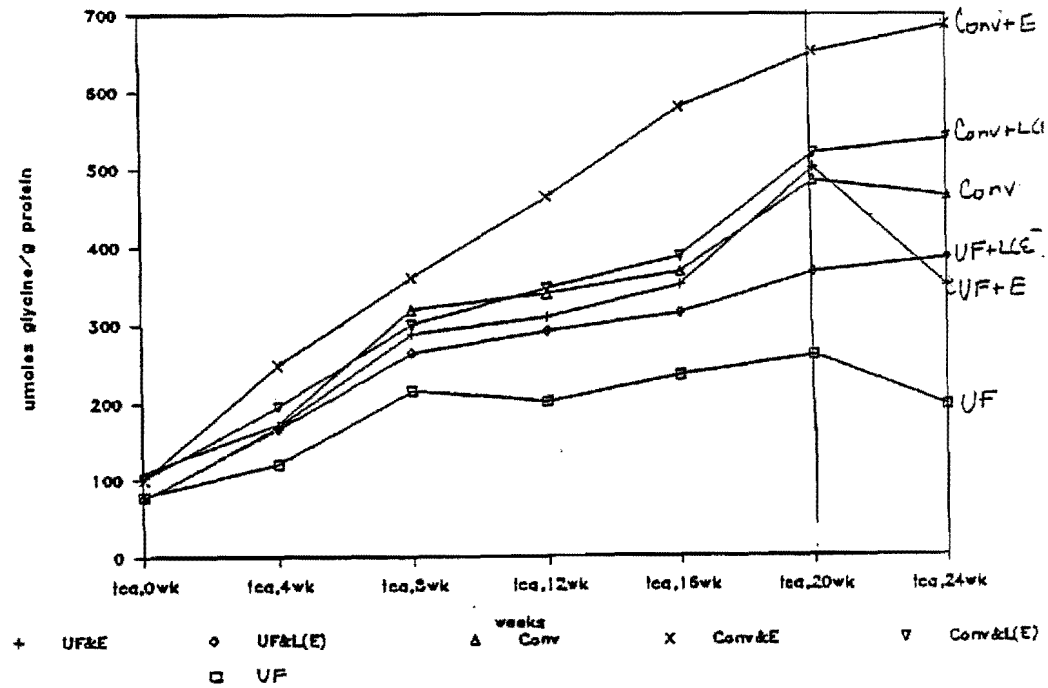


Figure 2. TCA soluble N of Gouda cheese.



Project Title

Increased flavor levels of cheese made from ultrafiltered milk.

Personnel:

N.F. Olson and C.H. Amundson, Professors, Food Science Dept.; M. El Soda, CDR visiting scientist; P.L. Spangler and M. Kim, graduate students, Food Science Dept; M.E. Johnson, Associate Scientist, CDR, L. Jensen, Associate Researcher, CDR.

Funding:

National Dairy Promotion and Research Board, Dairy Research Foundation, Wisconsin Milk Marketing Board (WITEP).

Project Objectives:

1. Use a commercial curd-forming device (Alfa-Laval Alcurd Coagulator) to manufacture Havarti, Gouda, and Cheddar cheeses from ultrafiltered milk.

2. Investigate various means to intensify flavor development of the selected cheese varieties made from ultrafiltered milk. Methods will include use of protease-peptidase preparations, microencapsulated enzyme systems and heat- or freeze-shocked strains of concentrated *Lactobacillus* sp.

3. Investigate physical properties of selected cheeses made from ultrafiltered milk using methods such as scanning electron microscopy, compression testing, and sensory analysis techniques.

Results:

A. Liposome Cheese Trials: Protease or cell-free extracts of *Lactobacilli* were entrapped in liposomes. Liposomes were added to UF Gouda cheese to increase proteolysis. A publication entitled: "A comparative study between several ripening agents for the acceleration of ripening of Gouda cheese made from ultrafiltered milk" is being prepared.

B. Split-plot design to study manufacturing variables for UF Gouda

Manufacturing variables investigated included preacidification level (none, pH 6.3), diafiltration level (10%, 7%), rennet concentration (.007% and .014%) based on unconcentrated milk volume), starter concentration (1%, 3%) and time between coagulation and cut (2 and 4 times the coagulation time). Preacidification and diafiltration were the whole plot variables. Repeat trials were done including the variables of rennet concentration, preacidification level, and diafiltration level. A total of 40 UF and 7 conventional Gouda cheeses were made. UF Gouda cheeses were made using 5X retentate.

Tables 1-3 compare the 40 UF cheeses with the 7 conventional cheeses.

From Table 1 it is seen that the UF cheeses have about 1% higher moisture than the conventional cheeses. Also, the pH tends to be about .1 higher throughout time. Table 2, which compares PTA and TCA levels, indicates UF to have slightly lower PTA and TCA levels at 4 weeks than the conventional cheeses. Table 3, which compares UF and conventional cheese taste panel results at 8 weeks, shows a slight preference for conventional cheese. Also, the UF cheese seems to have less flavor intensity.

Analysis of variance was done on the first 32 UF cheeses. Rennet concentration is a very significant variable having significance at the 99% level on hardness at 25 weeks, bitterness and overall preference at 8 weeks, and bitterness, flavor intensity, and overall preference at 25 weeks. Increasing rennet decreased hardness, probably due to alpha s1 casein hydrolysis. Increasing rennet increased bitterness and decreased overall preference. Increasing rennet decreased flavor intensity. Perhaps flavor intensity was masked by bitterness.

Starter concentration was a significant variable having significance at the 99% level on pH at 0 and 8 weeks. Increasing starter had the effect of

lowering pH which is desirable since the UF Gouda generally had a higher pH than the conventional cheeses. Starter had significance at the 95% level on Instron hardness at 4 weeks. This is probably due to its effects on moisture of the cheese at 4 weeks. Increasing starter decreased moisture. A faster rate of pH drop during cheesemaking increases syneresis of whey leading to a final lower moisture and increased hardness of the cheese. This is desirable since the UF Gouda tends to have higher moisture and to be softer than the conventional cheese. It appears that the 3% starter is better than 1% for UF Gouda composition.

Cut time by itself did not have a significant effect on any of the measurements at the 99% or 95% levels.

Diafiltration level and preacidification level were whole plot variables so we cannot claim significance of these variables on the measurements. However, diafiltration level appears to be a significant factor on pH at 0 weeks, hardness at 4 weeks, PTA at 4 weeks, and TCA at 0 and 4 weeks. A higher diafiltration level gave much higher PTA and TCA levels. We will investigate this further.

Preacidification level appears to be a significant factor on the pH of the cheeses. Preacidification to pH 6.3 seems to give lower cheese pH which are closer to the conventional cheeses.

Buffer capacity is decreased by preacidification. On the basis of the results of analysis of variance on the 32 UF cheeses, it appears that the starter is definitely a significant variable and that UF cheese should be made using the 3% starter level to improve composition. Also, rennet was very important and it would be further investigated in the repeat trials. Cut time does not appear to have much significance and we should use the shorter cut time of 2X to save time in manufacturing. Preacidification and diafiltration levels were also included in the repeat trials because of their interesting effects. Data from the repeat trials are undergoing analysis.

Impact of Research:

Our results show that rennet concentration is very important to the quality of the UF Gouda cheese. They also show that it is unnecessary to use longer than 2X cut time. Also, we found that increasing the starter concentration will be beneficial to cheese composition. In some cases, our results show inhibition of proteolysis in UF Gouda. It is important to study the effects of proteolytic enzymes or cell-free extracts on the acceleration of ripening and to study the means of incorporating the enzymes such as through liposomes.

Table 1. Comparison of UF and conventional cheese composition.

	UF (ave + sd)[1]	Conventional (ave + sd)[1]
% Moisture	39.57 + 2.12	38.62 + 1.80
% FDM[2]	48.4 + 1.6	47.6 + 1.2
% S/M[3]	3.82 + 1.1	4.61 + 2.2
% Protein	23.51 + 1.1	23.12 + 1.45
0 wk pH	5.24 + .13	5.12 + .23
25 wk pH	5.43 + .14	5.32 + .15

[1]Average + Standard Deviation
[2]Fat in Dry Matter
[3]Salt in Moisture

Table 2. Comparison of UF and conventional PTA and TCA solubleN.

	UF (ave + sd)[1]	Conventional (ave + sd)[1]
0 wk PTA[2]	30.39 + 22.22	29.18 + 21.29
4 wk PTA[2]	106.41 + 66.78	137.08 + 17.03
0 wk TCA[2]	47.02 + 25.49	56.48 + 30.81
4 wk TCA[2]	167.60 + 85.03	211.43 + 29.18

[1]Average + Standard Deviation
[2]umoles glycine equivalents/g protein

Table 3. Comparison of UF and conventional taste panel results at 8 weeks.

	UF (ave + sd)[1]	Conventional (ave + sd)[1]
Bitterness[2]	4.2 + 1.5	3.9 + 2.2
Off Flavor[2]	5.0 + 3.1	5.0 + 3.1
Flavor Intensity[2]	6.5 + 1.8	7.3 + 1.8
Overall Preference[2]	7.9 + 2.1	8.6 + 1.8

[1]Average + Standard Deviation
[2]Taste panels conducted with 6-8 experienced judges who rated cheeses for attributes on a scale of 0-18 cm (0=lowest,18=highest)

Project Title:

Safety of milk and milk products - *Listeria monocytogenes.*

Personnel:

H. K. Choi, S. E. El-Kest, S. Farrage, J.A. Olsen, M. M. Schaack, A.E. Yousef, and E.H. Marth, Professor, Food Science Department.

Funding:

Wisconsin Milk Marketing Board.

Objectives:

1. Determine behavior of *Listeria monocytogenes* in the presence of sanitizers and cleaning compounds.
2. Determine growth patterns of *L. monocytogenes* in the presence of other psychotrophic bacteria (e.g., *Pseudomonas* species).
3. Determine behavior of *L. monocytogenes* in the presence of lactic acid bacteria commonly used to produce fermented dairy products.
4. Determine the fate of *L. monocytogenes* in fermented milks.
5. Determine the fate of *L. monocytogenes* during the manufacture of and the subsequent storage of butter.
6. Determine the behavior of *L. monocytogenes* during the manufacture and ripening of Colby cheese.
7. Determine the heat resistance of *L. monocytogenes* at subpasteurization temperatures.
8. Investigate heat/acid injury in *L. monocytogenes*.

Results:

Inactivation of *L. monocytogenes* by chlorine and ultraviolet energy.

Chlorine:

Cells of *Listeria monocytogenes* strain Scott-A were harvested from cultures, washed, and then treated with a solution of sodium hypochlorite at 25°C and pH 7. The cells were more resistant to chlorine when they were (a) harvested from a 24- rather than 48-hour-old culture, (b) grown in tryptose broth rather than on a slant of tryptose agar, and (c) washed and suspended using a 20 rather than 0.312 mM phosphate buffer solution. Cells of *L. monocytogenes* were exposed for 30 seconds-4 hour to sodium hypochlorite solutions that contained 0.5-10 ppm available chlorine. Generally, the number of survivors decreased rapidly during the first 30 seconds followed by a slower decrease during the rest of the exposure time. The initial count of *L. monocytogenes* in the suspension ($1x10^8$-$3.2x10^8$/ml) decreased 0.49 to 6.4 orders of magnitude during the first 30 seconds of exposure to the chlorine solutions. The effect of the presence of organic substances on the strength of hypochlorite solutions was studied. Presence of 0.05 or 0.1% peptone caused a large and rapid loss of available chlorine. Glucose or lactose (up to 1%) had almost no effect on the concentration of available chlorine.

L. monocytogenes strain Scott-A was treated with 1 ppm available chlorine at different temperatures and pH values. Different strains of *L. monocytogenes* (California, Scott-A, and V7) were also exposed to 1 ppm available chlorine at pH 7 and 25°C. The initial population of *L. monocytogenes* was 1×10^8 to 3.2×10^8 CFU/ml of sodium hypochlorite solution. Survival of *L. monocytogenes* was measured by surface-plating (on tryptose agar) samples taken at intervals of 30 seconds to 1 hour of exposure to hypochlorite solution. Larger numbers of *L. monocytogenes* strain Scott-A survived at 25 than at 35°C. The smallest number was observed when cells were exposed to the hypochlorite

solution at 5°C. The higher the pH values, in the range of 5 to 9, the greater were the numbers of survivors of *L. monocytogenes* strain Scott-A. Of the strains studied, California was the most resistant, while V7 was the least resistant to the hypochlorite solution.

Ultraviolet energy:

Short-wave UV-energy (100 W/cm²) decreased the number of *L. monocytogenes* on Tryptose Agar (TA) ca. several orders of magnitude in 4 minutes. Age of culture (48 vs. 24 hours) did not alter the sensitivity of *Listeria* to this UV treatment. Increasing the intensity of irradiation (550 vs. 100 W/cm²) increased the rate at which *L. monocytogenes* was inactivated. Dry rather than moist cells of *L. monocytogenes* were most resistant to irradiation. In general, inactivation of *Listeria* with short-wave UV-energy yielded sigmoidal survivor curves. The population of *Listeria* on TA plates was not affected with long-wave UV-irradiation.

Growth of *L. monocytogenes* in the presence of *Pseudomonas* species.

Twenty-five milliters of autoclaved tryptose broth was inoculated with *L. monocytogenes* (strain Scott A or California), *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, or a combination of *L. monocytogenes* plus *Pseudomonas* species, and incubated at 7 or 13°C for 8 weeks. McBride Listeria Agar was used to determine numbers of *L. monocytogenes* (at 8, 7, 14, 28, 42, or 56 days), and Pseudomonas isolation agar to enumerate *Pseudomonas* species. Overall, results showed that both *P. fluorescens* and *P. aeruginosa* restricted growth of both strains of *L. monocytogenes* during incubation at either 7 or 13°C. Inhibition was greater at 13 than at 7°C and greater with *P. fluorescens* than *with P. aeruginosa*.

Behavior of *L. monocytogenes* in the presence of lactic acid bacteria.

Streptococcus lactis and *S. cremoris:*

The ability of *L. monocytogenes* to grow and compete with mesophilic lactic acid bacteria was examined. Autoclaved skim milk was inoculated with 10^3 cells of *L. monocytogenes* (strain V7 or Ohio)/ml, and with 5.0, 1.0, 0.5, or 0.1% of a milk culture of either *Streptococcus cremoris* or *Streptococcus lactis*. Inoculated milks were fermented for 15 hours at 21 or 30°C, followed by refrigeration at 4°C. Samples were plated on McBride Listeria Agar to enumerate *L. monocytogenes* and on either APT Agar or Plate Count Agar to enumerate lactic acid bacteria. *L. monocytogenes* survived in all fermentations, and commonly also grew to some extent. Incubation at 30°C with 5% *S. lactis* as inoculum appeared to be the most inhibitory combination for strain V7, causing 100% inhibition in growth based on maximum population attained. *S. cremoris* at the 5.0% and 0.1% inoculum levels, was slightly less inhibitory to *L. monocytogenes* at 37°C, but it was slightly more inhibitory to *L. monocytogenes* at the 1.0% inoculum level than was *S. lactis*. In general, *S. lactis* reduced the pH of fermented milks more than did *S. cremoris*. The population of *L. monocytogenes* began to decrease before 15 hours in only one test combination, which was use of a 5.0% inoculum of *S. cremoris* and 30°C incubation. In most instances, growth of the pathogen appeared to be completely inhibited when the pH dropped below 4.75.

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Streptococcus thermophilus and Lactobacillus bulgaricus:

Behavior of *L. monocytogenes* in skim milk and in yogurt mix during fermentation with thermophilic lactic starters was determined. Sterile skim milk was inoculated with ca. 10^3 *L. monocytogenes* cells/ml and with 5.0, 1.0, or 0.1% of a milk culture of *Streptococcus thermophilus, Lactobacillus bulgaricus*, or a mixture of the two species. The milk was incubated at 37 or 42°C for 15 hours, followed by refrigeration at 4°C. Yogurt mix was inoculated with ca. 5×10^3 *L. monocytogenes* cells/ml of mix and then was incubated at 45°C for 5 hours, followed by refrigeration at 4°C. *L. monocytogenes* survived the 15-hour fermentation with *S. thermophilus* in all combinations of level of inoculum and temperature of incubation, but inhibition of growth ranged from 96 to 100%. When incubated with *L. bulgaricus, L. monocytogenes* survived only between 9 and 15 hours of incubation; a decrease in pH to below 4.0 was accompanied by rapid death of the pathogen. The combination of the two species was more inhibitory to *L. monocytogenes* than was *S. thermophilus* alone but less inhibitory than was *L. bulgaricus* alone. In yogurt mix, *L. monocytogenes* grew during the fermentation and increased in number by about one order of magnitude.

Fate of *L. monocytogenes* in fermented milks.

L. monocytogenes added to milk before fermentation:

Skim milks containing *L. monocytogenes* were fermented at 21, 30, 37, or 42°C of 15 hours with *S. lactis, S. cremoris, S. thermophilus, L. bulgaricus*, or *L. bulgaricus* plus *S. thermophilus*. Inocula were 5.0, 1.0, 0.5, or 0.1%. Yogurt mix was inoculated with *L. monocytogenes* and fermented at 45°C for 5 hours. Cultured skim milks and yogurt were stored at 4°C and sampled weekly to monitor survival of *L. monocytogenes* and the pH. Yogurt was sampled ca. every 3 days for enumeration of *L. monocytogenes* and determination of pH. Results show that *L. monocytogenes* survived longest in skim milks fermented with *S. thermophilus*, ranging from 4 weeks in skim milk fermented at 42°C with a 5.0% inoculum to 37 weeks in skim milk fermented at 37°C with a 1.0% inoculum. When skim milks were fermented with *S. lactis, L. monocytogenes* survived from 2 weeks (5.0% inoculum, 30°C incubation) to more than 13 weeks (0.1% inoculum, 21°C incubation). *L. monocytogenes* survived from 4 to 13 weeks in milk fermented with *S. cremoris*. *L. bulgaricus* was the most detrimental to *L. monocytogenes;* the pathogen survived only 3 days to 1 week in the skim milk fermented at 37°C with 0.1% inoculum. Survival of *L. monocytogenes* in milk fermented with LBST culture (combination of *L. bulgaricus* and *S. thermophilus)* ranged from 1-12 weeks in those skim milks in which the organism survived the fermentation process. *L. monocytogenes* survived yogurt manufacture and survived from 1-12 days during refrigerated storage of the product.

L. monocytogenes added to finished products:

The ability of *L. monocytogenes* to survive in cultured buttermilk, plain yogurt, or vanilla-flavored yogurt was examined. Buttermilk was inoculated with ca. 10^3 cells of *L. monocytogenes* strains V7, Scott A, Ohio or California/ml and stored at 4°C. Yogurts were inoculated with 10^4 to 10^5 cells of strains Ohio or California/ml and stored at 4°C. Numbers of *L. monocytogenes* in products were determined at intervals using McBride Listeria Agar. Strains V7, Scott A, Ohio, and California survived in buttermilk for 26, 21, 26, and 18 days, respectively. Strains Ohio and California survived 20 to 27 days in plain yogurt and 13 to 27 days in vanilla-flavored yogurt. Of the stains tested, strain California was least hardy in the fermented milk products.

Behavior of *L. monocytogenes* during manufacture and storage of butter.

Cream (32-43% milkfat) was pasteurized and then inoculated to contain $1.0x10^4$ to $1.8x10^5$ CFU of *L. monocytogenes* (strain Scott A)/g. Butter (79.5-81% milkfat), made from the contaminated cream, contained 2.6-7.9% of the numbers of *L. monocytogenes* initially in cream. Contaminated butter was stored at 13, 4-6, or -18°C for up to 70 days. Numbers of *L. monocytogenes* increased 1.9 and 2.7 orders of magnitude in butter stored at 4-6 and 13°C, with maximum populations attained after 49 and 42 days of storage, respectively. This was followed by slight decreases in numbers, but populations in excess of 10^4-10^5/g remained in butter after 70 days of storage. Numbers of *L. monocytogenes* decreased slightly in butter stored at the sub-freezing temperature, but a population of ca. 10^3/g remained after 70 days of frozen storage.

Testing cheese and fate of *L. monocytogenes* in Colby cheese.

Testing cheese for *L. monocytogenes:*

Method of homogenization (Waring blender vs. Stomacher), type of diluent [Tryptose Broth (TB) vs. aqueous 2% trisodium citrate (CS)], and temperature of diluent (20 vs. 40°C) were compared for recovery of *L. monocytogenes* from freshly made and ripened Colby cheese. Using direct plating on McBride Listeria Agar, significantly higher numbers of *L. monocytogenes* were recovered when cheese samples were (a) homogenized for 2 minutes with the blender rather than the stomacher ($p<0.01$), (b) diluted in CS rather than TB ($p<0.01$), and (c) diluted in diluents at 40 rather than 20°C ($p<0.05$). Based on these results, a new diluent/enrichment medium was developed by adding 2% trisodium citrate to TB (TBC). Despite superior results from using the blender, biosafety concerns led to use of the stomacher for homogenization of cheese samples; hence, the stomaching time was increased to 3 minutes. Results obtained by direct plating, indicated that recovery of *L. monocytogenes* from Colby cheese and from curd samples taken during manufacture of Brick cheese increased when samples were diluted 1:10 in TBC at 45°C and stomached for 3 minutes, as compared to similarly treated samples diluted in TB at 25°C. A similar comparison of both diluents for recovery of *L. monocytogenes* from cold-pack cheese food yielded bacterial counts which were not significantly different. Recovery of *Listeria* from cold-enriched (at 4°C for up to 8 weeks) samples of Colby cheese and cold-pack cheese food was generally similar for samples homogenized in TBC or TB.

Fate of L. monocytogenes in Colby cheese.

Colby cheese was made from pasteurized whole milk to which *L. monocytogenes* (strain V7 or California) was added. Cheese was stored for 140 days at 4°C. Numbers of *L. monocytogenes* in newly made cheese were 1.27 orders of magnitude (average) higher than in milk from which cheese was made. This indicates that the bacterium did not grow to any appreciable extent during the manufacture of cheese. A minor portion (2.4%) of the population of *Listeria* in the cheese vat escaped in the whey and the rest was entrapped in the curd. Early during storage, numbers of *Listeria* in the cheese remained relatively constant for a time which depended on the strain used. Numbers of *Listeria* in cheese decreased steadily thereafter at a rate that depended mainly on composition of the cheese. After 140 days of storage, higher numbers of *Listeria* remained in cheese when (a) a higher rather than lower concentration of the microorganism was present in milk, (b) cheese with a higher rather than lower moisture content was produced, and (c) strain V7 rather than California was added to milk.

Inactivation and Injury of *L. monocytogenes*.

Cells of *L. monocytogenes* strain V7 suspended in reconstituted nonfat dry milk were placed in capillary tubes and heated at 50, 55, 60, 65, 70, and 75°C. Heated cells were plated on Tryptose Agar with and without 5.5% added sodium chloride. The pathogen had D-values of 1900, 270, 57, 6.0, 1.4, and 0.46 seconds, respectively, at the aforementioned temperatures when heated cells were plated on Tryptose Agar. The D'-value (decimal reduction time of injured cells as determined with the salt-containing medium) were 1600, 170, 36, 4.7, 1.2, and 0.46 seconds, respectively. Injury was most pronounced when heat treatment was at the lower temperatures, e.g., 55-65°C. At 75°C, it was difficult to distinguish between injured and dead cells, suggesting that heat injury of *L. monocytogenes* may be of greater concern when mild rather than high heat treatments are used to process foods. Our results indicate that pasteurization of milk as defined by FDA should inactivate freely suspended cells of *L. monocytogenes*.

The population of *L. monocytogenes* in a minimal medium (lacking a nitrogen source) at pH 5.5 decreased logarithmically during incubation of the culture. Rate of death of the pathogen, indicated by D-values, was greatly affected by changes in the temperature of incubation (4-35°C), but to a much lesser extent by the presence of benzoic acid (3000 ppm). *L. monocytogenes* had a faster rate of death at higher rather than lower temperatures of incubation, within the range we studied. Injury of *L. monocytogenes* during incubation in the minimal medium was equally detectable on Tryptose Agar containing 6% salt and McBride Listeria Agar containing 5 g of lithium chloride/L. A greater degree of injury was detected at the lower temperatures of incubation; the presence of benzoic acid (3000 ppm) did not appear to affect the extent of injury.

Impact of the Research:

Data gathered in this research have illuminated the behavior of *L. monocytogenes* during the manufacture and subsequent storage of a variety of dairy foods. The tenacity of *L. monocytogenes* is clearly evident. The susceptibility of *L. monocytogenes* to chlorine, UV-radiation, and heat is also evident. Use of this information should help processors of dairy foods to more clearly understand the nature of the problems that can be caused by *L. monocytogenes*, and then to exercise the appropriate precautions needed to produce *Listeria*-free products.

Project title:

Utilization of minerals in dairy products.

Personnel:

J.L. Greger, Professor, Nutritional Sciences Department; A. Behling, postdoctoral research associate; and C. Gutkowski, research specialist.

Funding:

Wisconsin Milk Marketing Board, National Dairy Council, National Cancer Institute, National Dairy Promotion and Research Board.

A project entitled "Sodium, potassium, calcium, magnesium, and chloride utilization by human subject" is being completed in July 1988. We have begun a project entitled "Evaluation of effects of lactose-hydrolyzed milk, yogurt, and calcium supplements on mineral metabolism of rats." Both projects were funded by Wisconsin Milk Marketing Board in association with the National Dairy Council. A National Cancer Institute training grant in "Nutrition and Oncology" has also supported work on the relationship of dietary calcium and fat to colon cancer in rat models. We will begin work on a project entitled "New dairy foods with added fiber and added calcium" funded by the Center for Dairy Research with National Dairy Promotion and Research Board funds in July 1988.

Objectives:

1) To determine the relative bioavailability of calcium from dairy products and calcium supplements.

2) To assess the significance of lactose's effect on calcium, magnesium, and zinc utilization in various physiological circumstances.

3) To assess the importance of dairy products as nutritional sources of other minerals, i.e., magnesium, chloride, and potassium.

4) To evaluate the interaction of butterfat and calcium in a DMH-induced rat tumor model.

Results and Significance:

Bioavailability projects

We have evaluated the bioavailability of calcium from more than eleven different commercially-available calcium supplements or food additives and from dairy products, such as yogurt and milk, with human subjects and animal models. We will soon be evaluating the bioavailability of calcium from new dairy products being formulated by food scientists.

Generally we have found that the bioavailability of calcium from calcium supplements fortified with magnesium was reduced. However, calcium bioavailability from milk products did not appear to be reduced by the presence of magnesium. Moreover, the use of certain calcium supplements or food additives was associated with renal damage, lower retention of calcium in bone, and reduced utilization of other nutrients, such as iron, in animal models. We are currently evaluating the importance of the lactose, protein, and phosphorus content of milk products in determining the bioavailability of calcium and other minerals from milk. This information is needed to formulate new dairy products with the highest possible calcium bioavailability.

Health-Concerns Projects

Dairy products are significant sources of a number of nutrients, besides calcium. We found that the isocaloric substitution of three glasses of milk for juices in the diets of human subjects increased their intake of chloride, sodium, and potassium by 18%, 12%, and 26% respectively. Moreover, human subjects absorbed potassium more efficiently when their diets were supplemented with calcium.

McCarron and others have reported that the consumption of milk and/or dairy products was correlated to a decreased incidence of hypertension. They assumed that this reflected only the calcium content of milk. However, the ingestion other dietary factors, including potassium, have been reported to decrease blood pressure. Perhaps ingestion of additional

dairy products has been correlated to decreased incidence of hypertension in previous studies partially because milk is a good source of potassium and because the calcium in milk improves potassium absorption. This deserves further study with a variety of subjects and animal models.

Several investigators have reported that the ingestion of increased amounts of dietary fat was related to an increased incidence of colon cancer. They have generally fed either vegetable oils or beef tallow, not butterfat, in their studies to animal models. Other investigators have reported that ingestion of supplemental calcium was protective against colon cancer. We hypothesized that the protective effect of calcium on colon carcinogenesis was partially due to calcium binding to fats within the gut and that calcium would bind the short-chain saturated fatty acids in butterfat more efficiently than other fatty acids. Thus the combination of fat type and calcium in certain dairy products may be fortuitous. To test these hypotheses, we conducted a 224-day study in rats in which carcinogenesis was induced by repeated injections with 1,2 dimethylhydrazine. Rats were fed four diets that contained 5 or 20% butterfat and 3 or 12 mg Ca/g diet. Food intake of animals were controlled so that energy intake of rats fed the various diets did not differ. We have not analyzed that data and can not report results yet.

Impact of Research:

Consumers are interested in the health benefits of foods that they consume. Several companies, notably cereal companies, have successfully utilized consumer interest in nutrition to market their products. This marketing approach requires careful and responsible evaluation of an extensive research base that is constantly updated. Projects of this sort provide some of the needed data.

Project Title:

Extraction of cholesterol from milkfat.

Personnel:

Dr. R.L. Bradley, Jr., Professor, Food Science Department, and Dr. V.F. Krukonis, President, Phasex Corp., Lexington, MA.

Funding:

Wisconsin Milk Marketing Board.

Research Objectives:

The proposed research will investigate the removal of cholesterol from butter and the potential application of new products produced with low cholesterol and modified fat structure.

A supercritical fluid extraction procedure using carbon dioxide as the extraction vehicle can remove cholesterol from milkfat. Butter and anhydrous milkfat will be treated with carbon dioxide under supercritical conditions. Consistent with the product specifications, fractions will be collected having the desired final composition. Some fractions possess high concentrations of volatiles that exhibit a true butter-like aroma. These volatile concentrates have potential application in enhancing butter-like flavor in enumerable foods. Collection of low-melting fat fractions also having low cholesterol will allow development and production of soft-spread butters.

Impact of Research:

These new products that will be derived from supercritical separation of milkfat are needed by the dairy industry. Low cholesterol products that are competitively priced fill an obvious need particularly when the medical community gives significant attention to the nutrition of daily diets. Further, soft-spread butter is a needed competitor to soft margarines. Flavor bases developed even from cultured butter offer further evidence of the potential of supercritical separation to produce highly competitive dairy products.

Eighteen-month report:

Research has continued on the supercritical CO_2 extraction of cholesterol from butter and butteroil. Table 1 indicates the results of cholesterol analysis for fractions generated from ascending pressure operation of bench-top supercritical equipment. Operating conditions are also shown.

Table 2 shows the selectivity of this extraction process compared to fatty acid distribution. The first and last fractions collected had about 0.5 g of butteroil extract. The fatty acid composition of each fraction shows a greater concentration of short-chain fatty acids in the first fractions compared to the last fractions. Higher molecular weight fatty acids were concentrated in the last fractions. Further evaluation of these results and some additional research may elucidate the mechanism of distribution of fatty acids on glycerol.

Scale-up information is being determined to allow projection of operation costs with greater reliability. In the annual report, distribution co-efficient and selectivities were calculated. The distribution co-efficient influences the solvent-to-feed ratio in the separation column. Recently, a computer program was developed that can accurately calculate the solvent-to-feed ratio and column design. The data for the program is based on material balances, equilibrium relationships that are functions of the separation, and levels of cholesterol in the extracted product and the cholesterol-rich fraction.

During the site visit by Dr. Joe O'Donnell, the cholesterol removal at 75% was suggested by him. This level might be acceptable for "cholesterol-free" designation based on the FDA guidelines of 2 mg cholesterol per serving (proposed in the Fed. Register). Since the inception of this project, the 75% and 90% removal level has been our objective. Based on these levels, the number of equilibrium stages and the solvent-to-feed ratio for a select number of extraction tests have been calculated; these data are in Table 3.

In previous reports, the distribution co-efficient (DC) and the solvent-to-feed ratio (R) have been defined. The most important information from the economic view is given in the last two columns of data in Table 3. These data show the number of total plates for the extraction. In fact most numbers indicate that the extraction vessels will have less than 20 plates with both 75% and 90% extraction efficiencies. The chemical process industries consider that vessels with 10-20 plates are quite small.

With the development of the computer program, the effect of such parameters as pressure and temperature can readily be determined. For example, at a constant temperature, raising the pressure lowers the solvent-to-feed ratio. This is an important consideration for calculating the cost of recycling CO_2. This computer program will be an important tool as we approach the final phases of this project when operating costs at optimal operating conditions will be computed with statistical confidence.

Table 1. Results of cholesterol extraction from butter and butteroil for fractions generated from ascending pressure operation of bench-top supercritical equipment.

Fraction	Pressure	Std. liters CO_2	Weight, g	Cholesterol mg/g
WM26, 80°, Butter				
1	2300	400	0.50	6.54
2	2500	2000	1.02	7.67
3	2800	400	0.35	3.08
4	3050	200	0.36	1.50
5	6000	300	2.29	0.37
Control			6.00	
WM27, 80°, Butter Oil				
1	2200	400	0.07	11.89
2	2500	2100	0.72	8.36
3	2800	400	0.23	6.19
4	3050	300	0.17	5.85
5	6000	200	4.50	1.46
Control			5.98	
WM29, 80°, Butter				
1	1400	80	0.43	nd
2	2100	3000	0.38	3.18
3	2800	400	0.36	6.79
4	6000	200	4.38	1.82
Control			6.05	2.03
WM30, 80°C, Butter				
1	1400	200	0.62	nd
2	2200	3000	0.49	13.85
3	3000	400	0.98	7.59
4	6000	180	4.09	1.25
Control			6.12	2.03
WM31, 80°C, Butter				
1	1350	200	0.53	nd
2	2250	2100	0.86	5.90
3	2700	250	0.69	2.38
4	6000	210	2.72	0.23
Control			6.05	2.03
WM32, 60°C, Butter				
1	1200	400	1.21	0.00
2	2100	1380	1.08	7.64
3	2100	1620	0.87	8.59
4	2700	250	0.89	4.08
5	6000	400	7.53	1.36
Control			12.00	2.02

Table 1. Continued.

WM33, 60°C, Butter				
1	1100	600	0.93	0.00
2	2200	1200	2.01	6.25
3	2200	1200	1.20	7.13
4	2700	250	1.03	3.41
5	6000	500	6.36	0.82
Control			12.03	2.04
WM35, 60°C, Butter				
1	1200	700	0.76	0.00
2	2000	1710	0.84	8.20
3	2000	2790	0.62	10.51
4	6000	400	7.40	1.66
Control			12.05	2.00
WM36, 60°C, Butter				
1	1900	1500	1.77	9.12
2	1900	3000	0.61	lost
3	6000	400	8.35	2.20
Control			12.06	2.04
WM39, 60°C, Butter Oil				
1	2000	3070	0.50	10.21
2	6000	400	10.69	1.92
Control			12.00	2.34
WM41, 60°C, Cultured Butter				
1	2000	100	0.47	NA
2	2000	1520	1.25	9.01
3	2000	1480	0.81	10.74
4	6000	400	9.04	2.21
Control			12.03	2.34
WM42, 40°C, Butter Oil				
1	1450	1100	0.10	4.25
2	1850	1150	2.54	4.19
3	6000	600	8.91	1.88
Control			12.17	2.36
WM43, 40°, Butter Oil				
1	1700	1050	0.66	1.81
2	6000	400	8.86	2.07
Control			12.22	2.26

Table 2. Butteroil fatty acid profile (mole %).

frac	C4:0	C6:0	C8:0	C10:0	C12:0	C14:1	C14:1	C15:0	C16:1	C16:0	C17:0	C18:1	C18:0
1	16.63	6.53	3.67	6.16	5.76	0.77	10.40	1.11	1.45	22.45	0.30	14.86	7.91
2	16.00	5.80	2.87	4.97	4.90	0.79	9.90	1.13	1.53	25.93	0.30	16.81	9.07
3	16.67	5.80	2.64	4.54	4.48	0.76	9.44	1.12	1.54	25.96	0.31	17.97	9.38
4	15.97	5.67	2.42	4.13	4.18	0.75	9.14	1.10	1.55	26.36	0.36	18.27	10.10
5	14.29	5.75	2.47	4.27	4.14	0.77	8.83	1.06	1.48	25.74	0.30	18.66	12.19
6	15.83	5.88	2.28	4.02	3.90	0.77	8.64	1.07	1.49	26.01	0.37	19.36	10.70
7	17.23	5.65	2.19	3.77	3.83	0.75	8.59	1.05	1.51	25.50	0.35	18.45	11.15
8	12.79	5.20	2.11	3.69	3.49	0.76	8.20	1.04	1.60	27.13	0.37	21.58	12.02
9	11.31	4.93	2.13	3.76	3.40	0.78	7.84	1.01	1.58	26.83	0.37	22.91	13.15
10	7.97	4.44	2.20	4.00	3.51	0.84	7.87	1.01	1.63	26.67	0.41	24.93	14.51
11	6.31	3.80	1.94	3.86	3.53	0.89	7.77	1.00	1.65	26.44	0.41	26.77	15.62
12	3.29	2.60	1.60	3.65	3.65	1.00	8.08-	1.02	1.77	26.67	0.47	29.22	16.96
13	1.29	1.52	1.16	3.10	3.55	1.05	8.31	1.05	1.91	27.05	0.51	31.79	17.68
14	0.00	0.60	0.63	2.10	2.93	1.00	7.98	1.06	2.01	27.33	0.54	35.20	18.61
15	0.00	0.03	0.08	0.56	1.18	0.54	4.86	0.78	1.63	24.18	0.53	40.99	24.63
Cont	8.36	3.48	1.61	3.20	3.35	0.73	8.04	0.98	1.59	26.57	0.43	26.27	15.39

Table 3. Initial extractor design parameters.

Test	DC	Selectivity	Pressure psi	Temperature °C	R	Plates (75%)	Plates (90%)
32	0.0014	3.89	2100	60	450	7	9
33	0.0024	2.51	2200	60	360	10	13
22	0.0007	1.75	2350	65	420	14	19
12	0.0026	1.91	2350	65	140	13	17
24	0.0018	1.59	2500	60	420	19	24
35	0.0008	3.02	2000	60	675	9	11
11	0.0056	2.65	2850	80	85	10	12
13	0.0027	1.57	2500	80	150	17	23
14	0.0011	2.63	2500	80	450	10	13
21	0.0040	5.73	2300	80	120	6	8
26	0.0019	4.57	2500	80	600	7	9

Project Title:

An economic assessment of the production from whey of acetic acid, acetone, butanol, ethanol, 2, 3-butanediol, gycerol, isopropanol, propylene glycol, and methyl ethyl ketone.

Personnel:

D. F. Rudd, Professor, Chemical Engineering Department, and A. A. Trevino, and A. Jimenez, graduate students, Chemical Engineering Department.

Funding:

Wisconsin Milk Marketing Board.

Objectives:

Several chemicals currently being produced and marketed by the petrochemicals industry were examined by economic analysis for possible production from whey. By using eight fermentation-based processes, the economics of utilizing whey through the manufacture of these commodity chemicals were evaluated.

Two situations are important. If the chemical is currently purchased for use within the food industry, it may be an advantage to manufacture it from byproducts (such as whey). That advantage does not exist if the chemical is manufactured within the food industry for sale on the outside markets. This research sets the pattern for further studies on the economic value of converting whey to marketable chemicals through biological and chemical methods.

Background:

It has been shown that a market can be developed for whey as a feedstock to produce commodity chemicals and fuels, including the conversion of the lactose into ethyl alcohol, methane, acetic acid, acetone, butanol, butylacetate, polyols, surfactants, and other chemical products. Many of these conversions have been demonstrated commercially and others have been suggested only in laboratory studies.

Unfortunately, whey must compete as a feedstock with other biomass sources, petroleum, coal, and natural gas liquids. Historically, for most commodity chemicals, petrochemical production is the most economical production. Thus, there is the strong chance that a market developed to utilize whey would be better served by the petrochemical industries.

To assess the impact of producing chemical feedstock from whey consider that all of the unutilized whey in the U.S. were gathered into one site and converted into a commodity chemical such as acetone/butanol solvent mixture. The 25 billion pounds of whey would produce 250 million pounds of the solvent. The 1980 U.S. production of acetone/butanol by the petrochemicals industry was 2 billion pounds. A single production facility manufactures 150 million pounds per year. We conclude that all of the unutilized whey available in the United States, if converted to an acetone/butanol solvent, would meet only 12% of the demand. Two typical petrochemical facilities have more than the capacity to produce all the acetone/butanol that could be produced by whey. Under the most favorable conditions the production of commodity chemicals from whey will have an insignificant impact on the commodity chemicals industry. Therefore in subsequent studies, the production of commodity chemicals from whey will not influence the target prices of those commodity chemicals.

Approach:

The general plan is first to establish a target value for the product by comparing the reported market price with the actual cost of manufacture by the petrochemicals industry. Then the detailed costs of production of the chemical by fermentation of whey are estimated. The difference between the target price and the costs of manufacture is a cost margin which should be sufficiently large for economically promising products.

The most readily available target price of a chemical product is the reported sales price.

This is an indication of the price at which sales have been made and which are likely to be made in the near future. However, the reported sale price gives no indication of the margin of profits made by the seller. If large profit margins are being made, the sales price could easily be lowered to maintain markets. A less accessible target price is the cost of manufacture of the chemical by the petrochemical industry. This is normally not available. Our extensive studies of the petrochemicals industry include the estimation of the cost of manufacture of most commodity chemicals.

Eight fermentation processes were included in this preliminary assessment study. For each process, a brief summary of its markets and production capacity is given. The process data includes fermentation reaction data such as fermentation conditions, type of process, organism, product concentration, and productivity. This information is summarized in Table 1. The process economics data include the size of a competitive plant, the consumption of deproteinized whey, the estimated conversion costs and plant investment, and the product recovery investment and energy requirements. All processes are assumed to follow a facility to deproteinize whey and include an anaerobic digestion waste treatment system to further lower the BOD of the aqueous waste streams.

The experimental, pilot plant, and process economics data for each assessed process was organized and consolidated into a technology catalog. Each entry in the catalog identifies and estimates the most important cost elements for the process. This information will then allow the assessment of a given process under given and projected economic scenarios. In producing chemical feedstocks, fermentation processes compete mostly with petrochemical sources.

The most important factor in the production cost of fermentation-based chemical feedstocks is the cost of raw materials. This is true of petrochemical processes as well. Since reaction (fermentation) rates are relatively slow, conversion costs are also high in fermentation processes. The process energy requirements of the fermentation sections are relatively low, but the product recovery section has high requirements due to the diluted solutions obtained in fermentation. This dilution is rather pervasive and results in the carrying of water throughout the plant, increasing the size of the necessary equipment.

The costs of fermentation can be estimated through a relationship linking the conversion cost to the product concentration and fermentation residence time data. One such formula is:

$$C = 0.167/P + 0.02B$$

where C is the conversion costs in 1987 $/lb and P is the productivity of the fermentation in l/g/h (Cockrem, 1986). This relationship is based on more detailed cost charts (Busche, 1986). The conversion cost includes all costs associated with the cost of manufacturing and the costs of sales, excluding feedstock costs and product recovery costs. It includes a pretax ROI of 30% on the permanent capital.

The product recovery cost can be divided into two parts: the investment related costs and the utilities cost. From the data reported on fermentation process economics, it is evident that the investment-related costs are relatively small when compared with the other costs in the process. A simple estimate of these costs is to assign a low, medium, and high cost to the product recovery section. The corresponding value of these three investment levels are 0.10, 0.20, and 0.30 $/lb product, which, translated into depreciation to be applied to the cost represent 0.01, 0.02, and 0.03 $/lb product respectively.

The effluent from the fermentation processes still exhibits a COD content of 7,000 mg/l. It is possible to install an anaerobic digestion facility to further process this waste and generate energy that can be used to offset part of the product recovery energy cost. The generation of fuel by anaerobic digestion is 250 BTU/lb of DPMS, assuming 80% of the maximum theoreti-

cal yield. Since the waste has lost nearly 90% of its BOD, it can only generate some 43 BTU/lb whey fed. The anaerobic digestion consumes some 0.003 KWH/lb whey, equivalent to some 30 BTU/lb whey. The net energy generation from the waste treatment option is then 13 BTU/lb whey fed.

The investment cost of the waste treatment facility is $0.0004/lb whey fed to the process. This amount can represent 10% of the total capital requirements, but its impact on the product cost is small, of the order of $0.002/lb product. This waste treatment facility is assumed to be included in the processes under consideration.

Results:

The potential attractiveness of each process is measured by its margin, i.e., the difference between the target price and estimated production cost. Table 2 summarizes the economic assessment for the most favorable of the whey processing alternatives. That situation occurs when the sales price is used as the target price. Only two processes pass this test: ethanol and glycerol.

Matching the petrochemical production costs is a more stringent test. The petrochemical cost is

Table 1. Biological Processes (process data summary).

Main Fermentation Product (s)	Process Code	Product Concentration (g/l)	Fermentation Time (h)	Productivity (g/l/h)	References
Acetic Acid	AcOOH	20	36	.55	Busche, Ghose, Ng.
ABE	ABE-1	24	40-48	.50-.60	Hobman, Lenz, Marlatt, Ng.
ABE	ABE-2	18	120	.15	Linden, Maddox, Phillips.
2,3 Butanediol	MEK	30	10	3	Dronamaraju, Kautola, Ng, Speckman, Underkofler (b), Voloch.
Ethanol	ETOH	70	8	8.80	Barry, Bailey, Collins, Demott, Izaquirre, Katzen, Maiorella, Moulin, Reed, Reesen, Rogosa, SERI, World Bank, Sandbach, Zadow.
Glycerol	GLYCER	20-30	48-60	0.33-0.5	Eveleigh, Underkofler.
Isopropanol	IPA	20	40-80	0.25-0.5	Ng, Peppler.
Propylene Glycol	PG	7	85	.82	Cameron.

Fuel Production

Product (s)	(BTU/lb DPMS)	(BTU/lb Waste)	References
Fuel Methane	250	43	Linden, Reesen, Hobman, Reesen.

A = Acetone
B = n-Butanol
E = Ethanol
IPA = Isopropanol
EtOH = Ethanol
MEK = Methyl ethyl ketone
DPMS = Deproteinized Milk Serum

calculated for acetic acid in Table 4. None of the processes exhibits a positive margin when this test is used, as shown in Table 3.

Improvements in fermentation productivity P can lead to reduced production costs. The effects of productivity changes on the economic competitiveness of a technology can be determined easily by the recalculation of the entries in Table 2. An important calculation is to set the production costs equal to the target price and calculate the productivity necessary to achieve that cost. This is shown in Table 4 for the processes with negative cost margins in Table 2. Also shown in Table 4 is the reported productivity. This shows the improvement in productivity needed to achieve economic feasibility.

Table 2. Economic assessment of fermentation processes market price as target.

	Process							
	AcOOH	ABE-1	ABE-2	ETOH	GLYCER	IPA	PG	MEK
Plant Size (Mlb/yr)	250	300	100	330	50	100	100	24
Annual Whey Req. (Mlb)	8,000	16,800	7,400	17,490	3,650	7,200	10,000	3,480
Target Price (Market,$/lb)	.25	.30(2)	.32(3)	.24	.89	.26(4)	.40	.24
Costs (1987 $/lb):								
Conversion	.33	.31	1.14	.05	.45	.45	.23	.08 (5)
Product Recovery								
- investment related	.029	.014	.011	.009	.027	.018	.009	.012
- utilities	.033	.009	.009	.030	.189	.030	.078	.054
- Total Prod. Recovery	.06	.02	.02	.04	.22	.05	.09	.07
Conversion + Recovery	.39	.33	1.16	.09	.67	.50	.32	.15
Whey (freight) (1)	.10	.17	.23	.16	.23	.22	.31	.45
Margin Before Credits	-.24	-.21	-1.07	-.01	-.00	-.46	-.23	-.36
Dry Slops Credit:	.05	.08	.11	.08	.11	.11	.15	.21
Margin After Credits	-.19	-.12	-.96	.06	.10	-.36	-.08	-.14

Fuel Cost: $3.00/MBTU

(1) $3.10/1000 lb-100 miles.
(2) Avg. = 26%(0.24)+65%(0.34)+9%(0.20).
(3) Avg. = 8.3%(0.24)+83%(0.34)+8.3%(0.20).
(4) Avg. = 27%(0.21)+60%(0.34)+13%(0.20).
(5) Dronamraju.

Table 3. Economic assessment of fermentation-processes petrochemical production cost as target price.

	Process							
	AcOOH	ABE-1	ABE-2	ETOH	GLYCER	IPA	PB	MEK
Plant Size (Mlb/yr)	250	300	100	330	50	100	100	24
Annual Whey Req. (Mlb)	8,000	16,800	7,400	17,490	3,650	7,200	10,000	3,480
Target Price ($/lb)	.19	.24(2)	.26(3)	.17	.62	.22(4)	.32	.20
Costs (1987 $/lb):								
Conversion	.33	.31	1.14	.05	.45	.45	.23	.08 (5)
Product Recovery								
- investment related	.029	.014	.011	.009	.027	.018	.009	.012
- utilities	.033	.009	.009	.030	.189	.030	.078	.054
- Total Prod. Recovery	.06	.02	.02	.04	.22	.05	.09	.07
Conversion + Recovery	.39	.33	1.16	.09	.67	.50	.32	.15
Whey (freight) (1)	.10	.17	.23	.16	.23	.22	.31	.45
Margin Before Credits	-.30	-.27	-1.13	-.09	-.27	-.50	-.31	-.39
Dry Slops Credit:	.05	.08	.11	.08	.11	.11	.15	.21
Margin After Credits	-.26	-.18	-1.02	-.01	-.16	-.39	-.16	-.18

Fuel Cost: $3.00/MBTU

(1) $3.10/1000 lb-100 miles.
(2) Avg. = 26%(0.24)+65%(0.34)+9%(0.20).
(3) Avg. = 8.3%(0.24)+83%(0.34)+8.3%(0.20).
(4) Avg. = 27%(0.21)+60%(0.34)+13%(0.20).
(5) Dronamraju.

Table 4. Target productivity to bring margin after credits to zero (1).

	Process							
	AcOOH	ABE-1	ABE-2	ETOH	GLYCER	IPA	PG	MEK
Current Productivity (g/l/h)	.55	.60	.15	8.80	.40	.40	.82	3 (4)
Target Price ($/lb):								
Market	.25	.30(1)	.32(2)	.24	.89	.26(3)	.40	.24
Petrochem. Cost	.19	.24(1)	.26(2)	.17	.62	.22(3)	.32	.20
Target Productivity Improvement (% over Current Productivity):								
Market Price	178	81	629	none	none	574	66	Infinite
Petrochem. Cost	548	195	1154	65	64	1719	375	Infinite

(1) Avg. = 26%(0.24)+65%(0.34)+9%(0.20).
(2) Avg. = 8.3%(0.24)+83%(0.34)+8.3%(0.20).
(3) Avg. = 27%(0.21)+60%(0.34)+13%(0.20).
(4) 2,3 Butanediol.

Project Title:

Development of a technology base for specialty cheeses: Wisconsin-style Havarti (TM) and Sweet Swiss.

Personnel:

M.E. Johnson, Associate Scientist, CDR, B.A. Riesterer, Associate Researcher, CDR, N.F. Olson, Professor, Food Science Department, and J. D. Beyer, graduate student, Food Science Department.

Funding:

Center for Dairy Research and the Wisconsin Milk Marketing Board

Research Summary:

In the two reports that follow this summary, we describe the manufacture of two specialty cheeses. They are similar yet unique enough to distinguish them from their European counterparts after which they were modeled. Our cheeses; Creamy Havarti and Sweet Swiss, have been shown statistically to be as preferred as Danish Havarti and Norwegian Jarlsberg (TM).

Havarti can be made by cheesemakers who now make Brick cheese without any new equipment other than a room to cure the cheese for two weeks at 60°F. Other cheesemakers would need new hoops. Without specific and special curd handling equipment, the manufacture of Havarti can be labor intensive. Havarti is probably best suited for the small cheesemaking companies.

Sweet Swiss can be made by Gouda, Edam, Swiss, and Baby Swiss cheesemakers without any new equipment or special curing rooms. A large amount of diluted whey would result from the manufacture of both Havarti and Sweet Swiss and could be an economic liability to the cheesemaker.

For the Creamy Havarti and Danish Havarti there are no similar cheeses to compare to. However, Sweet Swiss and Jarlsberg are similar to Iowa-style or Baby Swiss but are clearly different in taste. Sensory evaluation showed that Jarlsberg was preferred over Baby Swiss. Our Sweet Swiss cheese which lacks strong propionic acid flavor does offer the consumer an alternative to Baby Swiss.

In our investigations we found the following two points worthy of mention:

1) There was a wide variation in openness, color, taste, and quality in commercial samples of Havarti. We had to postpone or even cancel sensory trials until a quality Danish Havarti was procured. Commercial Jarlsberg samples showed much less variation in overall quality (all good quality) but exhibited a wide variation in intensity of flavor. This made it difficult to determine the direction we should take in trying to duplicate flavor attributes. We felt a milder cheese was more typical and chose to produce a cheese with this flavor intensity. Commercial Baby Swiss samples also varied widely in quality and intensity of flavor.

2) Consumers appear to accept different tastes in similar cheese. Niches exist for a wide variety of tastes from mild to rather pungent or acid. It is probable that the small cheesemaker could fill those needs. Results of the taste panel indicate that cheeses do not have to be exact copies of an imported cheese to be accepted by the consumer.

<u>Recommendations for future related research</u>

1) It was the intent of this study to use only commercially available starter cultures. However, it was difficult to find the exact commercial culture blends that gave us the flavor characteristics that we desired. This clearly hampered the progress of this investigation. A cooperative venture should be established with companies to develop the starter cultures.

2) Projects such as this might benefit by having interested cheesemakers act as consultants to the project or act as active participants by providing a commercial setting for the scale-up manufacture. This type of cooperation could also yield more information on the economic viability of manufacturing the new cheese.

3) A plan of action should be developed by WMMB to market the new technology or product(s) produced in coordination with the research personnel.

When these cheeses are developed commercially, strict adherence to quality must be given high priority or they will probably not be accepted. It is likely there will be less tolerance for diversity in a new cheese than for variation in an established cheese. These new cheeses should also be clearly described as new and not characterized as Havarti-like or Jarlsberg-like. They should develop their own identity and name!

Project Title:

Development of a technology base for speciality cheeses: Sweet Swiss.

Personnel:

M. E. Johnson, Associate Scientist, CDR, B.A. Riesterer, Associate Researcher, N.F. Olson, Professor, Food Science Department, J.D. Beyer, graduate student, Food Science Department, and T. M. Blattner, U.W. Dairy Plant manager.

Funding:

Center for Dairy Research and the Wisconsin Milk Marketing Board.

Procedures:

Milk

Milk is standardized to 3.2-3.3% fat with an approximate casein to fat ratio of .75-.86. The cheese will have a fat on the dry basis between .47 and .50. Milk is pasteurized after standardization at 163°F/17.5 seconds. The Cheese Research Institute's cheese yield equation is given near the end of this report. Remember that the efficiency of fat and casein recovery varies between plants. These values determine the yield equation as well as the milk composition needed to obtain the desired cheese.

Culture

Several starter combinations have been tried but a commercially available BD culture plus *Propioni* bacterium produced the cheese flavor desired. A 1% inoculum was used. The culture was grown in 12.5% reconstituted nonfat dry milk for 16 hours, pH 5.0-5.1. *S. lactis* did not produce the desired cheese but produced a typical Iowa-style or Baby Swiss. *S.thermophilus, Lactobacillus helveticus,* and *Propioni* bacterium sp. produced cheese with a more Swiss character, not unlike Baby Swiss.

We have experienced problems with the BD culture. The cheese may develop small eyes (sweet holes) near the cheese surface or throughout the cheese on occasion. The cause for this defect is probably too much activity of the *Leuconostoc sp.* and *Streptococcus diacetylactis* in the culture. We will be evaluating the reduction of the amount of starter in future work. The problem may also be exacerbated by our "less than perfect" pressing operation in the small cheese vats we use at the Cheese Research Institute. It may be that the BD culture is not the best choice of culture, but undoubtedly this culture possesses the strain(s) that produces a

cheese similar to Jarlsberg. There is a possibility that at times the numbers or ratio of each of the 4 types of cultures in the BD blend were not optimal to produce the cheese we desired. Chemical analysis of the cheese indicates that the BD culture produces far more ethanol in the cheese than is found in Jarlsberg, indicating that it is not the culture used in Jarlsberg. However to repeat, the cheese made with the BD culture as judged by the sensory analytical laboratory showed no significant difference between Jarlsberg and Sweet Swiss. Isovaleric acid is also higher in our cheese and Baby Swiss than in Jarlsberg culture metabolism is probably responsible.

Color

Level and type of color used depends on shade and intensity desired. Yellow ice cream color is used at a rate of .25 oz. per 1000 lbs. of milk. Color is added 10 minutes prior to rennet addition and is diluted about 10-fold.

Rennet Addition

Milk is ripened at 90°F for 20 minutes prior to the addition of rennet. There is little pH or TA change in the milk. Single strength calf rennet is used at the rate of 2.3 oz. per 1000 lbs. of milk. Less rennet could be used. More rennet is not recommended. We have not tried $CaCl_2$ addition but addition would help to firm the curd.

Cutting

Thirty to 35 minutes after rennet addition the curd is cut with 1/4 inch knives. The curd is soft at cutting. Cutting the curd soft insures adequate whey expulsion.

Healing

The cut curds are allowed to sit for 5 minutes before gentle agitation. The soft curds will shatter if not allowed to heal. Excessive fat losses will also result if the curds are agitated too abruptly.

Whey Removal

Fifteen minutes after cutting, 40% of the original milk volume is removed as whey. Whey is removed by setting up a dam at one end of the cheese vat and allowing the curd to settle before whey removal. Less whey removal will increase the "Swiss" flavor of the cheese. The whey may have an approximate fat level of .25 to .35%. Without whey removal the cheese would not develop eyes properly and the flavor would not be as sweet. Care must be taken to prevent shattering of the soft curd. As an alternative procedure, whey could be removed once the curd has firmed sufficiently during cooking. Another alternative could be to drain more whey and add less water.

Cooking

Hot water (125-140°F) equal to 30% of the original volume of milk is slowly added back to the remaining curd/whey mixture until the cooking temperature of 105-106°F is reached. Steam may have to be added to the vat jacket to heat the curd whey mixture to 106°F. The temperature is increased from 90°F to 106°F over 30 minutes to prevent case hardening of the rather large curd particles. The curd will become much firmer. The curd/whey is stirred for 40-45 minutes at 106°F. Lower cook temperatures may increase moisture content of the cheese resulting in a too soft and pasty cheese.

Pressing

The curd and whey is pushed or pumped into a pressing hoop. The curd is allowed to settle beneath the whey. A screen is placed over the curd and weights are placed on top of the screen. Fifteen to 25 lbs./60 lbs. of curd (less than .1 lb./inch^2) is used. The curd is pressed under the whey for 25 minutes and then all the whey is drained from the vat. The curd is cut to fit the hoop with about 1 inch space between curd and the sides of the hoop. A pressure of about .5 lbs./inch^2 is applied for 1 hour and then increased to about 5 lbs./inch^2. Total press time is about 6 hours. The pH at drain is about 6.4-6.5.

Brining

Cheeses are placed in 23% brine at 40-45°F for 2 days. The pH at brining will be about 5.25. Eyes may not develop properly if pH is less.

Drying

Cheeses are allowed to dry (45-45°F). This step may not be necessary. It is used only as a precaution to prevent brine or whey leakage in the wrapped cheese. If cheeses have developed proper pH at drain and at hooping this step is not necessary. Dry only to get rid of brine on outside of cheese.

Curing

Cheeses are cured at 40-45°F for 7-10 days and then stored at 72-74°F until eyes form (about 2 weeks). Cheeses are stored at 45°F until consumed at 2 or 3 months of age.

Cheese Composition

Composition consists of 30-31% fat, 37-38% H_2O, 1.0 to 1.2% salt, pH at 1 month 5.4-5.6, and fat in the dry matter of 47-50%.

The Cheese Research Institute's cheese yield equation is:

$$\frac{(.92F + .96C)\ 1.12}{1-W}$$

.92 = fat recovery
.96 = casein recovery
1.12 = other solids recovery
w = decimal of moisture in cheese
F = % fat in milk
C = % casein in milk

Results of the descriptive panel on Jarlsberg cheese are shown in Table 1 and voluntary comments by the panelists are listed in Table 2. Color scores were significantly different among the three samples with the commercial sample being perceived as pale yellow in color. Lower color scores received by the experimental samples indicated that the samples were perceived to be creamy white. Scores for properties of the cheese eyes were not significantly different for the commercial product and experimental sample 11-12. Experimental sample 10-8 received higher scores indicating that the eyes of the cheese had dull, irregular surfaces.

Aroma intensity, flavor intensity, and sweetness/nuttiness flavor were not perceived to be significantly different for all three samples at a 95% confidence level. However, scores for sharpness of flavor and aftertaste indicated that the experimental samples had a sharper flavor and a more pronounced aftertaste than the commercial product.

A difference between the texture of the experimental samples and the commercial product was also noted. The experimental samples were scored as being softer and less chewy than the commercial product. Experimental sample 11-12 received the highest absolute score for overall preference, but the mean score was not significantly different from the other two samples.

Results of the descriptive sensory of analysis of aged cheese with holes is presented in Table 1, and voluntary comments by the panelists are listed in Table 2. Mean scores for the intensity of difference component showed that the score (2.39) for the Jarlsberg sample was established at the normalization value for the samples evaluated since it was the selected reference sample. Experimental sample 11123 received a mean score of 3.79 for the degree of difference attribute, which was a significantly higher score than that for the Jarlsberg, but it was scored closest to the Jarlsberg reference of the samples tested. Mean scores for experimental samples 11121 and Baby Swiss were identical, and were significantly higher than the scores for the other two samples. A mean score of 4.79 would indicate that a moderate degree of difference was perceived between the reference (Jarlsberg) and these two samples.

No statistically significant differences at a 95% confidence level were observed in mean scores for sharpness of flavor intensity. However, the Jarlsberg sample received a higher mean score than the other samples, 4.45, compared to 3.86,

3.71, and 3.77 for experimental sample 11121. Experimental sample 11123 and Baby Swiss, respectively, would indicate a directional difference towards a sharper flavor. Although no statistically significant differences were noted for aftertaste intensity, mean scores which were highest for Baby Swiss cheese show a directional difference towards a more pronounced aftertaste. In the voluntary panelist comments section (Table 2), experimental sample 11121 was described by some panelists as "bitter," although mean scores for sharpness flavor intensity or aftertaste intensity were not significantly different for this sample.

Mean scores for overall texture were significantly higher for experimental sample 11123 indicating a firmer texture. Overall texture was perceived to be less firm for the Baby Swiss cheese as indicated by significantly lower mean scores. Jarlsberg cheese and experimental sample 11121 had mean scores that were in between those received by the two other samples and not significantly different from either one.

The Jarlsberg cheese received significantly higher mean scores for overall preference indicating that this was the most preferred sample. Experimental sample 11123 received mean scores that were slightly lower than but not significantly different from those of Jarlsberg cheese. Mean scores for this sample also were not significantly different from those of experimental sample 11121 and Baby Swiss. However, absolute mean scores, which were higher for the Jarlsberg sample (4.63) and experimental sample 11123 (4.11), indicate that these samples were preferred over the Baby Swiss cheese (3.55) and the experimental sample 11121 (3.77). Voluntary panelist comments (Table 2) did not provide additional information about the samples.

Table 1. Summary of mean scores for the descriptive sensory analysis of aged cheese with eyes.

Samples	Intensity of Difference[1]	Sharpness of Flavor Intensity[2]	Aftertaste Intensity[3]	Overall Texture[4]	Overall Preference[5]
Jarlsberg	2.39[a]	4.45[a]	4.14[a]	4.02[a]	4.63[a]
Experimental 11121	4.67[b]	3.86[a]	4.00[a]	4.34[a]	3.77[b]
Experimental 11123	3.79[c]	3.71[a]	4.11[a]	5.15[b]	4.11[a,b]
Baby Swiss	4.67[a]	3.77[a]	4.55[a]	3.01[c]	3.55[b]
F value	S	NS	NS	S	S
LSD (5% level)	(.77)			(.51)	(.61)

[1]Scale: 1 = none to 7 = extreme relative to the selected reference sample of Jarlsberg cheese.
[2]Scale: 1 = not sharp or biting to 7 = very sharp or biting.
[3]Scale: 1 = no aftertaste to 7 = pronounced aftertaste.
[4]Scale: 1 = very soft to 7 = very firm.
[5]Scale: 1 = dislike extremely to 7 = like extremely.

[a,b,c] Mean scores with the same superscript are not significantly different at the 5% level.

S = significant at the 5% level; NS = not significant.

n = 33

Date of evaluation: February 26, 1988.

Table 2. Summary of voluntary panelists' comments on the descriptive sensory analysis of aged cheese with eyes.

Characteristics	Comments	Samples: Jarlsberg	Expt. 11121	Expt. 11123	Baby Swiss
FLAVOR					
	Good flavor	1			1
	Good - similar to reference	1			
	Intense flavor-but stale			1	
	Bitter		3		1
	Sharp		1		
	Too much propionic acid				1
	Somewhat dry		1		
TEXTURE					
	Crumbly		1	1	
	Rubber-like			1	
GENERAL					
	All were very good.	1	1	1	1

Date of evaluation: February 26, 1988.

Project Title:

Development of a technology base for specialty cheeses: Wisconsin-style Havarti (TM).

Personnel:

M.E. Johnson, Associate Scientist, CDR, B.A. Riesterer, Associate Researcher, CDR, N.F. Olson, Professor, Food Science Department, J.D. Beyer, graduate student Food Science Department, and T.M. Blattner, U.W. Dairy Plant manager.

Funding:

Center for Dairy Research and the Wisconsin Milk Marketing Board.

Procedures:

Milk

Milk is standardized to at least 5.05% fat with cold-separated cream or an approximate casein to fat ratio of .48. The cheese must have a fat on the dry basis of at least .60. Higher fat on the dry basis (>.61) is not desirable. These cheeses will be too soft. Milk is pasteurized after standardization at 163°F/17.5 seconds. Remember that efficiency of fat and protein recovery play a critical role in establishing casein to fat ratios in milk necessary to give the desired cheese. The cheese yield equation which can be used to determine the proper casein to fat ratio may vary between plants. The yield equation established for the U.W. dairy plant is near the end of this report.

Culture

A commercially available culture (BD-CH-N-01 from Chr. Hansens Lab., Milwaukee, WI) containing *Streptococcus lactis, Streptococcus cremoris, Streptococcus lactis* var. *diacetylactis,* and *Leuconostoc cremoris* is grown in heat-treated, 12.5% reconstituted non-fat dry milk at 72°F for 16 hours (pH 5.0-4.6). Commercial cultures containing only *S. cremoris* and *L. cremoris* have also been used. One percent culture is added to the milk at 90°F. We have tried the addition of 1.6% starter with similar results.

Color

Level and type of color used depends on shade and intensity desired. Ice cream color is used at a rate of .5 oz. per 1000 lbs. milk. Color is added 10 minutes prior to rennet addition and is diluted about 10-fold.

Rennet Addition

Milk is ripened at 90°F for 40 minutes prior to the addition of rennet. The pH is about 6.50 and the T.A. at about .18. Single strength calf rennet is used at the rate of 2.3 oz. per 1000 lbs. of milk. Less rennet could be used. More rennet is not recommended. $CaCl_2$ addition may be used to firm curd.

Cutting

Thirty to 35 minutes after rennet addition, the curd is cut with 3/8 inch knives. The curd is soft at cutting. Cutting the curd soft insures adequate whey expulsion.

Healing

The cut curds are allowed to sit for 15-20 minutes before agitation. The soft curds will shatter if not allowed to heal. Stir gently, excessive fat losses will also result if the curds are agitated too abruptly.

Whey Removal

Thirty minutes after cutting, 20-25% of the original milk volume is removed as whey. More whey removal will result in a less acidic flavor. The whey may have an approximate fat level of .5 to .8%. Without whey removal the cheese may have a too acidic flavor. Care must be taken to prevent shattering of the soft curd. As an alternate procedure whey could be removed once the curd has firmed sufficiently during cooking. Another alternative could be to drain more whey and add less water.

Cooking

Hot water (our water is 140°F, equal to the amount of whey removed) is slowly added back to the remaining curd/whey mixture until the cooking temperature of 104°-105°F is reached. Steam may have to be added to the vat jacket to heat the whey to 105°F. The temperature is increased from 90°F to 105°F over 30 minutes to prevent case hardening of the rather large curd particles. The curd is cooked at 105°F for 1 hour. The curd will become much firmer and is easily pumped into hoops without shattering. Lower cooking temperatures will increase moisture content of the cheese resulting in pasty cheese.

Hooping

Whey is drained to about curd level and then the curd/whey mixture is pumped into standard brick hoops (6 x 5 x 10 inches). Dry curd could be bucketed into hoops. The whey will have .2 to .3% fat. Cheese will have more and larger openings if more whey is drained before hooping. Draining all the whey is preferred thus permitting the more desirable larger openings in the cheese. The pH of the curd should be about 6.1 - 6.2.

Turning

Once filled, the hoops are turned to prevent uneven openness in the cheese. Turning continues every 20 minutes for the first hour and then once every hour until the curd pH is 5.3 - 5.4 (about 3 to 4 hours after hooping).

Brining

The 7-10 lb. cheeses are placed in 23% brine at 40-45°F for 1 day.

Drying

Cheeses are allowed to dry (40-45°F) for 2 to 4 days. This step may not be necessary. It is used only as a precaution to prevent brine or whey leakage in the wrapped cheese. If cheeses have developed proper pH at drain and at hooping this step is not necessary. Dry only to get rid of brine on the outside of the cheese.

Curing

Cheeses are cured at 60°F for 2 weeks and then stored at 40-45°F until ready for consumption at 8-12 weeks.

Cheese Composition

Composition is described as 37 to 38% fat, 36.5 to 37.5% H_2O, 1.5 to 1.7% salt, at 4 days 5.1 to 5.2, and fat in the dry matter of .60 to .61. Yield is approximately 12 lbs. per 100 lbs. of milk. Although the cheese may appear firm initially, by 8 weeks the body will have softened. Higher moisture cheeses will become excessively soft and pasty and not easily sliced. Higher moisture cheeses tend to also have a whey taint flavor. This defect has been traced to inadequate whey expulsion and whey drainage.

The cheese yield equation established at the U.W. dairy plant is:

$$\frac{(.91F + .96C)1.09}{1-w}$$

F= fat in milk
C= casein in milk
.91= fat recovery
.96= casein recovery
1.09= other solids factor
w= decimal of % moisture in cheese.

Sensory Analysis

Descriptive and consumer preference panels were run on the cheese and directly compared Wisconsin Havarti to Danish Havarti. The results are shown in Table 1 and Table 2. The descriptive taste panel (32 people) is a direct comparison of several attributes and is useful in detecting differences between the cheeses. The flavor attributes are similar, however the body of our cheese is firmer than the Danish Havarti. This would also account for the differences in stickiness and creaminess. The Danish Havarti

might be older than our 8-week-old cheese. The overall preference of the cheeses showed no significant difference.

The consumer preference panel (208 people) showed that both cheeses were well liked. The Danish Havarti had more responses in the like very much category; however, the mean scores were not significantly different.

Conclusion:

The manufacturing schedule for creamy Havarti style cheese presented in this paper produced a cheese closely resembling Danish Havarti and equally acceptable to consumers. The method of manufacture did not involve exotic cultures and could easily be manufactured in most existing cheese plants.

The cheese described here is not Danish Havarti and cheese connoisseurs would likely readily separate the two cheeses. Consumer preference panels seem to indicate that a cheesemaker does not have to make exact copies of an existing European variety but can produce their own unique specialty cheese.

Note: Acceptability of unwashed and washed curd Havarti-Style cheese is compared in Table 3. Although the unwashed curd cheese is not as well liked as the washed curd, we feel such a cheese warrants some attention.

Table 1. Summary of mean scores for the descriptive sensory analysis of Havarti cheese.

	Sample Attributes						
Samples	Flavor Intensity[1]	Sharpness of Flavor[2]	Aftertaste[3]	Overall Firmness[4]	Stickiness[5]	Creaminess of Texture[6]	Overall Preference[7]
Experimental #4145	4.52[a]	3.96[a]	3.98[a]	3.72[b]	3.59[b]	4.70[b]	4.77[a]
Danish Havarti	4.71[a]	4.22[a]	3.96[a]	3.07[a]	4.27[a]	5.40[a]	4.61[a]
Value (LSD 5%)	NS	NS	NS	S(.50)	S(.54)	S(.40)	NS

[1]Scale: 1 = very weak to 7 = very sharp or biting
[2]Scale: 1 = not sharp or biting to 7 = very sharp or biting
[3]Scale: 1 = no aftertaste to 7 - pronounced aftertaste
[4]Scale: 1 = soft to 7 = firm
[5]Scale: 1 = not sticky to 7 - very sticky
[6]Scale: 1 = not smooth, not creamy to 7 = very smooth, very creamy
[7]Scale: 1 = dislike extremely to 7 = like extremely

a,b Mean scores in the same column with the same superscript are not significantly different at the 5% level.

S = significant at the 5% level; NS = not significant.

Date of evaluation: July 7, 1987.

Table 2. Response frequencies and mean scores for the consumer preference panel evaluation of Havarti cheese.

Preference rating	Assigned numerical score	Samples	
		Commercial	Experimental
		(—Frequency of responses—)	
Like very much	7	96	79
Like moderately	6	75	85
Like Slightly	5	24	29
Neither like nor dislike	4	4	6
Dislike slightly	3	8	4
Dislike moderately	2	1	3
Dislike very much	1	0	2
Total responses: n=208			
MEAN SCORES:		6.17[a]	6.02[a]
STATISTICAL ANALYSIS:			
F value		NS	
LSD (at 5% level)		.20	

S = significant at 5% level; NS = not significant
a, b Mean scores with same superscripts are not significantly different at 5% level.

Date of Evaluation: July 16, 1987

Table 3. Response frequencies and mean scores for the consumer preferences panel evaluation of Havarti cheese.

Preference Rating	Assigned Numerical Score		WASH Samples	NO WASH
		Commercial	181202 Exp.	9014 Exp.
		(----Frequency of responses----)		
Like very much	7	68	51	32
Like moderately	6	64	76	74
Like slightly	5	36	40	44
Neither like nor dislike	4	11	18	23
Dislike slightly	3	16	12	24
Dislike moderately	2	7	5	4
Dislike very much	1	2	2	3
Total responses: n = 204				
MEAN SCORES:		5.63[a]	5.55[a]	5.21[b]
STATISTICAL ANALYSIS:				
F value			S	
LSD (at 5% level)			.25	

S = significant at 5% level; NS = not significant

[a,b]Mean scores with same superscripts are not significantly different at the 5% level.

Date of evaluation: October 22, 1987.

Project Title

Expression of bovine alpha S(1) casein in milk and tissues of transgenic mice.

Personnel:

R. D. Bremel, Professor, Dairy Science Department and N. L. First, Professor, Meat and Animal Science Department.

Funding:

Center for Dairy Research and the Wisconsin Milk Marketing Board.

Objectives:

1) A determination will be made as to whether or not a cloned cDNA gene for bovine casein, fused to the mMT-1 promoter can be expressed in transgenic mice.

2) It will be subsequently determined if the milk protein composition of mice can be altered by the transfer into mice of a casein gene fused to a promoter specific for mammary tissue.

3) Finally, it will be determined if transgenic mice, carrying the cloned cDNA for bovine casein fused to the mMTV promoter, can be used to establish lines of descendants in which the casein genes are stably integrated and transmitted through the germ line and consistently expressed in mammary tissue and milk.

Modifications of objectives :

With research done since the proposal was written it is now clear that Objective 1 (above) of the original proposal, to study the expression of bovine casein gene fused to mMt-1 promoter, is no longer appropriate. The mMT-1 promoter may not be mammary-specific, but predominantly specific to liver, intestine, kidney, and other tissues. Therefore, we decided to concentrate upon the study of the expression of mMTV promoter (Objective 3) which has been shown to be mammary specific. As a test system we have constructed the mammary specific recombinant DNA; CAT (chloramphenicol acetyl transferase) gene fused to mMTV LTR (long terminal repeat). We are in the process of engineering a genetic construct containing the bovine casein gene fused to mMTV LTR.

Most recent research results:

As a test system we have made the recombinant DNA of the chloramphenicol acetyl transferase (CAT) gene fused to the mouse mammary tumor virus long terminal repeat (mMTV LTR) and micro-injected this gene construct into several hundred mouse embryos. This gene is widely used in research because it is only found in bacteria and therefore easy to assay in animals. The embryos were transferred to recipient mice who are now pregnant and have already produced about 10 pups. We anticipate a transgenic rate of 1-3% of live births. The screening methods are at hand and when all the animals have been born we will conduct a test to see how many are transgenic.

We are also in the process of making the recombinant DNA of bovine casein gene fused to mMTV LTR. We transformed the casein gene in *E. coli* and isolated few hundred mg of plasmid containing the gene. Also we obtained the plasmids containing the mMTV promoter and isolated few hundreds mg of this plasmid. Both plasmids were cut with appropriate restriction enzymes. The resulting fragments were isolated and ligated. The *E. coli* cells are currently being screened for positive gene constructs.

In addition, we improved the efficiency of microinjection and rate of embryo survival. We also improved the milking procedure for lactating mice and analytical methodology for bovine and mouse milk proteins.

Project Title:

Behavior of *Listeria monocytogenes* in the presence of *Streptococcus lactis* in a medium with internal pH control.

Personnel:

E.H. Marth, Professor, Food Science Department and J.M. Wenzel, graduate student, Food Science Department.

Funding:

Wisconsin Milk Marketing Board and Chr. Hansen's Laboratory, Inc., Milwaukee, WI.

Objectives:

1) Overall objective of this project is to develop additional information which will be useful in improving the safety of cheese and other cultured dairy foods.

2) The present study sought to answer the question: How will *Listeria monocytogenes* behave if present when a starter culture is prepared using an internal pH controlled medium?

Procedures:

Preparation of internal pH control medium.
1. 15.0 g added to 200 ml tap water
2. pH determination (7.0-7.3)
3. Medium heated (100°C) for 45 minutes then cooled rapidly
4. Medium held at 21 or 30°C for 14 hours

Preparation of Lactic culture.
1. *Streptococcus lactis* frozen culture thawed
2. Culture added to 1 ml sterile skim milk; frozen until time of use
3. 200 ml sterile skim milk inoculated at 21°C for 14 hours

Treatments:
Cultures (in internal pH control medium)
1. *Listeria monocytogenes*
2. *Streptococcus lactis* (0.25 or 1.0%)
3. *Listeria* or *S. lactis* (0.25 or 1.0%)

Incubation temperatures:
1. 21°C
2. 30°C

Sample plated on appropriate culture medium:
1. *Listeria* – Mcbride's Listeria agar.
2. *S. lactis*– APT agar

Conclusions:

1) The medium supported growth of all three strains of Listeria.

2) Research has shown that some starter cultures are antagonistic to various foodborne pathogens as well as food spoilage organisms. This study supported that.

3) The fact that a percentage of lactic or strain of Listeria make no difference in the hydrogen ion concentration at varied temperature indicates that some other factor is contributing to the inhibition differences.

4) Factors that have been suggested include antibiotics produced by lactic cultures such as nisin and lactostreptins and volatile compounds. Whatever the factor or combination of factors, they are enhanced by greater percentage of lactic and higher temperature.

5) California strain was more sensitive to the effects of *S. lactis*.

6) Even though there was inhibition, no total inactivation occurred at pH 5.5; the pH at which the medium can be used.

The following abtract has been accepted for presentation at a conference:

Growth of *Listeria monocytogenes* strains V7, Scott A, or California (initial inoculum ca. 10^3/ml) in the presence of *Streptococcus lactis* (initial inoculum 0.25 or 1.0%) was determined using a medium with internal pH control. Thirty-hour incubation of all cultures was at 21° or 30°C. The pH of the substrate and the control was 7.0 before and after incubation. All three strains

behaved similarly at 21°C with inhibition starting after 18 hours of incubation with 0.25% *S. lactis*, and 12 hours with 1.0% lactic inoculum. At 30°C, inhibition of strains V7 and California began at 12 hours of incubation with 0.25% *S. lactis* and 10 hours with the 1.0% level. Greatest inhibition of *L. monocytogenes* was seen at 30°C with strain California. Inhibition began after 9 hours and 6 hours of incubation with 0.25 and 1.0% *S. lactis*, respectively. At this temperature with 1.0% *S. lactis* the population of strain California dropped below that of the initial inoculum after 30 hours of incubation. The final pH values at 21° (5.7) and at 30°C (5.0) were independent of the strain of *L. monocytogenes* or percentage of lactic culture but different degrees of inhibition were seen. Since pH at either temperature was not affected by size of lactic culture inoculum or by strain of *L. monocytogenes*, some other factor(s) contributed to differences in degree of inhibition. Although growth of *L. monocytogenes* was inhibited, it was complete only under one set of conditions (strain California with 1.0% *S. lactis* at 30°C).

Project Title:

Effect of calcium and other salts on both the primary and secondary phases of enzyme-induced coagulation of milk.

Personnel:

R. W. Hartel, Assistant Professor, Food Science Department; D.B. Hyslop, Senior Lecturer, Food Science Department, and J.M. Johnson, graduate student, Food Science Department.

Funding:

Center for Dairy Research/Walter V. Price Cheese Research Institute.

Objectives:

1) To study the effects of calcium salts on the enzymic hydrolysis of casein.
   a. Perform equilibrium binding studies of calcium on alpha-, beta-, and kappa-casein as well as on rennin.

   b. Monitor conformational changes during binding using absorption and/or circular dichroism.

2) To study the effects of calcium salts on the aggregation of casein micelles.

   a. Using immobilized enzymes, prepare solutions of varying concentrations of hydrolyzed casein.

   b. Investigate the effects of adding calcium to above solutions on coagulation rate using rheometric tests.

3. To study the effects of calcium salt addition on the coagulation rates of non-fat milk.

   a. Determine rate of enzymic hydrolysis by monitoring the rate of macropeptide production using HPLC.

   b. Determine the rate of coagulation by rheometric changes.

Results:

During this first year of the project, much of the effort has gone into an extensive literature search to delineate the areas of research requiring further effort. The main outcome of this search has lead to the incorporation of the experiments involving the equilibrium binding studies of calcium on casein as outlined in the first objective. This appears to be an area where there has been only limited study yet may have a large impact on the understanding of the effects of calcium salts on milk coagulation. It

also is an area that will complement the second objective and yield some useful mechanistic information.

The equlibrium binding studies are currently underway. A constant temperature dialysis apparatus has been set up for studies of calcium binding at 31 d pH 6.7. Protein samples are dissolved in a 20 mM imidizole solution and placed in one half of the equilibrium dialysis apparatus. At specified time intervals, samples are withdrawn from the cell half side containing the calcium-only solution. Results indicate at equlibrium of calcium is reached after 5 hours while the sample is rotating at 20 RPM.

This coming year will see the completion of these equilibrium binding studies as well as the coagulation rate studies.

Impact of Research:

It has long been a goal in the dairy industry to design continuous cheese manufacturing processes, for example, by way of immobilized enzyme reactors or by extrusion processes. So far, this goal has eluded us. If the dairy industry is ever to come to grips with this problem, as well as other clotting problems, further understanding of the role of calcium salts on the coagulation process will be required.

Project Title:

Enzymatic modification of butterfat in supercritical CO_2.

Personnel:

R.W. Hartel, Assistant Professor, Food Science Department, K.L. Parkin, Assistant Professor, Food Science Department, and T.M. White graduate student, Food Science Department.

Funding:

Center for Dairy Research/Walter V. Price Cheese Research Institute and the Wisconsin Milk Marketing Board.

Objectives:

1) Assess the feasability of using supercritical (SC) fluids as a means to control lipase action on butterfat.

2) Determine the specificity of lipase action on butterfat in SC CO_2 as influenced by the reactor operating temperature and pressure, as well as the level of an aqueous entrainer.

3) Develop a fundamental understanding of how lipase action on butterfat in SC CO_2 may be controlled by determining the reaction kinetics and specificities under various processing conditions.

Results:

The primary focus for the year was the design and assembly of a supercritical reactor/extractor for this project. The equipment has been successfully constructed and testing of the apparatus is essentially complete. However, some experimental difficulties have arisen that will cause progress in the immediate future to be rather slow. These problems are associated with the difficulty in sealing high pressure fittings using rubber O-rings since SC CO_2 is very corrosive. The solution to this problem is continuous monitoring of the equipment and periodic replacement with new O-rings.

The solubilities of free fatty acids and triglycerides in SC CO_2 is the main topic of our current research. We have begun solubility experiments for the individual triglycerides and our preliminary results indicate that we can reproduce quite closely some of the limited data in the literature on stearic acid. This work will continue on shorter chain triglycerides.

We are collaborating with Dr. Pat McMahon in the Chemical Engineering Department on this project in terms of predicting solubilities of the various triglycerides. The model developed by Dr. McMahon and Bess Hwang, his graduate student, will be tested on the experimental data

being generated at this time.

Future work on this project will continue under a WMMB research contract recently awarded to R.W. Hartel and K. L. Parkin. This work will involve the study of the enzymic reactions of various lipases on butterfat and its fractions in the supercritical phase.

The current work on triglyceride solubilities is an important first step in understanding the modifications of butterfat that will be possible in SC CO_2. The solubilities of the individual triglycerides and mixtures thereof will determine the ability to fractionate these compounds using SC CO_2. These fractions then form the substrate for the enzyme action and determine the final modified product from this process.

Project Title:

A linear programming model integrating resource allocation and product acceptability for process cheese products.

Personnel:

J.P. Norback, Professor, Food Science Department and K.L. Craig, graduate student, Food Science Department.

Funding:

Center for Dairy Research/Walter V. Price Cheese Research Institute.

Objectives:

The objective of this research was the application of an operations research methodology to the manufacture of pasteurized process cheese. We wanted to identify and analyze the critical factors in the operation that have a high impact on costs and profits. A linear programming model was constructed to integrate the cheese manufacturing, blending and aging, and flavor components for a process cheese manufacturing operation.

Results:

Using a linear programming model, the input resources for the process cheese products have been identified and defined. These input resources include the direct, single purpose ingredients such as emulsifiers and whey protein concentrate. Other ingredients such as Cheddar cheese and cream are considered intermediate products since they are manufactured by a dairy plant and can be sold or processed further into the value-added process cheese product. The decision alternatives regarding the most profitable use of the manufactured intermediate products are an integral part of this systems approach model. Using legal, quality, and management guidelines, constraints on the process cheese formulation have been constructed and refined. Such a model may then be optimized with respect to a function which maximizes the profits of pasteurized processed cheese.

Sensitivity analysis conducted during model development revealed several critical factors impacting on the optimum formulation. Several of the ingredient costs were quite sensitive to price fluctuations. When these costs were altered by a small amount, a different optimum formulation blend was obtained. These ingredients were identified and their economic impact on the objective function noted. The advantage of process cheese as a value-added product was quantified when several fat and casein sources were allowed into the manufacturing formulation for natural Cheddar cheese. With the profit coefficients and constraints used in this model, the increased Cheddar cheese yield obtained from allowing the solids content of the cheese milk to increase resulted in higher objective function value.

An unexpected problem was discovered during the model development stage. An operations research approach to process cheese manufacture involves two design steps, the natural cheese manufacture and the process cheese manufacture. The natural Cheddar cheese used

in this example is a function of Xi, the ingredients available for use to make Cheddar cheese. Each Xi has a known and defined coefficient for fat, moisture, casein, cheese yield, whey cream yield, and solids. The process cheese product is a function of Yi, the direct ingredients available for use during processing as well as the Xi's. Again, each Yi has a known and defined coefficient for fat, moisture, casein, cheese yield, whey cream yield and solids. Because the optimum formulation of the latter depends on an unknown optimum formulation of the former, the model cannot automatically tie the two operations together in a linear format. To circumvent this linear-induced problem, parametric analysis is used to reveal the affect increasing Cheddar cheese prices are varied with the changing fat values. The optimum process cheese formulation varies with the higher fat Cheddar cheeses, the retail price of Cheddar cheese that could be purchased on the market and the cheese milk solids and process cheese fat constraints.

Impact of Research:

It is not uncommon for the many components of an organization to become autonomous units, with their own goals. The goals of the organization as a whole may get overlooked by this specialization.

This research has shown the value of an operations research/systems approach for coordinating operations or activities within an organization. It is possible to identify the best or optimal solution to a problem while keeping the objectives of the components consistent with those of the entire organization.

It is inefficient to study an ingredient or formulation constraint that is not critical to the optimal solution. This model identifies those critical factors that have high impact on the costs and profitability of a process cheese manufacturing operation. The decision makers can thus focus their attention and time on the factors that influence the profitability of the manufacturing operation.

Project title:

Temperature-sensitive liposomes; a controlled release system for the acceleration of cheese ripening.

Personnel:

M. El Soda, CDR visiting scientist, Professor, Dept. of Agriculture Industries Faculty Alexandria University, Egypt, M.E Johnson, Associate Scientist, CDR, and N.F. Olson, Professor, Food Science Department.

Funding:

National Dairy Promotion and Research Board and Center for Dairy Research (WITEP).

Objectives:

Liposomes technology which was recently proposed as an effective tool for the acceleration of cheese ripening (5,6,7,8) offers the possibilities of preparing a wide range of vesicles varying in size, net charge, stability and sensitivity to pH and or temperature. Phospholipid vesicles showing differences in sizes, net charge or stability were previously evaluated for the acceleration of cheese ripening (3,9,11). The aim of the present investigation was to use liposomes that would deliver their content in the cheese at a well defined temperature (temperature-sensitive liposomes).

Procedures and Methods:

Cheesemaking

Three batches of a feta-type curd were manufactured according to Abou Donia (1) but with some modifications; salt was not added to milk

but the cheese was salted in a 23% brine for three days at 100°C. Renneting was accomplished at low temperature (28°C) for three hours in order not to destabilize the temperature-sensitive liposomes. Corolase PN, a neutral proteinase from *Aspergillus* sp. (Rohm Tech, West Germany) was added to the cheesemilk of the first batch while calcein (a fluorescent dye) was added to the second batch, the third batch was considered a control. All the cheesemaking steps were performed at temperatures not exceeding 300°C in order not to destabilize the vesicles. Cheese from each vat was treated in one of three ways:

1. Ripened at 12°C and was not subjected to further treatments.
2. Heated to 40°C/2 hours in order to destabilize the vesicles and then ripened at 12°C.
3. Samples were kept at 12°C for 48 hours, heated to 40°C for two hours and then returned to 12°C.

Preparation of liposomes

Multilamellar vesicles were made according to the method of Wilschut (12) using dipalmitoyl phosphatidyl choline (DPPC) (Avantic polar lipids USA). The lipid shows a transition temperature of 41°C and DPPC liposomes are only stable at temperatures below 35°C. Vesicle formation was accomplished at 50°C for two hours. For the entrapment of Corolase PN, 25mg of the enzyme were dissolved in 5ml, 0.05 M phosphate, buffer pH 7.0. The aqueous enzyme solution was then entrapped in 100mg of the lipids. Calcein dye (5ml) was prepared according to Allen (2) and was also entrapped in 100 mg DPPC.

Proteolysis measurement

TCA and PTA soluble nitrogen determinations were accomplished as described by Bartels et al. (4).

Fluorescence measurement

Forty grams of cheese were mixed with 100ml phosphate buffer 1M in a mortar, the resulting cheese paste was then centrifuged at 6000 rpm for 1/2 hour and then filtered through Whatman 42 filter paper. The clear filtrate was then subjected to fluorescent measurement on an SLM 8000 fluorometer (excitation maximum 488mm emission maximum 520mm).

Results and discussion

Table 1 (see next page) shows that the initial fluorescences value for all cheeses are similar. The readings are probably due to the fluorescence of riboflavin in the cheese. After heating the cheese for two hours at 40°C a significant increase in fluorescence could be measured in the calcein-entrapped liposome treated cheese. This indicates that heating the cheese will lead to liposome leakage and to the release of the dye. Calcein was also released in cheese held for two days at 12°C. This is probably a result of vesicle disruption due to the joint action of salt, pH (3), and phospholipase. However, heating the samples increased dye release. After seven days of ripening, most of the calcein was released from the vesicles indicating total disruption of the liposomes and that heat treatment is not necessary for release of calcein from the vesicles after only one week of ripening.

The rate of protein breakdown during a four-week ripening period is given in Table 2. There are very little differences in the TCA sol N and PTA sol N values between the control cheese and the various treatments. This is probably due to the denaturation of the Corolase PN enzyme during liposomes preparation (two hours at 50°C).

Although temperature-sensitive liposomes might be a good candidate for targetable drug delivery (10,13,14). They do not seem to be the appropriate controlled release system for accelerated cheese ripening due to the fact that relatively high temperatures are to be used during vesicle formation which can lead to partial or total denaturation of the enzyme to be entrapped. The obligation to use low coagulation and manufacturing temperature during the cheesemaking process is also another limitation for the application of temperature-sensitive liposomes for the acceleration of cheese ripen-

Table 1. Calcein release from temperature-sensitive liposomes in Domiati cheese.

	Fluorescence Values		
	Liposome + Calcein	Liposomes + Proteinase	Control
Cheese after manufacture	24135	23137	21847
Cheese heated 40°C/2hr.	75644	22005	20732
Cheese after 2 days/12C	45743	25876	25000
Cheese 2 days/12C then heated 40°C/2hr.	72814	24300	23282
Cheese after 7 days/12°C	69333	n.d.	24899

n.d.= not determined

ing. On the other hand, it seems that liposomes engineered to have a phase transition at a pH that is reasonably well defined (pH 5.5-5.0) will probably be more appropriate for enzyme delivery under cheesemaking conditions and work in this area is currently in progress.

Summary:

Temperature-sensitive liposomes made of Dipalmitoyl phosphatidyl choline were evaluated as a controlled release system for the acceleration of cheese ripening. Although enzyme was released at a precise temperature the system faces many problems and is not recommended as an effective tool to shorten the cheese ripening period.

References:

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Table 2. Effect of temperature-sensitive liposome-entrapped proteinase on the rate of protein breakdown in cheese as measured by PTA and TCA soluble nitrogen.

	Age (weeks)	PTA soluble nitrogen Treatment			TCA soluble nitrogen Treatment		
		1	2	3	1	2	3
Liposomes + calcein	0	11.9			15.6		
	1	9.6	8	7.3	21.8	19.9	20.3
	2	11.0	10.4	10.6	30.8	27.4	28.8
	4	19.9	17.5	15.9	44.5	47.1	44.6
Liposomes + proteinase	0	7.4			12.3		
	1	8.2	8.8	7.7	20.5	21.2	26.7
	2	9.2	11.0	11.2	31.2	30.8	33.7
	4	11.9	20.3	12.4	37.3	41.0	35.2
Control	0	9.8			12.2		
	1	10.0	9.4	7.9	19.7	17.3	16.7
	2	9.1	10.6	13.9	28.2	29.7	32.9
	4	15.4	20.2	14.7	46.7	44.7	44.8

Treatment 1= Cheese ripened at 12°C and not subjected to further treatments.

Treatment 2= Cheese heated to 40°C/2 hours and then ripened at 12°C.

Treatment 3= Cheese ripened at 12°C/2 days, heated to 40°C/2 hours and then returned to 12°C

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Project Title:

Development of lowfat Cheddar cheese: Procedures and acceleration of flavor development.

Personnel:

N.F. Olson, Professor, Food Science Department; M. E. Johnson, Associate Scientist, CDR, B.A. Riesterer, Associate Researcher, CDR, and S. Lutzke and P. Freund, Chr. Hansen's Laboratory, Inc., Milwaukee, WI.

Funding:

Center for Dairy Research, Wisconsin Milk Marketing Board, and Chr. Hansen's Laboratory, Inc.

Objectives:

The objectives of this project were to produce a good flavored lowfat Cheddar cheese with a shelf life of at least 6 months; to evaluate different cheese manufacturing procedures to determine the most efficient and economical; to determine the effect of increasing the curing temperature (forced aging) on the body, flavor, and appearance of the cheese. The protein breakdown and free fatty acid (FFA) levels were monitored for all the vats. This provided a reference between the manufacturing procedure and the effect of ripening enzymes. Addition of the ripening enzymes is another part of this project but the results for 1987 are not sound enough to report. The final approval of the lowfat Cheddar cheese developed will be put to a consumer sensory taste panel and compared to other available lowfat and full-fat Cheddar cheeses.

Results:

It is very difficult to produce a lowfat Cheddar cheese that has a shelf life of at least 6 months. The problems of poor body, and poor flavor and appearance usually start to show up after three to 4 months. Some of the defects are bitterness, rancidity, meaty-brothy flavor, gas holes, lack of flavor, unclean flavor, and a soft pasty body.

A lowfat Cheddar cheese has been developed which doesn't develop bitterness until after 5 months of age, but the body is slightly firmer than that of a full fat cheese. The data for the 4 cheese trials are given in Table 1.

Table 1. Four cheese trials

	Trial 1	Trial 2	Trial 3	Trial 4
FAT	18.13	19.1	19.6	21.35
MOISTURE	46.66	45.75	44.94	41.82
SALT	1.62	2.11	1.51	1.34
pH	4.98	5.00	5.02	5.08
FDM	34	35.2	35.59	36.69
S/M	3.04	4.61	3.36	3.20
MNFS	57.00	56.55	55.89	53.17
COOK	96^{0}F	96^{0}F	96^{0}F	98^{0}F
PAST. TEMP	168.5^{0}F	167.0^{0}F	163.5^{0}F	163.5^{0}F

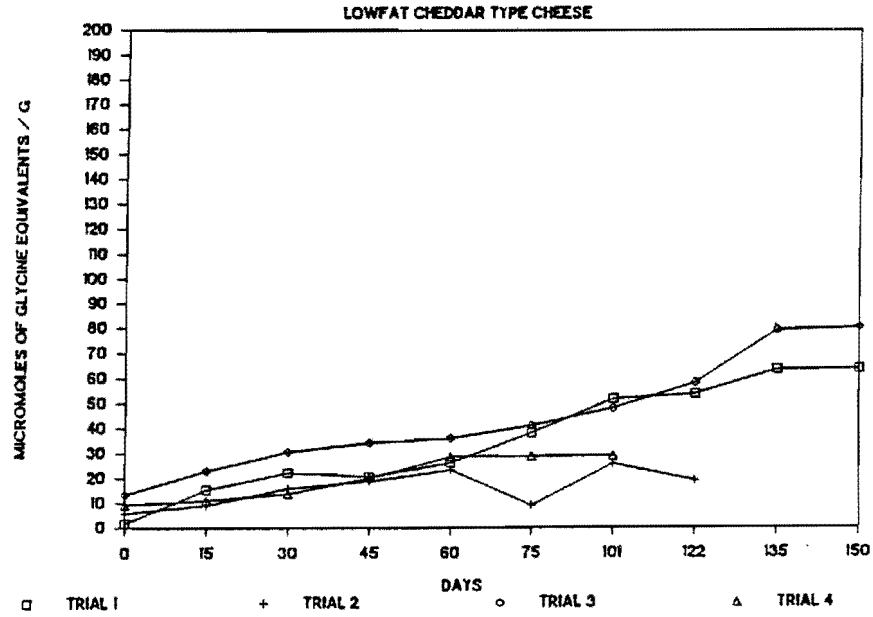
Table 1 indicates that the lower the moisture, the better the moisture in the non-fat substance (MNFS) ratio improves. According to R.C. Lawrence et.al. *J. Dairy Science* vol 67, 1984, the optimum MNFS is .53 for a cheese to be aged 6-7 months. This ratio can be increased to .56 but the Cheddar cheese quality will deteriorate rapidly after reaching its optimum. It is too early in our research on trial 4 to make a prediction. In trial 3 the cheese had excellent quality up to 4 months of age, but by 5 months it was very bitter. Trial one had a MNFS ratio of .57 and developed the most defects the earliest of all the four trials.

In trial 1 and 2 a high moisture cheese was desired. The curd after milling was soaked in 70 - 75°F water for 15 minutes to rehydrate the curd. This step was omitted for trial 3 and 4, because it produced an inferior cheese. In trials 3 and 4, 20% of the whey was removed after cooking and then 10% (if original volume) water was added to help reduce the amount of lactose present. This allowed better control of final pH.

The amount of color was reduced 40% and no difference was observed in final cheese color. Because a lowfat cheese has a higher proportion of protein than a full fat cheese, more proteins in a lowfat cheese will be stained with the cheese color.

In Figure 1, a similarity between trial 1 and 3 and trial 2 and 4 is observed. The activity of the starter and non-starter bacteria can be controlled by either salt or low moisture. Trial 2 had the highest salt in the moisture phase (s/m) ratio (4.61) and trial 4 had the lowest MNFS ratio (.53). This effect is demonstrated by the reduced amount of proteolysis observed at 7.2°C as measured by PTA (33% phosphotungstic acid) analysis. Trial 1 and 3 had low s/m ratios 3.0 and 3.3 and a MNFS ratio of .57 and .56 respectively. These cheeses indicated less control over the starter and non-starter bacteria which resulted in an increased amount of proteolysis.

Figure 1. PTA at 7.2 °C



In Fgure 2 and 3, the proteolysis was measured using 12% tricholroacetic acid (TCA). This measures the amount of peptides in the cheese. Trial four has the slowest rate of proteolysis, but all the trials are similar at 101 days.

In Figure 3, a cheese from the same vat was stored at a higher temperature (10°C). As the temperature was increased, the rate of proteolysis also increased. Examples from trial 3 and 4 PTA values at 101 days of storage are listed below:

Trial 3:
7.2°C 50 micromoles of glycin equivalents/g
10.0°C 70 micromoles of glycin equivalents/g

Trial 4:
7.2°C 25 micromoles of glycin equivalents/g
10.0°C 50 micromoles of glycin equivalents/g

Figure 2. TCA 7.2°C

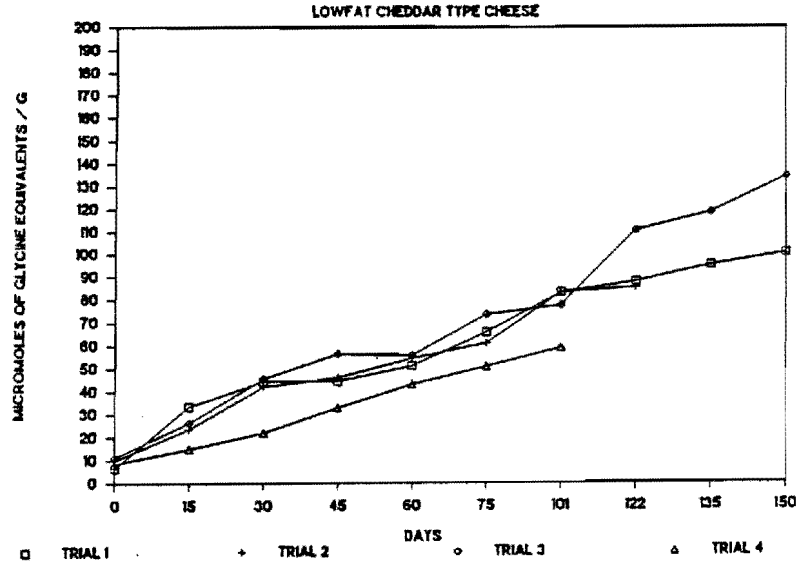
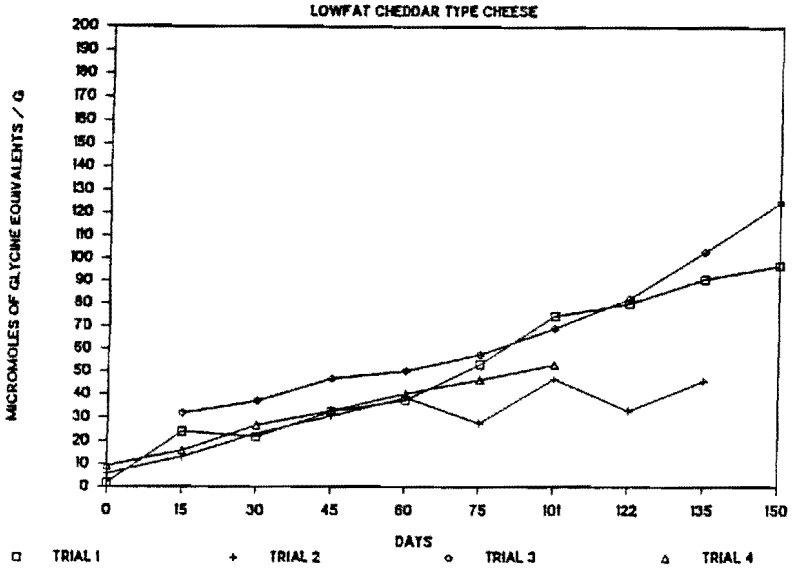


Figure 3. PTA at 10.0°C



As shown in Figure 4, again an increase in temperature caused an increase in proteolysis as measured with TCA. Examples of cheese ripened 101 days and analyzed for TCA are listed below.

Trial 3:
7.2°C 50 micromoles of glycin equivalents/g
10.0°C 70 micromoles of glycin equivalents/g

Trial 4:
7.2°C 25 micromoles of glycin equivalents/g
10.0°C 50 micromoles of glycin equivalents/g

In Figures 5 and 6 the same trends were observed such that as the temperature was increased so did the rate of lipolysis or free fatty acid (FFA) development. Also trial 2 and 4 showed a slower break down of the C10 and C12 chain fatty acids.

Figure 4. TCA at 10.0°C

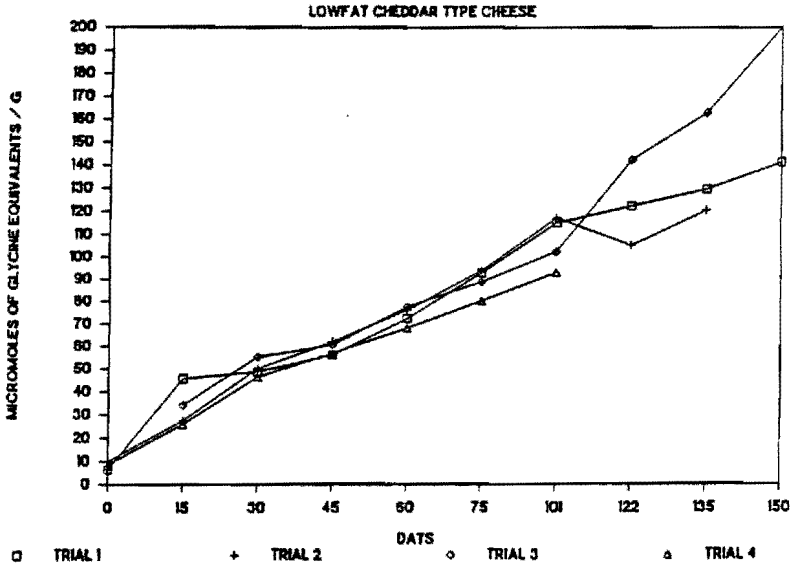


Figure 5. Free fatty acid concentration at 7.2°C

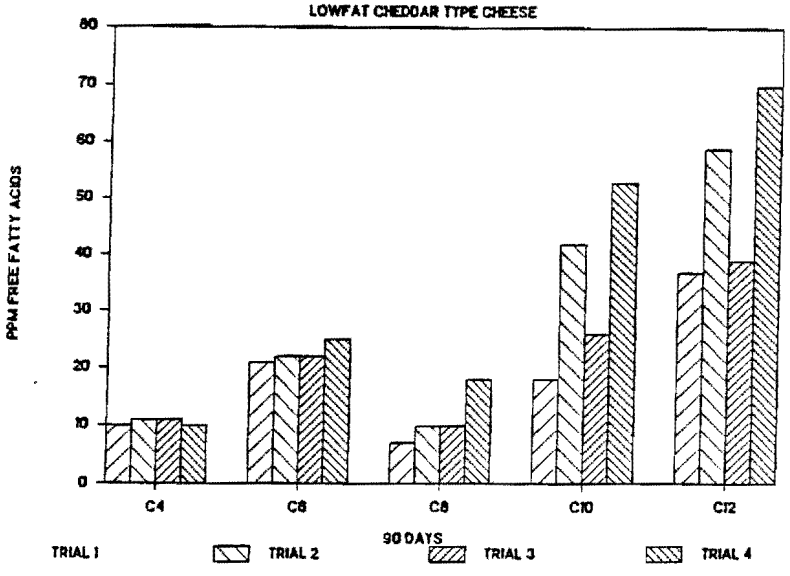
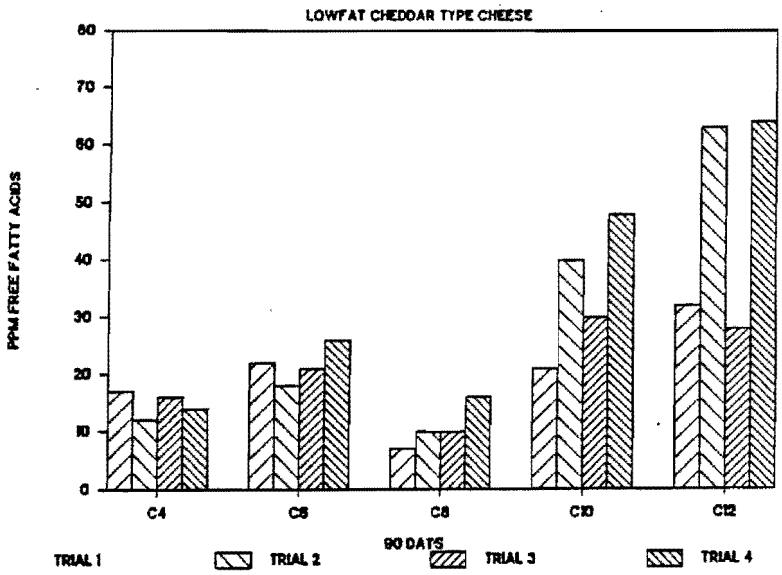


Figure 6. Free fatty acid concentration at 10.0°C



Impact of Research:

This research adds to the knowledge base we need to produce the flavorful, quality lowfat cheeses that consumers are looking for. In the July 15, 1988 *Cheese Reporter*, it was stated that probably the hottest cheese category is the so called "dietary" cheese products, including those reduced in fat, cholesterol, and sodium. One of the guidelines of the National Academy of Sciences for cancer prevention is to reduce the current national average of calories obtained from fats from 40 to 30 percent. Ways to reduce dietary fat levels include consumption of lowfat cheeses. In the September 24, 1986 issue of *The Progressive Grocer*, a study cited that 16.2% of the customers used lowfat cheese. Of these customers only 4.3% were classified as heavy users yet they consumed 59.2% of the total lowfat cheese sold.

The low levels of consumption by the majority of customers who use lowfat cheese may be due to the lack of variety and lack of consistent flavor quality of the lowfat cheese offered. The demand for quality lowfat cheese is there. The challenge for the cheese industry is to give the consumer a variety of these cheeses to choose from and to produce a cheese where the consumer does not have to sacrifice quality.

Project Title:

Citrate inhibition of aminopeptidase in commercial fungal protease preparations used to accelerate cheese ripening.

Personnel:

N.F. Olson, Professor, Food Science Department and M.S. Kim, graduate student, Food Science Department.

Funding:

National Dairy Promotion and Research Board.

Objectives:

The purpose of this study was to examine the possible inhibition of commercial fungal protease and peptidase preparations from *Aspergillus* species by citric acid and to discuss the possible implications of the inhibition on cheese ripening.

Results and Discussion:

Citrate-Na_2 HPO_4 buffer(McIlvaine buffer) was used initially to measure proteolytic, leucine-aminopeptidase, dipeptidase, and carboxypeptidase activities because of its good buffering capacity at pH 5.4 (buffer range: pH 2.6-7.0), but enzymatic activities, especially aminopeptidase, were lower than with phosphate buffer. This suggested that citric acid chelated metallic ions and reduced activities of metalloenzymes such as leu-aminopeptidase.

Subsequent experiments demonstrated that leu-aminopeptidase activities in the fungal commercial enzymes were reduced to varying degrees at pH 5.4 in four different buffers (Table 1). Enzymatic activities in tris-malate buffer decreased about 50% of that in phosphate buffer whereas enzyme activities were markedly inhibited by using acetate and citrate-phosphate buffer. This variability may be related to the stability constant of the bond between the metal atom in Leu-aminopeptidase molecule and buffer ligand and the concentration of ligand.

Table 1. Leu-aminopeptidase activities of commercial proteases extracted from *Aspergillus* species at 37°C in different buffers at pH 5.4

	commercial enzymes					
buffer	Rhozyme P-41		Corolase PN		Corolase PS	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
	---(μM substrate released/min/mg protein)---					
phosphate	245	30	156	11	124	12
citrate-phosphate	12	2	6	3	0	0
tris-maleate	102	20	70	19	38	4
acetate	23	2	15	2	8	3

Citric acid in citrate-Na_2HPO_4 buffer at pH 5.4 probably is present as equimolar divalent and trivalent conjugate bases since it has three pK's of 3.1, 4.7, and 5.4. This will promote complexing of Zn ions fromthe leu-aminopeptidase active site since the general order of complexing ability of metal ions is alkali metals (Na^+, K^+, etc.) < alkaline earths (Mg^{++}, Ca^{++}, etc.) < transition metals. For the divalent ions of the transition metals the complexing ability usually follows the Irving-Williams Series, $Mn^{++} < Fe^{++} < Co^{++} < Ni^{++} < Cu^{++} > Zn^{++}$.

Acetic acid does not have a strong stability constant compared with citric acid. However, the acetic acid concentration (.099 M) in the buffer solution was approximately 32 times higher than that of citrate (.0031 M). Maleic acid caused less inhibition because of the low concentration (.0084 M) in the buffer and its high pKa, 6.22. At the buffer pH of 5.4, the apparent stability constant of maleic acid and zinc was: log = pKa - pH =6.22 - 5.4 = 0.82; log K(app.) (Zn^{++}) = 2.0 - 0.82 = 1.18.

EDTA is known to be a very strong metal chelating agent; 10^{-14} M of EDTA inhibited more than 90% of leu-aminopeptidase activities (Table 2).

The potential for citrate to inhibit aminopeptidase in cheese depends upon the concentration in milk, the proportion recovered in cheese, and the amount solubilized into the cheese serum. The average citric acid concentration in milk is 170 mg/100 ml. However citric acid concentration of milk varies seasonally; it is usually higher in summer milk (152-264 mg/100 ml) than winter milk (83-193 mg/100 ml).

Acidification of milk, whether by direct addition of acid or by bacterial metabolism causes a progressive solubilization of the colloidal Ca-P-citrate in the early stages and a progressive removal of Ca, and other cations from casein. More than 97.6% of citric acid, 68% of total Ca, and 78.5% of Mg is in serum phase at a pH of 5.6. Most of the whey is expelled from curd when the curd pH is above 6.0. A portion of the citrate would be solubilized and removed with the whey. The remainder would be solubilized as the pH of the cheese drops to 5.0-5.2 during the first few days of storage.

Citrate levels in cheese remaining during ripening depends upon the presence of citrate-fermenting bacteria. *Streptococcus lactis* or *S. cremoris* do not ferment citrate during ripening

Table 2. Reduction of leu-aminopeptidase activities at 37°C of commercial proteases extracted from *Aspergillus* species in the presence of EDTA in tris-maleate buffer at pH 5.6.

EDTA concentration	commercial enzymes		
	Rhozyme P-41	Corolase PN	Corolase PS
--(M)--	---(μM substrate released/min/mg protein)---		
0	256	167	207
10^{-4}	16	16	14
10^{-3}	13	14	10

so it remains fairly constant at 110 mg - 140 mg/100 g cheese in rindless Cheddar cheese. However lactic starters with citrate-fermenting organisms utilize virtually all the citrate within 2-3 days. If it is assumed that the citrate content was approximately 120 mg - 200 mg/100 g cheese and that the moisture content of the cheese was 40-45%, the citrate concentration in the moisture phase of cheese was approximately .012 M - .018 M. Leu-aminopeptidase activities are almost totally inhibited at .015 M of citrate (Table 3). This suggests that the leu-aminopeptidase in cheese would be inhibited since cheese contains, in addition to citrate, considerable lactate (approximately .4 - .5 M in moisture phase of Cheddar), acetate, minor metabolites such as pyruvate, succinate, malate, etc., and amino acids which also have metal chelating properties.

The potential adverse effect of citrate on cheese ripening has been suggested by previous studies. Schrijver et al. reported that cheese ripening was delayed as shown by low free amino acid nitrogen content when *S. lactis* and *S. cremoris* were used rather than BD-type culture which contains a citrate fermenting organism. Nakanishi reported that good-flavored, ripe, Dutch type cheese had a low citric acid content whereas cheese with higher citric acid content had usually flat and sour flavor. Qvist et al. observed that BD type starter fermented citric acid rapidly in Harvarti cheese made from ultrafiltrated milk and resulted with better flavor quality at 6 and 16 weeks ripening than cheese made with starter containing only *S. lactis* and *S. cremoris*.

Since we hypothesized that carboxylic acids bound or chelated to Zn atom in leu-aminopeptidase to inhibit the enzymes, enzyme activities should be restored by removing ligands from Zn^{++} in the active site of the enzyme. Thompson and Carpenter reported the similar result in bovine lens leu-aminopeptidase where Co^{++} and Zn^{++} compete reversibly for two independent binding sites per subunit. When Co^{++} was substituted at activation site, specific activity of leu-aminopeptidase was increased more than 10 times.

Table 3. Reduction of leu-aminopeptidase activities of commercial fungal protease at 37°C in presence of different citric acid concentrations in tris-maleate buffer at pH 5.6.

	commercial enzymes					
citric acid	Rhozyme P-41		Corolase PN		Corolase PS	
concentration	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
--(M)---	---(μM substrate released/min/mg protein)---					
0	236	20	151	20	102	9
5.0×10^{-3}	11	2	9	3	1	0
1.0×10^{-2}	7	0	5	2	1	0
1.5×10^{-2}	2	0	3	1	0	0
2.0×10^{-2}	2	0	3	0	0	0

To test if chelation of essential metal ions was the mechanism of peptidase inhibition, Mg^{++} was added to leu-aminopeptidase in citrate buffer. This cation was tested because of its relatively high concentration in milk and its ability to activate peptidases albeit to a lesser extent than Zn^{++} or Co^{++}. There were 120.5% enzyme activities obtained when approximately .9 M of Mg ion were added (Figure 1), which was a much higher concentration than milk total divalent cation concentration (approximately .04 M). However 90.9 % of enzyme activities were still inhibited when 10 mM of Mg ion, the same concentration in milk, was added to the solution which contained .01 M citric acid. This result suggested that divalent cation present in cheese may remove ligands to restore the leu-aminopeptidase activities but the concentration of divalent cation in cheese was very low, so leu-aminopeptidase may be still inactive. This present data also suggested that the understanding of carboxylic acids during cheese ripening is important in the role of enzyme reaction as well as flavor development which relates to many recent studies that focus on accelerated cheese ripening by using the enzyme preparations from *Lactobacillus* species and fungal sources.

References:

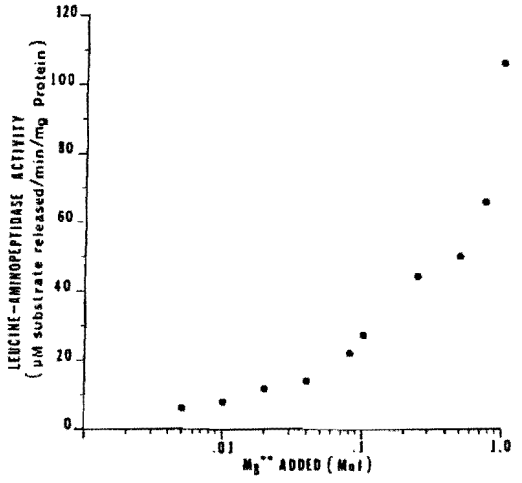
Nakanishi, T. and F. Tokida. 1958. Citric acid content of cheese and its behavior during ripening. Int. Dairy Congr. 2:907.

Qvist, K.B., D. Thomsen and E. Hoier. 1987. Effect of ultrafiltered milk and use of different starters on the manufacture, fermentation, and ripening of Havarti cheese. J. Dairy Res. 54:437

Schrijver, R. DE., R. Martens and M. Weckx 1972. Manufacture of rindless Cheddar cheese with single strain starters. Revue de l'Agriculture 25:935.

Thomson, G.H. and F.H. Carpenter. 1976. Leucine aminopeptidase (bovine lens). The relative binding of cobalt and zinc to leucine aminopeptidase and the effect of cobalt substitution and specific activity. J. Biol Chem. 251:1618.

Figure 1. Effect of $MgSO_4$ addition on leu-aminopeptidase activity of Corolase PN in TRIS-Maleate buffer, pH 5.4, containing .01 M citric acid.



Project:

Proteolysis of cheese treated with commercial fungal protease as monitored by gel electrophoresis procedures.

Personnel:
N.F. Olson, Professor, Food Science Department, M.S. Kim, graduate student, Food Science Department, and L.A. Jensen, CDR Associate Researcher.

Funding:

National Dairy Promotion and Research Board.

Objectives:

The purpose of this study was to compare the use of SDS-PAGE (sodium dodecylsulfate-polyacrylamide gel electrophoresis) and alkaline PAGE to monitor the proteolysis of UF (ultrafiltration) and commercial fungal protease-treated cheese.

Procedures and Results:

In Figure 1, the pattern of the PAGE gel shows 7 or 8 clear bands on the control cheese and 11 or 12 bands on fungal enzyme-treated cheese. The main differences were first, an extra band of γ-casein region on fungal protease-treated cheese which did not appear in the control cheese, and secondly rapid α_{s1}-I casein hydrolysis on the fungal protease-treated cheese, which degraded slowly in the control cheese.

<u>β-Casein</u>
The rate of β-casein hydrolysis of fungal protease-treated cheese during ripening was faster than that of the control cheese.. Approximately 25% of β-casein was already hydrolyzed during the manufacture of high level fungal protease-treated cheese and it resulted in a gel pattern showing a lighter β-casein band and darker γ-casein regions at 0 weeks compared to the control cheese (Table 1). β-casein was resistant to hydrolysis in the control cheese during

Figure 1. Alkaline PAGE pattern of experimental cheeses at 6 weeks of ripening.

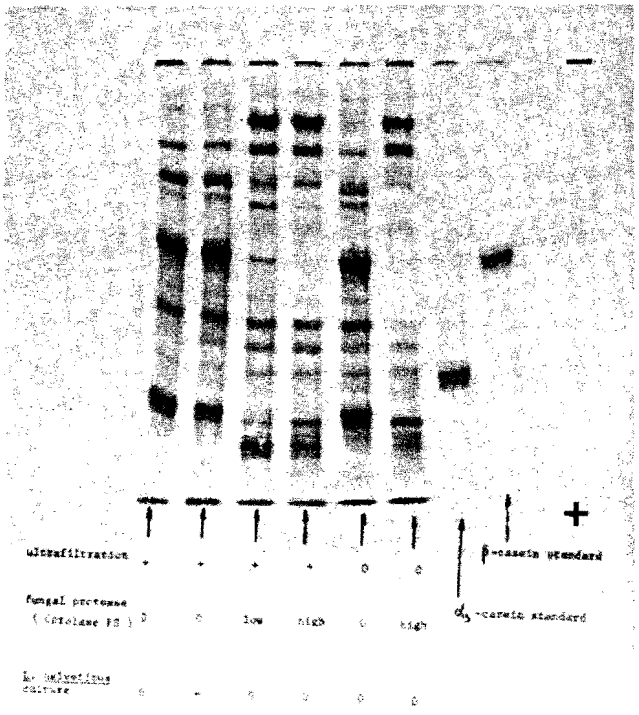


Table 1. β-casein hydrolysis of experimental cheese during ripening determined by PAGE.

Ripening Time	Control		UF *	
	Control	PS 30 mg**	Control	PS 30 mg**
--(Wk)--	----------Hydrolysis(%)----------			
0	N.D	24.4	N.D.	18.9
1	N.D.	60.0	N.D.	56.9
3	23.7(2.6)	81.5	13.1(5.3)	76.3
6	29.5(4.5)	97.0	15.0	85.9
12	41.6(14.5)	100	18.8(3.8)	96.0
18	N.D	100	29.4(4.3)	100
24	64.3(10.0)	100	N.D.	100

* Cheese made from 5 times concentrated milk by ultrafiltration.
** Corolase PS was standardized to proteolytic activity equivalent to 30 mg Neutrase per Kg milk or retentate.
N.D. not determined.
Numbers in blank indicate standard deviation of three trials.

ripening, and approximately 40% of β-casein was hydrolyzed after 12 weeks ripening in conventional cheese. However β-casein was completely hydrolyzed in fungal protease-treated cheese after 12 weeks ripening.

We observed three γ-caseins on control cheeses; the relative mobilities to α_{s1}-casein was 0.285, 0.399, and 0.452. Four bands were observed in γ-casein region of fungal protease-treated cheese, three γ-casein bands showed the same mobility with the control cheese but one extra band, we called, γ-W (relative mobility to α_{s1}-casein, 0.200), had the slowest mobility and appeared only in fungal protease-treated cheese. This suggests that γ-W casein was not cleaved by plasmin, but by fungal protease, and that enzymatic specificity of plasmin and fungal protease to β-casein was different. It appears that γ-W peptide was cleaved from β-casein because it appeared in the γ-casein region and the darkness of this band in the gel was proportional to disappearence of β-casein. The gel pattern of SDS-PAGE on fungal protease-treated cheese accumulated the approximate molecular weight of 11,000 dalton peptide while β-casein was excessively hydrolyzed, and was close to the γ_3-casein position. However further study is necessary to characterize the γ-W band.

Hydrolysis of β-casein in the control cheese during ripening on SDS-PAGE gave poor resolution with γ_s-casein and its breakdown products and κ-casein. When the β-casein band was extracted from the SDS-PAGE gel of the 0 weeks control cheese by electroelution in a dialysis tube and reinjected to PAGE, the result showed two distinct bands. One has the same mobility as β-casein and the other was a band which had slightly higher mobility than α_s-casein. β-casein concentration seemed to go up slightly at the initial stage of ripening on SDS-PAGE gel because of the migration of α_s-casein breakdown product to the β-casein region. β-casein hydrolysis of fungal protease treated cheese was much faster than the control in

patterns of PAGE and SDS-PAGE. But using the SDS-PAGE technique to observe the β-casein hydrolysis resulted in underestimation of β-casein hydrolysis.

α_s-casein.

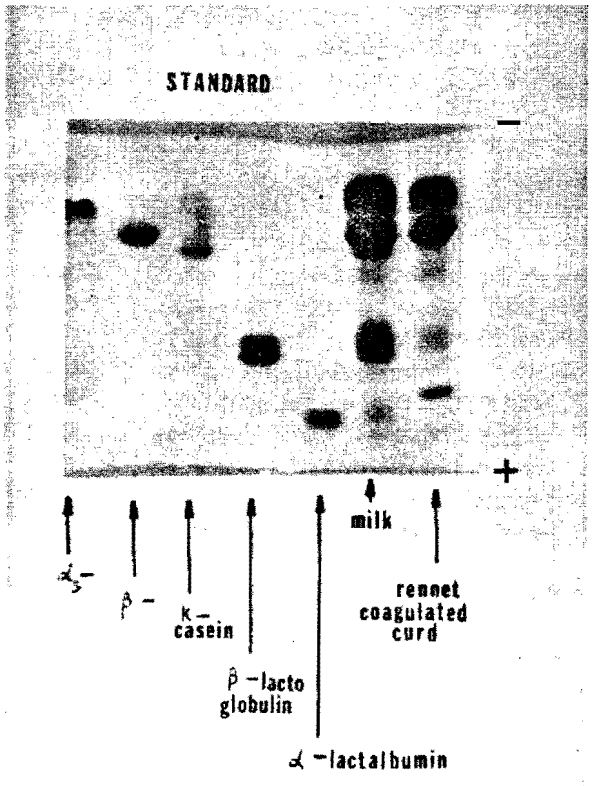
α_{s1}- and α_{s2}-casein were not resolved under the experimental conditions, we considered these as α_{s1}-casein and used it for calculations.About 15% of the α_s-casein of fungal protease treated cheese was hydrolyzed during manufacture compared to the control cheese. Almost all of α_s-casein was hydrolyzed on the control and fungal protease-treated cheese at 12 weeks ripening by using alkaline PAGE. However the rate of α_s-casein hydrolysis of fungal protease-treated cheese (PS 30 mg equiv.) was much faster than the control. Excess and different patterns of hydrolysis of β-casein by fungal protease resulted in peptide which migrated to α_s-casein region at alkaline PAGE.

We observed fungal protease-treated (PS 30 mg equiv.) cheese showed less hydrolysis of α_s-casein than the control cheese. Treated cheese showed 80.7% hydrolysis for Corolase PS 10 mg equivalent, UF cheese showed 81.7% for Corolase PS 30 mg equivalent, while α_s-casein of the control cheese was completely hydrolyzed at 18 weeks.

In contrast with the above results, SDS-PAGE showed α_s-casein hydrolysis of fungal protease-treated cheese was approximately 95% at 12 weeks ripening whereas that of the control cheese was 66% in UF cheese, and 88% in the conventional cheese at 18 weeks of ripening. It suggested that the α_s-casein hydrolysis product by chymosin might not be separated from the α_s-casein region on SDS-PAGE, which when using the SDS-PAGE procedure resulted in an underestimation of α_s-casein especially for the control cheese.

The localized large number of negatively charged amino acids residues of α_s-casein (26 negative charge at pH 8.0 for Asp 43 -Glu 78) induced a considerable amount of inter- and intrasegmental electrostatic repulsion. This lead to an expanded or extended structure in the presence of SDS and moved slower than expected on the basis of its known molecular weight. Figure 2 shows the electrophoretic pattern of milk proteins in SDS-PAGE.

Figure 2. SDS-PAGE pattern of standard caseins and whey proteins.



α_s-casein hydrolysis of the control cheese was faster than the UF cheese, which was also observed by other researchers. This could be explained by the amount of rennet retained in cheese due to different procedures of manufacture (less rennet used for UF cheese manufacture), different cheese pH and buffer capacity depending on the preacidification during ultrafitration of milk, loss of essential minerals during ultrafiltration of milk which catalyze the metalloenzyme activities, etc.

When we compared alkaline PAGE and SDS-PAGE as a tool of observation of α_s-casein and β-casein hydrolysis during ripening, PAGE was a better technique to observe the β-casein and its hydrolysis products of both control and experimental cheese. The SDS-PAGE technique should not be used to observe β-casein in milk because of poor resolution with κ-casein and the underestimation of α_s-casein hydrolysis in cheese due to the same migration with its breakdown products. We are still studing the heterogenesity of the α_s-casein band on SDS-PAGE pattern to prove the above. However SDS-PAGE was a useful technique to observe the β-lactogloblin in cheese especially that made from ultrafiltered milk and para-κ-casein which appeared close to the origin on PAGE because of its high hydrophobisity (non-polar nature).

The relative concentration of α_{s1}-I casein of the control cheese was not proportional to α_s-casein hydrolysis; it increased for the first 3-6 weeks of storage and then began to decrease slightly. Fungal protease-treated cheese showed a different pattern of α_s-casein hydrolysis. α_{s1}-I peptide was rapidly hydrolyzed to form 3-4 of the fast-moving peptides which did not normally appear in the control cheese but accumulated at the same relative peptide mobility as As1-II in the protease-treated cheeses. The rate of accumulation of this peptide seemed different depending on the level of protease applied. That is, when cheese was treated with a lower level of fungal protease, it accumulated more slowly during ripening.

Project Title:

Physiology of D(-) lactate formation by a *Lactobacillus* sp. (LB26).

Personnel:

E.A. Johnson, Assistant Professor, Food Research Institute, and S. Rengpipat, graduate student, Food Research Institute.

Funding:

Center for Dairy Research/Walter V. Price Cheese Institute.

Objectives:

The objective of this project is to understand the physiology of D(-) lactic acid of ripening organisms in Cheddar. The formation of excess quantities of D(-) lactate results in an undesirable white haze on the surface of the cheese which consumers often interpret as mold growth.

Procedures and Results:

The white haze crystals are comprised of L(+)-lactic acid, D(-) lactic acid, Ca, and water (Mark Johnson, pers. comm.). The L and D types of lactic acid are produced by lactobacilli and pediococci during ripening. The biochemical mechanism(s) of D-lactate formation is mainly believed to result from racemization of L(+) lactic acid to D(-) lactate. L(+) lactate is formed in high quantities during starter culture growth. In this study, we examined the physiology of D(-) lactate synthesis in order to understand and hopefully control its formation in cheese.

Several lactic acid bacteria produce L(+) and/or D(-) lactic acids (Table 1). We isolated a pure culture of a ripening bacterium from a mixture of organisms found on cheese with white haze defect. The *Lactobacillus* sp. produced both D and L lactic acids. Properties of the organism are presented in Table 2.

Table 1. Homo- and heterofermentative lactic acid bacteria and the configuration of lactic acid produced.

genera and species	homo-fermentative	hetero-fermentative	configuration of lactic acid
Lactobacillus			
L. delbrueckii	+	−	D (−)
L. lactis	+	−	D (−)
L. bulgaricus	+	−	D (−)
L. casei	+	−	L (+)
L. plantarum	+	+	DL
L. curvatus	+	−	DL
L. brevis	−	+	DL
L. fermentum	−	+	DL
Sporolactobacillus			
S. inulinus	+	−	D (−)
Streptococcus			
S. faecalis	+	−	L (+)
S. cremoris	+	−	L (+)
S. lactis	+	−	L (+)
Leuconostoc			
L. mesenteroides	−	+	D (−)
L. dextranicum	−	+	D (−)
Pediococcus			
P. cerevisiae	+	−	DL
Bifidobacterium			
B. bifidum	−	−	L (+)

Table 2. Properties of *Lactobacillus* sp. LB26

Morphology:

Non-motile rods, Gram-positive, occur singly or in clusters.
Colonies on MRS are white, raised, and circular in form.

Physiology:

Catalase negative, grows in pH range 5.6 to 7.3.
Halotolerant, can grow in 4% NaCl.
Homofermentative–utilizes sugars including glucose, fructose, lactose, mannose, melibiose, and galactose.
Produces D(-) lactic acid and L(+) lactic acid.

We attempted to identify the isolate to species using standard identification methods for the lactobacilli. Taxonomically, the organism is related to *Lactobacillus plantarum* and *Lactobacillus casei* but cannot be classified precisely within either species. Careful determination of its mol% G+C by the thermal melting method was carried out (Figure 1).

Calculation of mol% G+C for LB26 by the equation of Marmur and Doty using *E. coli* DNA as reference gave a mol% G+C of 40 + or - 0.5, which lies outside of the species ranges for *L. plantarum* (44-47 mol % G+C). For now, we are calling the organism *L. pseudoplantarum*. DNA homology studies are being done to accurately identify the organism to species.

To investigate white haze formation, we prepared Cheddar and determined various parameters during ripening including formation of L(+) and D(-) lactic acids and precipitation of white haze (Fig. 2). During starter activity, L(+) lactate was produced rapidly, then dropped in concentration shortly after curd ripening had begun, and then started to be produced again (Fig.2). D(-) lactic acid was produced continuously following the initial starter fermentation. It continued to be produced even after the total bacterial counts in the cheese started to fall (Fig.2). White haze was distinctly noticeable after 2 months ripening at 40°F.

Cheese involves a complex mixed-culture fermentation and the preliminary data could support several means of D-lactate formation. The mechanism believed to occur most commonly is racemization of L(+) to D(-). This may actually occur in cheese where a variety of organisms are present, but physiological studies of our purified *Lactobacillus* isolate (LB26) and enzymic studies in cell-free extracts did not support racemization as the means for D-lactic formation. In vitro, we examined the D- and L-NAD linked dehydrogenase and found that reduction of pyruvate to L(+) lactic acid had obligate

Figure 1. Mol %G+C of *Lactobacillus* LB26

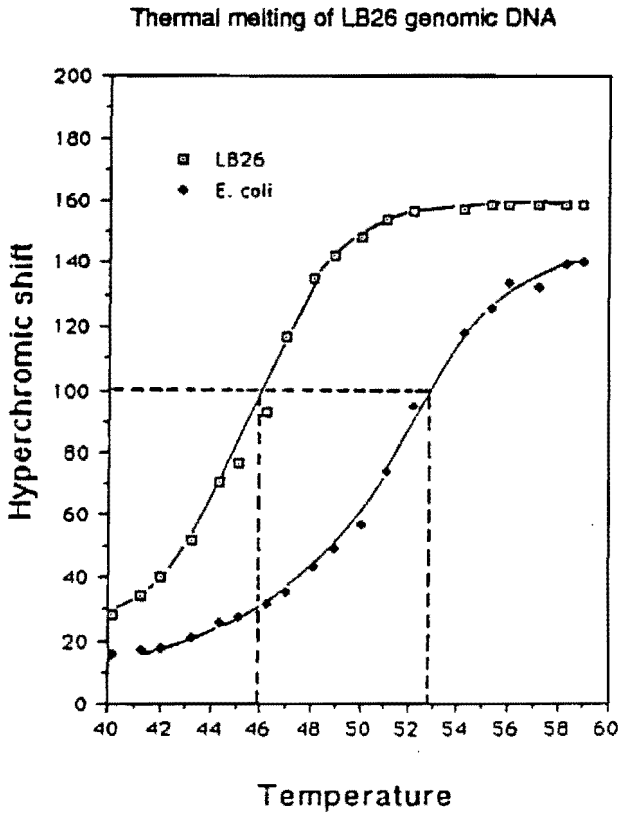
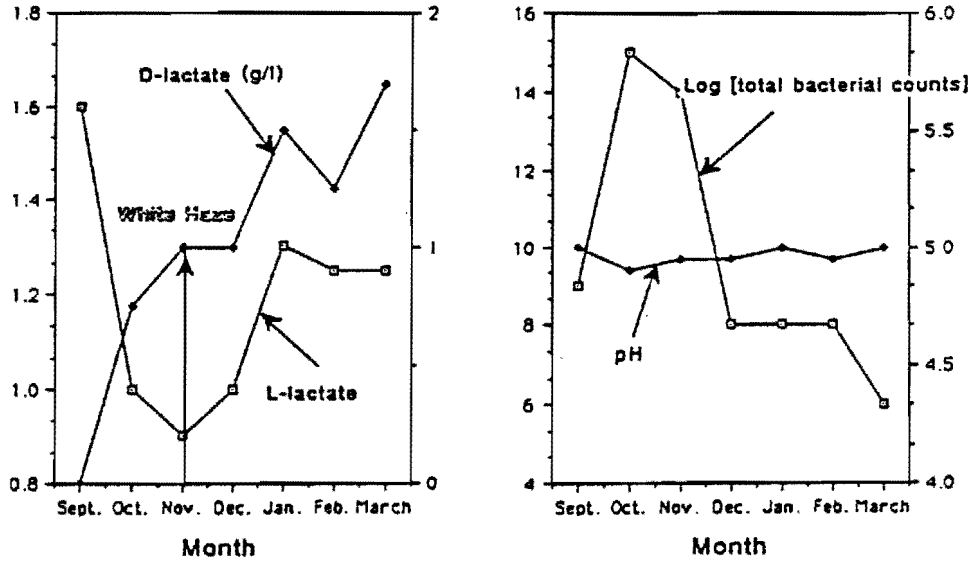


Figure 2. White haze formation during Cheddar ripening.



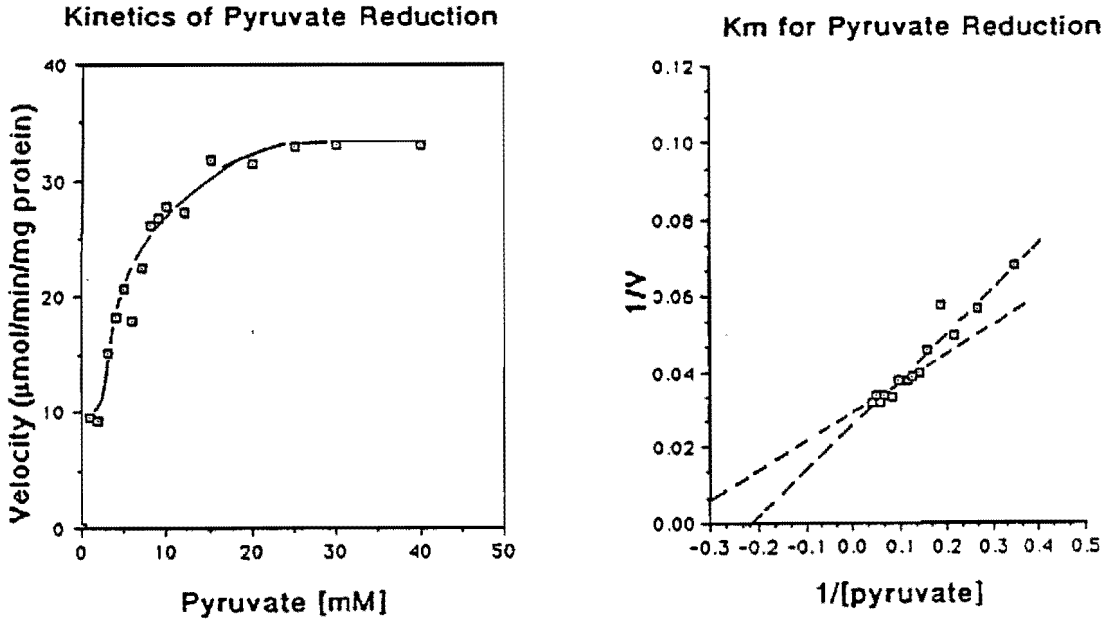
requirements for fructose-1, 6-bis-phosphate, and manganous ion as activators. Interestingly, manganous was bound loosely and could be replaced with magnesium or iron with correspondingly lower activities. The loose binding of the metal suggests that availability of metal may be of importance in activation or control of the enzymes during ripening. Kinetic measurements of pyruvate reduction (Figs. 3 and 4) indicated that the Km for pyruvate was approximately 5-6 mM and that possibly there was a second Km near 1 mM. More data is needed for the lower pyruvate concentrations to determine if the velocity is sigmoidal with two Kms.

As mentioned above, we feel that racemization of L to D lactate is not occurring in the LB26. Characterization of fructose-1, 6-bis-phosphate activated L-lactic dehydrogenase have shown that these enzymes usually do not catalyze the opposite reaction, i.e., the oxidation of lactate. We have tried unsuccessfully to detect oxidation by native polyacrylamide gel electrophoresis followed by a coupled enzyme assay. Also, we have fed L(+) lactate to LB26 but have not seen an increase in the concentration of D(-) lactic acid. Therefore, in the experimental conditions tried, the organism probably does not convert L to D. If this is so, then why is it necessary for the organism to have two lactate dehydrogenase? We considered two hypotheses: 1) either the D-specific enzyme serves to detoxify a toxic metabolite in the same manner that *Escherichia coli* uses a flavin-linked D-lactate dehydrogenase to detoxify methylglyocal, which is highly toxic to aerobic cells, or 2) the D-specific enzyme responds to specific intracellular or extracellular environmental signals. We tried exposing cells of LB 26 to potential toxic metabolites including methylglyocal and D-alanine, but the D-lactate dehydrogenase did not appear to transform these compounds.

We feel that the simplest explanation for onset of D(-) lactate formation is due to the homofermentative organism's requirement to dispose of excess NADH which is generated by sugar or

Figures 3 and 4. Kenetics of pyruvate reduction by cell-free extracts from LB26.

Kenetics of pyruvate reduction by cell-free extracts from LB26



amino acid oxidations that generate energy for growth (Fig. 5). When sugar is exhausted in cheese, which occurs soon after the starter fermentation, it would be expected that fructose-1,6-bis-phosphate would decrease to low levels and L(+) lactic acid dehydrogenase would not be active. To dispose of hydrogen under conditions of low FDP, the organism would require an alternative enzyme system which could be the D(-) lactic-specific dehydrogenase.

As a first test of this hypothesis, we examined whether the exhaustion of FDP in growing cells might stimulate D-lactate formation. We examined acid formation and cell growth under strictly anaerobic or aerobic conditions. It would be expected that in the aerobic environment it would grow more rapidly, quickly use its available sugar and rapidly deplete its intracellular content of FDP. D(-) lactate formation should then be induced. These are the results which we found (Fig.6). Calculation of the carbon balance showed that all could be accounted for in the aerobically-grown cells, but only 88% was recovered anaerobically (Table 3). Anaerobically, other fermentation products may also be formed.

In conclusion, we feel that D(-) lactate formation provides an escape valve for disposal of hydrogen during energy limitation. Other stresses in the cheese environment including low pH and relatively low Aw which probably also contribute to increased formation of D-lactate. These related stresses and the involvement of other organisms, including species of *Lactobacillus* and *Pediococci*, will be studied in future experiments.

Figure 5. Homofermentative pathway of glucose fermentation by *Lactobacilli.*

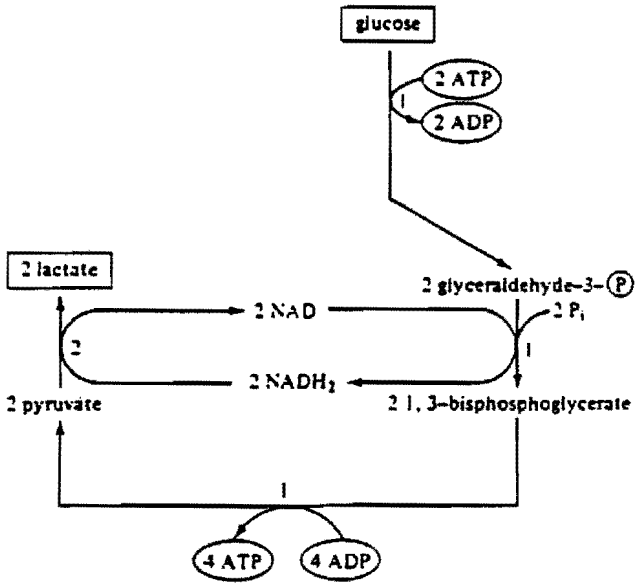
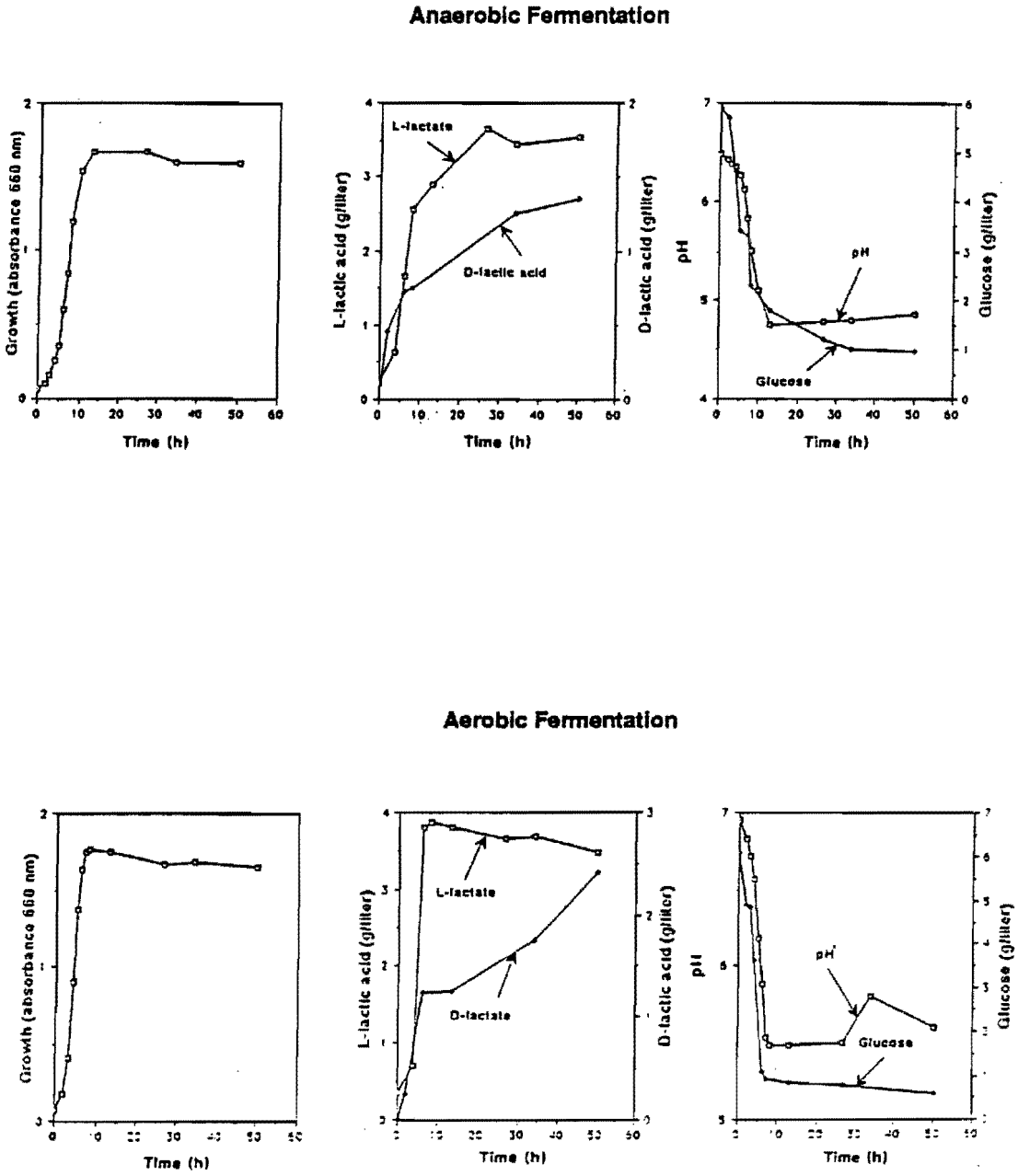


Table 3. Glucose fermentation of *Lactobacillus* sp. LB-26.

Condition	Glucose Consumption (mmol/flask)	Lactic Acid (mmol/flask)		Carbon & Electron Recovery (%)
		L-form	D-form	
Aerobic	11.32	18.2	6.4	108.2
Anaerobic	12.34	18.4	3.6	88.9

Figure 6. Aerobic and anaerobic fermentations by *Lactobacillus* LB26.



Project Title:

Protease and peptidase activity of *Micrococcus* species.

Personnel:

E.H. Marth, Professor, Food Science Department and T. Bhowmik, graduate student, Food Science Department.

Funding:

Center for Dairy Research/Walter V. Price Cheese Research Institute.

Objectives:

To examine the intracellular protease and peptidase enzyme activity of different strains/species of micrococci, the pattern of proteolysis during their growth in skim milk or ultrafiltered milk, and proteolysis caused by the action of crude cell-free extracts on skim milk.

Procedures and Results:

Three cultures of *Micrococcus caseolyticus* ATCC 13548, *M. freudenreichii* ATCC 407, *M. luteus* ATCC 10240, *M. varians*, *Micrococcus* sp. ATCC 8459, *Micrococcus* sp. MK, *Micrococcus* sp. C1, *Micrococcus* sp. ATCC 398 and *Micrococcus* sp. LL3 were used in this study. The cells were grown in 1% tryptone broth for 24 hours at 30°C with continuous agitation. The pH of the broth was maintained at 6.5 by addition of 1 N HCl using a Chemtrix type 45 AR pH controller. Cells were harvested by centrifugation at 6000 x g for 10 minutes at 4°C, washed twice with cold saline (0.85%) solution and deionized water, respectively. The pellet was resuspended in PGM buffer (50 mM potassium phosphate, 5% glycerol, 10 mM $MgCl_2$), mixed with an equal volume of glass beads (0.10 mm) and homogenized in a Braun MSK homogenizer for 8 minutes at 4°C to break the cells. The glass beads were removed by filtration, the filtrate was centrifuged at 15,000 x g for 15 minutes at 4°C and the supernatant fluid was used as crude cell-free extract.

The protein content of the crude cell-free extract was determined by the Bio-Rad method using bovine serum albumin as the standard. Aminopeptidase enzyme activities were determined by measuring the liberated p-nitroanilide from L-lysine-p-nitroanilide, L-leucine-p-nitroanilide, L-alanine-p-nitroanilide, and L-methionine-p-nitroanilide used as substrates. Iminopeptidase activity was measured using L-proline-p-nitroanilide as substrate. Protease, endopeptidase, and dipeptidase enzyme activities were measured using N,N dimethyl casein, Benz-arg-p-nitroanilide and Glycyl-Tyrosine as substrates respectively. Results of intracellular protease, amino/imino peptidase, dipeptidase and endopeptidase enzyme activities are presented in Table 1.

Fresh pasteurized skim milk was sterilized by adding 1% (w/v) hydrogen peroxide (30%) and inoculated with about 6×10^6 cells/ml from a 24-hour-old culture previously grown in sterile skim milk and incubated at 30°C in a rotary shaker at 200 rpm. A flask containing sterile skim milk without inoculum served as a control. The samples were taken at a predetermined time interval and were analyzed for total count, pH, and proteolysis. The ultrafiltered milk (5-fold concentration) was sterilized in the same way except 5% (w/v) hydrogen peroxide was added. Proteolysis of skim milk and ultrafiltered milk was evaluated by polyacrylamide gel electrophoresis using a 15% running gel. Disappearance of original protein band as determined by densitometric scanning was considered as evidence of proteolysis. Time required for complete degradation of casein (αS1 and β), aerobic plate count and pH value at the end of proteolysis by the different strains of micrococci grown in skim milk and ultrafiltered milk are given in Table 2.

One milliliter of crude cell-free extract (filter sterilized) was added to 5 ml of sterile skim milk and incubated at 30°C. Samples were taken at a predetermined time interval and analyzed for proteolysis by polyacrylamide gel electrophoresis. It was observed that ß-casein was preferentially degraded by the action of crude cell-free extracts on skim milk.

Table 1. Protease and peptidase specific activity[a] of different strains of micrococci grown in 1% tryptone broth at 30°C.

Strain	LYS-p-NA[b]	LEU-p-NA[c]	PRO-p-NA[d]	ALA-p-NA[e]	MET-p-NA[f]	B-ARG-p-NA[g]	CASEIN[h]	GLY-TYR[i]
M. freudenreichii ATCC 407	3505 ±55	251 ±15	253 ±20	283 ±20	±344 11	29 ±14	54 ±22	403 ±54
Micrococcus sp. ATCC 398	291 ±61	24230 ±387	2547 ±547	774 ±32	2732 ±258	144 ±29	22 ±3	125 ±23
M. caseolyticus ATCC 13548	25 ±1	7 ±2	4 ±0.4	39 ±9	3 ±0.1	— —	3 ±1	— —
Micrococcus sp. ATCC 8459	479 ±26	67 ±5	32 ±7	49 ±11	166 ±19	— —	34 ±9	60 ±3
M. variana	155 ±4	57 ±6	30 ±4	154 ±10	148 ±13	26 ±1	21 ±3	34 ±2
Micrococcus sp. MK	26 ±5	24 ±7	— —	150 ±2	28 ±4	— —	24 ±2	55 ±4
Micrococcus sp. LLJ	1018 ±24	240 ±24	212 ±78	330 ±30	390 ±16	— —	132 ±12	211 ±16
Micrococcus sp. C1	189 ±19	84 ±13	48 ±8	134 ±9	166 ±6	33 ±8	123 ±7	159 ±10
M. luteus ATCC 10240	6 ±0.2	1 ±0.1	5 ±0	19 ±5	4 ±1	5 ±0.5	10 ±3	16 ±2

[a]Data based on 6 analyses made on cells from each of 3 fermentations. Specific activity is expressed as umoles of substrate released/min/mg protein at 37°C.

[b]L-Lysine-p-nitranilide
[c]L-Leucine-p-nitroanilide
[d]L-Proline-p-nitroanilide
[e]L-Alanine-p-nitroanilide
[f]L-Methionine-p-nitroanilide
[g]Benz-arginine-p-nitroanilide
[h]N,N,dimethyl casein
[i]Glycyl-cryrosine

Table 2. Time required for proteolysis, aerobic plate count, and pH values at the end of proteolysis by different strains of micrococci grown in skim milk and UF milk at 30°C.

Strains	Time for proteolysis[a] (h, α_{s1}, and β)	Plate count (Log CFU/ml)	pH at end of proteolysis[c]
Micrococcus sp. ATCC 8459	120	8.75	4.46
M. luteus ATCC 10240	120	8.60	6.90
M. freudenreichii ATCC 407	72	7.45	5.87
M. varians	60	9.38	7.16
Micrococcus sp. LL3	36	8.81	7.35
Micrococcus sp. C1	21	9.49	6.53
M. caseolyticus ATCC 13548	12	9.38	6.30
Micrococcus sp. MK	9	8.99	6.49
M. caseolyticus ATCC 13548[b]	23	9.71	5.93

[a]Based on disappearance of original protein bands as observed by polyacrylamide gel electrophoresis.

[b]Grown in UF milk.

[c] Initial pH value = 6.51

Impact of Research:

Micrococci occur in raw milk as well as in the secondary cheese flora. This study indicates the possible role this group of microorganisms might play during the cheese ripening process and these results would be helpful to select a strain/species on the basis of protease/peptidase enzyme activity for use as an agent in accelerated ripening of cheese.

Project Title:

Regional milk supply response in the United States.

Personnel:

E.V. Jesse, Professor, Department of Agricultural Economics, Jean-Paul Chavas, and A.F. Kraus (Research Assistant), Department of Agricultural Economics.

Funding:

USDA, SEA (Hatch)

Objectives:

Evaluate factors affecting milk supply response on a regional basis. Relate ongoing shifts in regional comparative advantage to changes in relative milk prices, costs of production, dairy farm structure, and productive efficiencies. Simulate changes in regional milk production given likely changes in economic conditions and proposed changes in U.S. dairy policy.

Results:

State-level supply relationships were estimated for both cow numbers and milk production per cow. Both relationships included milk/feed price ratio, milk/slaughter cow price ration, and risk as explanatory variables along with other relevant factors. States were combined into 9 U.S. regions. Six scenarios were defined under which the effect on milk production of specified changes in milk prices, feed prices, slaughter cow prices, and risk were simulated over a 30 year interval.

The results showed substantial regional differences in dairy farmers' response to included variables. Milk production increased most rapidly in response to higher milk prices and decreased most rapidly in response to higher feed prices in the Southwest region. In general, regions with low sunk costs, high resource mobility, and many alternative uses for capital and labor exhibited a more rapid response to changing economic conditions in dairy. The Upper Midwest responded slowly because of generally opposite characteristics.

Impact of Research:

The model of regional supply response was used to assess the possible impact of regionalizing the dairy price support program along the lines proposed by Senator Patrick Leahy. Through producer assessments, Leahy's proposal would severely penalize dairy farmers in the Pacific Coast states because of their disproportionate share of surplus dairy product sales in recent years. Other regions would have smaller assessments, with the Southeastern states practically unaffected. Under the assumptions employed, the proposal would cause milk production to fall rapidly in the Pacific region. Other regions would gain market share proportional to their current share.

Chapter 3 Worldwide Information and Technology Exchange Program

Introduction

The objectives of the Worldwide Information and Technology Exchange Program are to facilitate the exchange of information and technology between CDR and other dairy research organizations and between CDR and the dairy industry. From July 1987 to June 1988, the following mechanisms were employed to reach these objectives: a scientist exchange program, a seminar series, an annual conference, and dairy industry workshops and seminars.

Scientist Exchange Program

WITEP gives CDR research program participants the opportunity to invite researchers from other organizations to come to CDR and collaborate with them during a research visit that can last from days to several months. Funding for scientist exchanges enables CDR to have a stronger, broader research team and produces valuable cooperative research ties with other organizations.

WITEP also provides funds to send CDR staff members and CDR research program participants to go to other research organizations for research training or visits. CDR's scientist exchange program is a means of developing a network of commmunication with other research organizations.

Scientist Exchanges

To UW-Madison:

Dr. Morsi El Soda, Professor, Department of Agricultural Industries, Alexandria, Egypt. Research Area: Use of enzymes in cheese ripening. Period of stay: July 1987 - September 1987.

Demetrios Papageorgiou, Professor, Department of Food Hygiene and Technology, Aristotelian University, Thessaloniki, Greece. Research area: *Listeria monocytogenes* in the manufacture of Blue cheese and Feta cheese. Period of stay: October 1987 - September 1988.

Dr. Moustafa El-Shenawy, Professor, Department of Food and Dairy Science, National Research Center, Cairo, Egypt. Research area: The inhibition and inactivation of *Listeria monocytogenes* by preservatives. Period of stay: April 1988 - September 1988.

Betty DeHaan, M.S. degree student, Food Science and Technology, Wageningen Agricultural University, The Netherlands. Research Area: The use of enzymic reactions in supercritical fluids. Internship with Dr. Kirk Parkin. Period of stay: September 1987 - November 1987. See Dr. Parkin's synopsis of Ms. Dehaan's report on page 109.

From UW-Madison:

Brian Riesterer, CDR Research Associate. Institution visited: Bernese Dairy Institute, Switzerland. Research Area: Manufacture of European cheeses. Period of stay: August 4 - 31, 1987. See the scholar exchange report on page 111.

Sending CDR program participants to workshops and training seminars:

Lee Jensen, CDR Research Associate. Seminar attended: Food and Biochemical Engineering with special emphasis on applications of ultrafiltration. University of Illinois at Champaign-Urbana. October 28 and 29, 1987.

Dr. Ken Lee, Associate Professor, Food Science Department and Lauren Jackson, PhD graduate student, Food Science Department. Shortcourse attended: Gum Chemistry and Technology in the Food Industry. November 18-20, 1987. Chicago, Illinois. Sponsored by: American Association of Cereal Chemists. See the scholar exchange report on page 110.

Scientist Exchange Reports

This section includes research reports for some of the CDR scientist exchanges. Research reports for exchange scientists that are in the form of a published research paper are found in Chapter 2. On the following pages are the reports for Betty deHaan, Dr. Ken Lee, and Brian Riesterer.

Scientist Exchange **Report for Betty deHaan**

Betty deHaan came to work in this department through the CDR-WITEP program. She received her B.S. degree in Food Science and Technology and Wageningen Agricultural University in The Netherlands. Upon her arrival in Madison, she was in the process of pursuing her M.S. degree in Dairy Technology from the same university and this internship fulfilled a portion of the degree requirements. She successfully completed her studies for the M.S. degree in Spring, 1988 and is currently employed in the food industry in The Netherlands. One of her first assignments was a processing plant site visit to Bangkok, Thailand, where she expects to be for several months.

Betty initiated some of our work on the lipase modification of butterfat in supercritical carbon dioxide. Her primary responsibility during her three month stay in our department was to set up analytical procedures for measuring free fatty acid release from butterfat treated with lipase. She was successful in improving upon an existing HPLC method for free fatty acid analysis in dairy projects. Previous methods were founded on the conversion of free fatty acids into their bromophenacylesters derivatives followed by separation on a reverse phase column. The previous methods did not allow complete separation of all free fatty acids in dairy projects; different conditions had to be used to efficiently separate the long-chain or short-chain fatty acid. Therefore, a complete analysis of the free fatty profile in dairy products by HPLC required two HPLC runs. Betty modified the method such that all of the free fatty acids in lipase-hydrolyzed butterfat could be quantified in a single chromatographic separation. The HPLC method was improved by modifying the elution gradient pattern and exercising strict control of column temperature during the chromatographic separation.

Betty also had the opportunity to run some preliminary experiments on the nature of the action of lipase on half-and-half as influenced by reaction temperature. This set of studies was designed to provide background information on lipase activity in aqueous systems to allow for the comparison of the effect of using supercritical carbon dioxide as the reaction medium. Her preliminary results indicated that temperature appeared to affect the specificity of lipase action toward stearic acid. Increasing proportions of stearic acid were hydrolyzed as the temperature was increased form 5° to 35°C. The significance of this observation, which to our knowledge has not been previously reported, resides in the negative impact that free stearate has on the flavor of hydrolyzed diary products (cheese).

Jerry Elliott, an M.S. student in this department is continuing these studies. He is in the process of repeating some of Betty's experiments to provide verification. His next step will be to examine lipase action on butterfat in non-aqueous media (hexane, cyclohexane, chloroform) and finally in supercritical carbon dioxide.

Scientist Exchange **Report for Dr. Ken Lee and Lauren Jackson**

Gum Technology Short Course, Chicago

The 14th annual short course on gum chemistry and technology was attended by Ms. Jackson and Dr. Lee with support from WITEP. The course was presented by the American Association of Cereal Chemists on November 18-20 in Chicago. The course was taught by experts in hydrocolloid chemistry from both industry and academia and was targeted towards R&D applications of gums in the industry.

Our interest is in applications of soluble hydrocolloids in dairy products. The course faculty included Dr. BeMiller who discussed the rheological properties of gums and mechanisms of gelation. Dr. Whistler (Purdue) gave information on properties of polysaccharide polymers. Dr. Pettitt (Kelco) presented microbial gums, xanthan, gellan and alginate, a red seaweed gum. Gellan is awaiting FDA approval and forms brittle gels that are desired in reduced calorie foods. Alginates react strongly with calcium and other ions to form gels which may limit their use in milk systems. Propylene glycol alginates are more hydrophobic and less calcium reactive, properties which enable them to be used in oil-in-water emulsions and some dairy products. Dr. McIntyre (Hercules) presented CMC, guar and locust bean gums. CMC is chemically modified cellulose that is more soluble, and is commonly used in ice cream to control crystal growth. Guar and locust bean gums are inexpensive, viscous food thickeners. Dr. Barfoot (FMC) and Dr. Coffey (Dow Chemical) presented microcrystalline cellulose and methyl cellulose. Hydrated microcrystalline cellulose forms dispersions that have textural properties of an oil/water emulsion. Microcrystalline cellulose may be used in reduced fat products and for anticaking in shredded cheese. Methylcellulose derivatives have a unique ability to form gels when heated. Dr. Hoefler (Hercules) described pectin and carrageenan uses in food. Pectins form gels in the presence of calcium ions. Carrageenans interact strongly with proteins and calcium ions to form gels and is used in chocolate milk to suspend cocoa particles. Starches are by far the most commonly used thickener due to low cost and wide food compatibility. Dr. Rutenberg (National Starch) demonstrated physical properties of some starch solution. An interesting new product is a starch with hydrophobic side chains.

This short course was a useful survey of polysaccharide gums in processed foods. Gum chemistry and nutritional effects were not discussed. Possible applications of soluble hydrocolloids in new dairy foods may be a focus of future CDR research.

Scientist Exchange **Report for Brian Riesterer**

Acknowledgements
This research leave was made possible through the cooperation of Center for Dairy Research Worldwide Information and Technology Exchange Program, and Dr. Heinz Rothlisberger, Director of the Bernese Dairy Institute. Dr. Rothlisberger was a visiting professor at UW-Madison for the first six months in 1986. Through Dr. Rothlisberger's efforts, I was able to tour, study, and work in various dairies, museums, cheese factories, and schools. Below I have listed dates, places, and people I met during my trip.

8/5/87	Professor H.U. Markwalder - Bernese Dairy Institute. Discussed Starter Cultures, quality control programs, feeding programs, milk composition, laboratory equipment.
8/6/87	Rolf Nufer, Director of Emmental AG-Exporter of Swiss Cheeses, Zollikofen-Bern. Discussed the grading, payment, and exporting processes.
8/7/87	Hans Schar - Federal Institute of Dairy Research, Liebefeld-Bern. Discussed Swiss Cheese, research manufacturing procedures, and techniques.
8/7/87	Bernese Dairy Institute - Bern. Spoke with the cheesemakers and students about the manufacturing procedures for Cheddar cheese.
8/10/87	Erich Hirshi - Master Cheesemaker, Amsoldingen (South of Thun). Emmentaler factory which also produced yogurt, butter and Ziger. I worked there from August 10th to the 25th everyday for room and board.
8/12/87	Karin Ayer - Microbiologist, Verbandsmolkerei, Thun. Discussed fluid milk processing and Edam cheese manufacturing.
8/15/87	Studied a mountain cheese factory and a Simmental cattle auction near Gstaad.
8/17/87	Bergi AG, Bern. Discussed grading and exporting of Emmentaler.
8/19/87	Toured Emmental Museum in Kiesen. Studied the origin of Emmentaler cheese.
8/29/87	Traveled by train from Bern, Switzerland to Ede, Netherlands. Met Theo Jeurnink a Researcher at NIZO - The Netherlands Research Institute.
8/31/87	Dr. Wout Heinen - NIZO - surface fouling researcher.
8/31/87	Dr. M.D. Northolt - Microbiologist - NIZO. Discussed the starter culture systems for Gouda cheese.

This program has provided me with a lot of new information and valuable practical experience. Dr. Rothlisberger and I discussed the possibility of continuing an exchange program between Switzerland and Wisconsin and he was highly in favor of the program. I see this as an excellent opportunity to bring the best of two worlds together for a common cause, to increase the consumption of dairy products.

I landed in Zurich, Switzerland not able to speak the language (German, French, Italian, or Romanich), and realized there was no turning back. I found Dr. Heinz Rothlisberger and proceeded toward Bern, Switzerland. This is where I would stay for a few days until I could start at the Hershi Kaserei (cheese factory). The Hershi Kaserei is located in the Bernese Oberland, south of Thun in a town called Amsoldingen, which is located at the foot of the Stockhorn Mountain.

Before I report on what I learned about Emmental, I will describe the system of milk production and handling and also explain how the composition and production of milk differ in Switzerland and the Upper Midwest of the United States. In Switzerland, milk production is heavily regulated and subsidized by the government. If the milk is for Emmentaler cheese, the farm can't have any silos, because *Clostridium tyrobutyricum* will grow in improperly fermented silage. This bacteria can cause blowers or very large areas of gas production which ruins the cheese, so by eliminating silos, this defect is eliminated. The farmers have a quota system, where each farm can only produce a certain amount of milk. If the farm produces more milk, the farmer will receive a reduced amount of money for the extra milk. The price of milk is the same for everyone so the farmers only get deducted when they have lower quality milk. If the Standard Plate Count (SPC) was > 80,000 c.f.u./ml of milk or Somatic Cells Counts (SCC) are > 200,000/ml of milk, the farmer would receive a deduction in their paycheck. The milk is tested for antibiotics only if there has been a problem with the cheese manufacturing. If antibiotics are found, the farmer must pay for the damages to the cheese factory or the loss of all the cheese made with the milk containing antibiotics that day. These regulations are required to make sure the country of Switzerland produces the highest quality Emmentaler cheese possible.

A brief explanation of Emmentaler should be given to give a better understanding of the cheese. Emmentaler is better known as Swiss cheese. It has a sweet nutty-like flavor and has holes or eyes about 1 inch in diameter. The fat in the dry matter is a minimum of 45%, with a fat on a wet basis of 30 - 31%. The moisture is 35 - 37% and the cheese has a final pH of 5.2 - 5.3. The origin of Emmentaler can be traced to a small town of Kiesen in the Emmental Valley, from which the cheese was named. The early cheese factories were very simple and usually were run by one man or family. The raw milk was heated in a copper kettle over a fire to 88°F. The bacterial starter consisted of old coagulated milk from the day before; this is better known as back slopping. Rennet is an enzyme used to coagulate milk and is found in the calf stomach. The early cheesemakers would soak rolled calf stomachs in the milk to extract the rennet for the milk coagulation. These early cheeses were round in shape and weighed 180-200 pounds. But before I give any more details about Emmental, it would be better to look at the differences in milk composition between the United States and Switzerland.

The differences in milk composition between the United States and Switzerland are based on the breed of cattle and the type of feed. The Simmental cattle in Switzerland are quite different from the Holstein cattle in the U.S. The Simmental is a larger and much more beefy animal than the Holstein, and produces less milk but has higher milkfat (4.0*%) and protein (3.2 - 3.4%) content. The Simmental are raised in the Alps (Mountains) and are purchased for 3,000 - 6,000 dollars depending on the buyer. The buyer does not only look at the milk production but also the beef or meat potential, because later the older animals or low producers will be sent to market to be slaughtered for food. The Swiss have done some crossbreeding between the Holstein and the Simmental but the won't crossbreed and further than 50:50 Simmental:Holstein. The Holstein is fine boned and doesn't endure the ruggedness of the Alps; so the Simmental cross can't be greater the 50% Holstein. The Swiss feed very little grain and very few dairy cows fed concentrated protein or soybean meal. Instead, the animals are allowed to graze most of the year and fed dry hay only during the winter or rainy seasons. The hay consists of grasses and clovers, with little alfalfa, so the protein level of the hay is lower than that of the alfalfa hay in the U.S. The Swiss methods is just the opposite of the process for the dairy cows in the United States. The cows are confined fed alfa and high protein energy rations.

The milk is handled differently in Switzerland. There the milk is hauled in cans while it is still warm and brought to the factory twice a day. The handling of warm milk (greater than 59°F) reduces damage to the fat globule membrane. The warm milk has a more pliable fat globule membrane and so it will not break as easily as it would if the milk were cold. The damage to the fat globule membrane will increase when the milk is pumped or handled cold (40°F or less), because the fat globule membrane is more fragile. The milk must have a SPC of < 80,000 c.f.u./ml of milk and a SCC of < 200,000/ml of milk. In the United States, for Grade A milk, the SPC is < 100,000 c.f.u./ml of milk and a SCC OF < 1,000,000/ml of milk. The Hershi cheese factory had 35 patrons (farmers) with production from each patron ranging from 28 - 900 pounds per day.

The manufacture of Emmental cheese at the Hershi cheese factory began the night before with the intake of the patron's milk. The fresh raw milk was filtered through a fine wire mesh screen into a scale. The weight of the milk was recorded in one of two ways, either the weight was written down on paper, or a computerized scale gave the farmer a print out of their milk weight. After weighing, the milk was pumped through an ice bank to cool the milk to 36°F; filtered through a sock type filter, and then run into the copper lined vat where the milk sat until morning. In the morning, water was added at a rate of 9% of the final volume of milk for that vat of cheese milk. The final pH of the cheese can be controlled by the addition of water. There are two factors involved for the addition of water. The first is to dilute the amount of lactose in the cheese milk, because lactose will be converted to lactic acid by the starter bacteria. The second reason is to control any acid development that may have occurred over night while the milk sat in the vat. The fresh morning milk was run through a clarifier/separator to remove cream which was later used to make butter. The skimmed morning milk was mixed with the evening milk to standardize the cheesemilk to 3.0% milkfat. The cheesemilk was then heated to 88°F before the starter bacteria were added.

The starter culture system for the Emmental cheese has evolved with the development of Emmental cheese. The factory receives the starter culture once a week from the Central Research Laboratory in Liebefeld. Each day the cultures are propagated in sterile skim milk and used for the next day of cheese making. The starter system consists of six strains of *Streptococcus thermophilus*, five strains of *Lactobacillus helveticus* and *L. bulgaricus*, and four strains of *Propionibacteria*. The inoculation rate was .1 % of *thermophilus* and .1 % of *Lactobacillus* with only a small

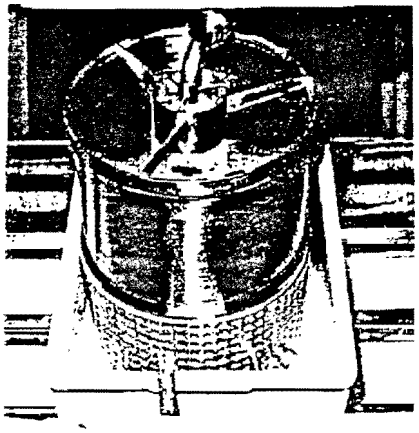
amount of *propioni-bacteria* (10 ml/10,000 lbs. of milk) added. A fermentation test sample of the inoculated cheesemilk was removed from the vat. The fermentation test is a post cheesemaking indicator of the cheesemilk quality and potential cheese development. If the fermentation is good, the milk coagulate forms a solid core in the test tube. The acid produced in the test tube should be .18 - .22% T.A. at 10.5 hours and .38 - .42% T.A. after 22 hours. If there is a problem, the cheesemaker then tests the individual patron's milk to determine which patron's milk is the problem. For the patron fermentation test, add 10 ml of patron's milk to a sterile test tube and add 0.1 ml of starter, then allow the tube to incubate at 99°F overnight (2). Good quality milk will form a solid coagulum.

The rennet is added to the cheesemilk 45 minutes after adding the starter. Enough rennet is added to obtain a forty minute set. The coagulum firmness is tested by moving some of the top coagulum away and a knife is inserted and lifted to break the coagulum. If the coagulum is ready to cut it will break clean with no particles of milk sticking to the knife. The next step is to cut the coagulum with vertical wire knives attached to the rotating arms in the round vat. The speed of the cutting knives will increase until the coagulum is cut evenly or about the size of a • inch cube. The curd and whey slurry is stirred for 30 min. before cooking begins.

Cooking the curd means increasing the curd slurry temperature from 88°F to 127.5°F over a 30 minute interval. When the temperature reaches 104°F another 3.5% water is added to the cheese vat. The addition of water will control the rate of cooking, the lactose removal, and prevent case hardening of the curd. If the curd is cooked too fast, the curd will become hard on the outside which traps lactose and water on the inside of the curd, and the cheese will be low in pH and high in moisture. The curd slurry is cooked to 127.5°F and held until the proper firmness is reached. This is determined by removing some of the curd and firmly squeezing the whey out and then breaking the squeezed curd to determine how well the curd is cooked. If the curd breaks apart easily and retains its curd identity, it is thoroughly cooked. Once the proper firmness is reached the curd slurry is cooled to 123°F and held there for 15 min. before it is pumped over into the hoops. The curd slurry was cooled to 123°F because this is the optimum growing temperature for *S. thermophilus*.

The curd is ready to be pumped into the hoops. The hoops are made of stainless steel consisting of two major parts (Figure 1) (1).

Figure 1. Hoops.



This design of hoop allows the free drainage of whey while retaining the curd. The retained curd will settle to the bottom and be pressed under the whey. The whey in the top portion of the hoop provides enough pressure to press the curd in the bottom of the hoop. After 15-20 minutes, the top section is loosened slightly so the whey can drain, leaving the curd in the bottom section. It is important to have a uniform filling rate for each hoop so that the cheeses will weigh approximately the same. The bottom third of the hoop is insulated to prevent rapid cooling of the cheese. If the cheeses are different sizes, have uninsulated hoops, or the room temperature varies greatly, the cooling rate will vary from cheese to cheese. These variations result in cheese defects in uniformity of flavor, body and appearance. The ideal situation is to have a constant room temperature with the same amount of cheese in each insulated hoop. The differences in cooling rates can be examined below (Figure 2) (1).

Figure 2. Cooling Rates.

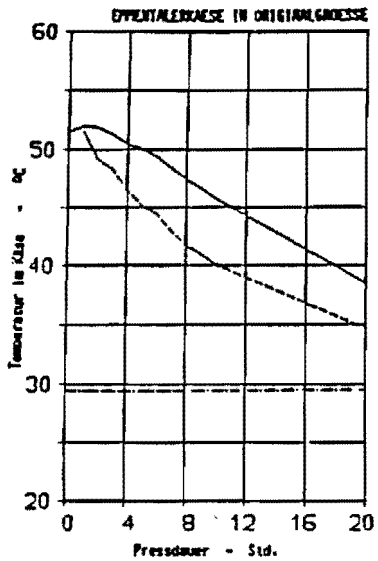


Abbildung 19: Temperaturverlauf in Emmentalerkäse während des Pressens (Laibgewicht = 85 kg)
Temperatur im Zentrum
Temperatur in Randzone
Pressraumtemperatur

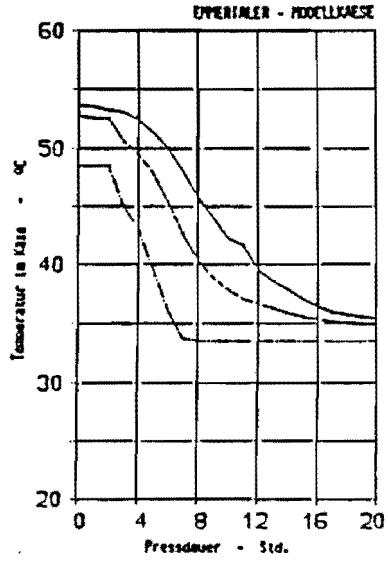


Abbildung 20: Temperaturverlauf in Emmentaler - Modellkäse während des Pressens (Laibgewicht = 11.5 kg)
Temperatur im Zentrum
Temperatur in Randzone
Pressraumtemperatur

Before the cheese is pressed, the factory number, vat number, and date, which is made of casein, is placed on the top of the wet curd. They are embedded into the cheese for later identification. The hoop cover is placed on top of the hoop and locked tight. Pressing begins at 1.35 psi and is gradually increased to 3.69 psi in 2.5 hours. The cheese hoops are turned every 15 minutes for the first hour then once every half-hour for the next 17 - 18 hours automatically. This allows

proper whey drainage and reduces the chance of whey entrapment. The pressing is run by a computerized program which determines the amount of pressure on the hoops and the intervals of turning. Marked acid development in the cheese takes place while the cheese is pressed overnight. After two hours of pressing the acid in the cheese should be .09 - .10% T.A., after four hours .18 - .20% T.A., and after twenty hours all the lactose should be converted to lactic acid.

The next morning the cheese is removed from the hoops and a sample is cut off the outside for testing. The strip is about two inches wide and three-eighths inch deep. This sample is then taken to the laboratory and examined for L(+) and D(-) lactic acid, aminopeptidase activity and composition. *S. thermophilus, Streptococcus lactis* and *Streptococcus cremoris* produce 100% L(+), while *L. helveticus* only produces 70% L(+). *Lactobacillus lactis* and *bulcaricus* produce 100% D(-) and *L. helveticus* produces only 30% D(-). The final balance in a good cheese should be L(+) 40 - 50% of the total acid and the D (-) being 50 - 60% of the total acid, where the total lactic acid is 130 micro moles/g cheese (3). When the leucin aminopeptidase (LAP) is measured, the desired level of activity is less than three, because if the LAP is above three the cheese will be white and nonelastic (6). The desired composition on the cheese should be: fat 31.0%, moisture 37.0% and pH 5.2 - 5.3 (4,7).

The whey cream obtained from separating the whey is pasteurized and churned into whey cream butter. The remaining whey is pasteurized before the farmers pick it up and is then fed to their hogs. A soft cheese called Ziger or Ricotta is made by heating the skimmed whey to 195°F and adding enough lactic acid to precipitate the albumin proteins (7). Once the protein is precipitated, it is gathered in a cheese cloth and hung so the whey can drain freely. By evening the cheese is firm, cooled, and ready to be sliced.

The 180 pound wheels of Emmental cheese are stacked in a vertical stainless steel rack and submerged in a 22% salt brine for two days. When the cheeses are removed, the exterior is scrubbed with coarse stainless steel wool, to make the outside of the cheese smooth. The smooth cheeses stay in the cool room (50 - 57°F) for three weeks before they are moved to the warm room (68 - 73°F). The cheese will ripen in the warm room for 4 - 6 weeks, depending on the rate of eye development. The eyes should develop to be about one inch in diameter and evenly distributed throughout the cheese. The cheeses that are shipped to France have smaller eyes, while cheeses shipped to Italy have large eyes. Once the proper eye size is developed, the cheeses are moved to the cold room (50 - 55°F) to finish aging. The cold room must be cold enough to prevent growth of propionic bacteria or else the gas formation will split the eyes. This is not the end for the Emmental cheese; it must still be graded and distributed.

A cheese buyer will visit the cheese factory once a month and select the best quality cheeses that are over 172 pounds with good eyes, flavor, appearance, and texture. These are taken to the central warehouse and stored for final grading and aging. The cheeses that are not selected by the buyer are kept by the cheesemaker and if the family has a store, the cheeses will be sold there. Before the cheeses are exported, the cheeses in the warehouse are graded again by three official graders. They use the 20 point system, which consists of four categories (flavor, texture, eyes, and appearance) at five points each. The cheesemaker is paid more for each quality point over 17 points per cheese. If the cheese is less than 17 points, it will be rejected for exporting

and used for processed cheese. If cheeses of 17 points or less exist, fieldpersons from the warehouse and Liebefeld will go to assist the cheesemaker and help correct the problem. If the problem is not corrected by the cheesemaker, their cheesemaking licence can be revoked.

Many defects are possible in Emmental or Swiss cheese; for more details refer to Dr. Reinbold's work in the Pfizer monologues (4). Some of the possible causes of surface splits are: high acid or low pH in the cheese, and cooking the curd too high and too long, which may alter some of the milk proteins, resulting in an inelastic curd. If the curd is cut so fine, that when hooping there are a lot of fines or dust, the fines will hold and trap whey. This will give areas of poor body and the cheese will have many small holes (5). Poor quality milk may also cause surface splits and gas formation leaving undesirable eyes. If a cheese is considered "fine," it means the body is long or weak, sometimes caused by high moisture or a high fat content of the cheese. This type of cheese will usually have an atypical flavor.

The next time we see the Emmentaler cheese, it is in the deli section of our supermarkets. One exporting company in Switzerland exports 9% of their Emmental cheese to the United States. In the United States, Wisconsin produces 42 million pounds of Swiss cheese per year which is 18.8% of the total U.S. Swiss cheese production. Of the total Wisconsin cheese production (excluding cottage cheese) only 2.3% is Swiss cheese (8). The style of production is 83.8% block and 16.2% in wheels. The percentage of cheese made in wheels dropped from 28.8% in 1984 to 16.2% in 1986 (8). This may have been due to the lack of handling and packaging equipment. A piece of new equipment which may prove to be useful to the Swiss cheese industry is the Caseomatic. The Caseomatic can produce Swiss, Gouda, Edam or Jarlsberg cheeses.

Section II.

I received an invitation from Theo Jeurnink to visit NIZO or the Netherlands Institute for Dairy Research. The manufacture of Gouda cheese is a symbol of the Dutch as the Emmentaler is a symbol of the Swiss. I was only able to spend one day at NIZO, but I was able to talk with microbiologist, Dr. M.D. Northolt about Gouda cheese. In Gouda, the formation of carbon dioxide (CO_2) gas is early after the cheese is pressed verses several weeks later as in Emmentaler. The gas production in Gouda is influenced by the decomposition rate of the citrate in the milk. Citrate is metabolized by *Leuconostoc citrovorium* and *Streptococcus diacetylactis*. These organisms produce CO_2. The level of citrate should start at > 2 g/kg of cheeses and be metabolized to 0.5 g/kg of cheese in 24 hours after pressing the cheese. Gas production must be fast so the CO_2 can saturate the cheese curd and start to gather at weak regions. This should yield good eyes. Factors to be considered in the manufacture of Gouda are the CO_2 production in the first 24 hours after press and the air content of the milk. If the air content is too high, the starter bacteria will not grow very well. If it is too low, there will be no weak spots for the CO_2 gas to collect. As the CO_2 gas is produced by the starter bacteria, it will attach to hydrophobic particles (dirt) in the milk. The CO_2 molecules will collect in weaker areas of the cheese curd and eyes will result. After the eye development, the cheese is put into the cooler for aging and stored until it appears on the dinner table.

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CDR Seminar Series

WITEP sponsors seminars on topics of interest to the dairy industry and to the dairy foods research community. Seminar speakers are suggested by participants in the CDR research program and by the dairy industry.

Dr. Morsi El Soda, Department of Agricultural Industries, Alexandria, Egypt. CDR exchange scientist. "Acceleration of Cheese Ripening by Liposome-Entrapped Enzymes." September 18, 1987.

Brian Riesterer, CDR Associate Researcher. "Comparison of U.S. and Swiss Technologies for Making Emmental Cheese." October 14, 1987.

Dr. Dale Rudd, Department of Chemical Engineering, University of Wisconsin - Madison. "Economic Analysis of the Conversion of Whey to Commodity Chemicals." November 11, 1987.

Dr. George Robertson, USDA, Western Regional Research Center, Albany, California. "Secondary Metabolites from Plant Tissue Cultures." December 8, 1987.

Dr. Roy Rong-Yue Chao, Department of Food Science, Cornell University, Ithaca, New York. "Application of Supercritical Fluid Extraction in the Food Industry." December 11, 1987.

Fred Wegner, Stoelting Bros. Co. "Specialty Cheese Equipment." January 6, 1988

Dr. Eric Johnson, Department of Food Microbiology and Toxicology, University of Wisconsin - Madison. "Antimicrobial Effects of Lysosymes Against Milkborne Pathogens." March 10, 1988.

Dr. John Nelson, Department of Food Microbiology and Toxicology, University of Wisconsin - Madison. "The Role of IDF in Dairying." March 31, 1988.

Dr. Demetrios Papageorgiou, Department of Food Hygiene and Technology, Aristotelian University, Thessaloniki, Greece. CDR exchange scientist. "Technology for Making Feta Cheese

CDR-Sponsored Workshops, Seminars, and Annual Conference

WITEP has been involved with the coordination of seminars which address issues of concern in the Wisconsin dairy industry. In fiscal year 1987-1988, CDR sponsored *Total Quality Systems, A Workshop for the Dairy Industry* and *What is Milk Worth, A Seminar for the Wisconsin Dairy Processing Industry.*

Total Quality Systems, A Workshop for the Dairy Industry
November 19, 1987. Madison, Wisconsin. Sponsored by the University of Wisconsin - Madison, Center for Dairy Research and the University of Wisconsin - Extension, Cooperative Extension Service. In cooperation with The National Cheese Institute. This workshop was designed for supervisory personnel of dairy processing plants. The workshop emphasized training participants of systems used to help ensure quality dairy products, namely, Hazard Analysis and Critical Control Points (HACCP) and Good Manufacturing Practices (GMP).

What is Milk Worth? A Seminar for the Wisconsin Dairy Processing Industry
March 21, 1988. Madison, Wisconsin. Sponsored by the University of Wisconsin - Madison, Center for Dairy Research and the University of Wisconsin - Extension, Cooperative Extension Service. In cooperation with the Wisconsin Cheesemakers Association, and the Wisconsin Dairy Products Association. This seminar was designed to present an overview of the technical and economic issues affecting milk prices.

CDR Annual Conference
The objectives of CDR's annual conference are to address issues of concern to both the dairy industry and to the dairy research community. To best satisfy these goals, on odd-numbered years CDR hosts a conference that focuses on cheese research and technology (the *Cheese Research and Technology Conference*). On even-numbered years CDR hosts a conference that focuses on a specific dairy research topic (the *Dairy Research Conference*).

1988 Dairy Research Conference, Milkfat: Trends and Utilization. April 20-21, Madison, Wisconsin. Sponsored by the University of Wisconsin-Madison, Center for Dairy Research, and the University of Wisconsin-Extension, Cooperative Extension Service. Conference speakers addressed a great array of issues regarding milkfat from the foods manufacturing and research perspective to the nutritional and economic perspectives.

Chapter 4 Dairy-Related Research Publications at the University of Wisconsin - Madison

Introduction

At the University of Wisconsin-Madison, there are numerous academic departments which have research projects investigating dairy-related topics. This chapter provides lists of publications produced by several departments. This is not an inclusive listing but should prove to be a large percentage of what has been done in the past couple years. The intent of this chapter is to indicate the wide array of dairy research being done at the University of Wisconsin-Madison and to illustrate the large resource of personnel and skills the Center for Dairy Research potentially has available to it. Many of these projects are not receiving funds through CDR.

Dept. of Agricultural Economics

Taylor Hall
Chairperson, Edward V. Jesse

The following is a selected list of publications published by the Department of Agricutural Economics. This list is comprised of most, but not all, of the dairy-related publications.

Cambell, Gerald R., Bruce Marion, and Thomas Cox. "Agricultural Marketing: Linking Farmers and Consumers." Bulletin R3400-9. Madison, Wisconsin: U.W. College of Agricultural and Life Sciences (1987): 6 pp.

Chavas, Jean-Paul and Richard Klemme. "Aggregate Milk Supply Response and Investment Behavior on U.S. Dairy Farms." American Journal of Agricultural Economics 68:1 (February 1986): 55-66.

Connor, J .M., D. Heien, J. Kinsey and R. Wills. "Economic Forces Shaping in Food Processing Industry." American Journal of Agricultural Economics 67:5 (December 1985): 1136-1148.

Connor, John, R.T. Rogers, Bruce W. Marion and Willard F. Mueller. The Food Manufacturing Industries: Structure, Strategies, Performance, and Policies. Lexington, Massachusetts: Lexington Press (1984): 472 pp. [Japanese and Spanish editions published in 1985.]

Connor, John M. and Robert L. Wills. "Trends in Marketing and Market Structure in the U.S. Food Processing Industry." Chapter 4 in Economics of the Food Processing Industry, C. McCorkle (editor). San Diego, California: Academic Press (1987).

Conner, J., R. Rogers, B. Marion, W. Mueller, and R. Wills. "The Organization and Performance of Food Manufacturing Industries." Chapter 4 in The Organization and Performance of the U.S. Food System, Bruce Marion (editor). Lexington, Massachusetts: Lexington Books (1986): 533 pp.

Cropp, Robert A. and Edward V. Jesse. "A Single National Milk Marketing Order." Marketing and Policy Briefing Paper No. 10. Madison, Wisconsin: U.W. Department of Agricultural Economics (June 1986): 12 pp.

Cropp, R.A. and E.V. Jesse. "The Minnesota-Wisconsin Price Series: Its Validity and Alternatives." Marketing and Policy Briefing Paper No. 18. Madison, Wisconsin: U.W. Department of Agricultural Economics (September 1987): 17 pp.

Culbertson, John and Willard F. Mueller. "The Influence of Market Structure on Technological Performance in the Food-Manufacturing Industry."In Review of Industrial Organization 7: pp. 40-54 (Spring 1985).

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Graf, Truman F. "Economic Projections for Midwest Dairy Industry." AMPI Dairymen's Digest (Mid-States Region) 16:1 (January 1985): 1 pp.

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Graf, Truman F. "A Mixed Bag for Midwest Dairy Farmers." Land 0'Lakes Mirror 15:6 (June 1985): 1 pp.

Graf, Truman F. "Dairy Price Support Impacts." AMPI Dairymen's Digest (Mid-States Region) 16:6 (June 1985): 18 pp.

Graf, Truman F. "Effect of Price Support Elimination on Farm Milk Prices." Hoards Dairyman 130:17 (September 1985): 994.

Graf, Truman F. "Is One Grade of Milk a Solution to the Price Squeeze?" Hoards Dairyman 130:23 (December 1985): pp. 1277-1296.

Graf, Truman F. and others. "Increasing Returns to Dairy Farmers by Generic Promotion of Milk and Dairy Products: The Issue of Which Products to Promote." ESO 1162. Ohio State University: Department of Agricultural Economics (April 1985): 93 pp.

Hughes, W. and Edward V. Jesse. "Producer Milk Pricing Practices in Wisconsin, 1985." Marketing and Policy Briefing Paper No. 14. Madison, Wisconsin: U.W. Department of Agricultural Economics. (May 1987): 17 pp.

Jacobson, R., E.V. Jesse, et al. "Dairy Outlook: 1986-1990." ESS 1250. Columbus, Ohio: Ohio State University Department of Agricultural Economics (January 1986).

Jesse, Edward V. "Economic Efficiency and Marketing Orders." Economic Efficiency in Agriculture and Food Marketing, R. Kilmer and W. Armbruster (editors). Ames: Iowa State University Press (1985): 315 pp.

Jesse, Edward V. and Aaron C. Johnson, Jr. "An Analysis of Cooperative Over-Order Pricing of Fluid Milk." Technical Bulletin No. 1708. Washington D.C.: U.S.D.A. Economic Research Service (August 1985): 45 pp.

Dept. of Chemical Engineering

2014 Engineering Bldg.
Chairperson, Stuart L. Cooper

The following lists the majority of the dairy-related research publications in the Chemical Engineering Department.

D.E. Romero-Ferrer, C.H. Amundson, and C.G. Hill; Jr. "The Effects of Gas Injection on the Efficiency of Thermal Energy Utilization in Spray Drying."Journal of Food Process Engineering, 8, 171 (1986).

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A. Morales, C.H. Amundson, and C.G. Hill, Jr. "Comparative Study of Different Reverse Osmosis Membranes for Processing Dairy Fluids: I Permeate Flux and Total Solids Rejection Studies." Paper submitted for publication in Journal of Food Processing and Preservation.

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P.L. Spangler, M. El Soda, M.E. Johnson, N.F. Olson, C.H. Amundson and C.G. Hill, Jr. "Accelerated Ripening of Gouda Cheese Made from Ultrafiltered Milk Using a Liposome-Entrapped Enzyme and Freeze-Shocked Lactobacilli." Paper submitted for publication in Milchwissenschaft.

J.H. Flatt, T.A. Cooper, D.C. Cameron, and E.N. Lightfoot. "Utilization of dairy waste: Microbial production of galactose-containing polysaccharides from lactose in whey." Paper presented at AIChE 1988 Annual Meeting, Washington D.C.

Dept. of Dairy Science

266 Animal Science Bldg.
Chairperson, David P. Dickson

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Food Research Institute

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Dept. of Food Science

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The following is a list of dairy-related publications published by the Food Science Department in 1987.

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Dept. of Nutritional Sciences

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The following is a list of the dairy-related publications of the Nutritional Sciences Department for 1987.

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Behling, A.R., Khazen, R.R., Krashoc, C.L. and Greger, J.L. 1987. Mineral utilization in mature, weanling and anemic rats fed various levels and forms of calcium. Fed. Proc. 46:3329. (Abstr.)

Behling, A.R., Lewis, N.M., Marcus, M.S. and Greger, J.L. 1987. Fecal ß-glucuronidase activity and fecal pH in rats and humans fed various levels and forms of calcium. Institute of Food Technologists, Abstract 369.

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