2003 Annual Report

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Western Dairy Center
Annual Report
2003

Western Dairy Center
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Western Dairy Center
Activities Summary
2003

The Western Dairy Center is a consortium of researchers devoted to improving the dairy industry in the United States by conducting research in all areas of dairy foods. The Center includes researchers from Utah State University, University of Idaho, Oregon State University, Brigham Young University, Washington State University and Weber State University. This report summarizes the research activities from January 1, 2003 through December 31, 2003.

The Center conducted two sessions of the 18th Annual Cheese Making Short Course in February 2003, at Utah State University with 12 attendees in each session. We limit the number of attendees to ensure a "hands on" learning experience. Our short course was able to use our new cheese making facilities, specifically two automated Scherping cheese vats. Our Scherping cheese vats have a 1500 lbm capacity and we also have a new finishing table with a 150 lbm capacity.

We will be conducting our 17th Biennial cheese Industry conference in Sun Valley Idaho on August 11, 2004. The focus of this conference will be use of concentrated milk in dairy products. The continued success this conference is due to the excellent speakers, the teaming with the Idaho Milk Processors Association Annual Meeting, and our sponsors.

In 2003, the number of new competitive grants awarded by Dairy Management was 4. We have 2 other DMI funded projects in progress which resulted in $210,000 research dollars. The Western Dairy Center funded 5 seed proposals for a total of $39,000. In addition we had two Western Dairy Center funded projects for a total of $30,000. Project progress reports of all research projects active in 2003 are included in this report.
WESTERN DAIRY CENTER
OPERATIONAL ADVISORY COMMITTEE

Pursuant to the Western Dairy Center proposal and contract with the National Dairy Promotion and Research Board, the voting members of the Operational Advisory Committee are:

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Financial Summary of Approved Projects for 2003

Research projects funded by DMI

Effect of oxidation-reduction potential on growth of lactic acid bacteria
PI: Jeff Broadbent .......................................................... $33,038

Dried whey minerals as an antioxidant in processed meats
PI: Daren Cornforth .......................................................... $45,900

An objective test for measuring stretch properties of Mozzarella cheese
PI: Don McMahon .......................................................... $17,000

Pressure processing to improve milk freshness and refrigerated shelf-life
PI: Antonio Torres .......................................................... $27,464

Cultivation of mushroom mycelia using whey permeate
PI: Conly Hansen .......................................................... $58,140

Analysis of capsul production in Streptococcus thermophilus by Comparative genomics
PI: Jeff Broadbent .......................................................... $28,560

Research projected funded by the Western Dairy Center

Rehydration and structure of reconstituted casein micelles
PI: Don McMahon .......................................................... $20,000

Identify the role of milk and milk products on nutrition and health issues of importance for adults fifty years of age and older, year 2
PI: Ann Sorenson .......................................................... $10,000

Oxidation-reduction potential in Cheddar cheese
PI: Carl Brothersen .......................................................... $10,000
Western Dairy Center

Reporting Period January 1, 2002 — June 30 2004

Principal Investigators: Daren Cornforth, Utah State University

Co-Investigators:

Project Title: Dried whey minerals as an antioxidant in processed meats

Institution’s Project #: 01126

Project Completion Date: December 31, 2003


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

Objective 1: Determine the effectiveness of dried whey mineral (WM) as inhibitor of rancidity in fresh pork sausage (an uncured sausage cooked immediately before serving). Rancidity will be measured by a chemical method (Thiobarbituric acid or TBA test) and by trained panel sensory evaluation.

Objective 2: Determine the effectiveness of dried WM as an inhibitor of rancidity in Italian sausage (an uncured, precooked sausage used as a pizza topping). Rancidity will be measured by the TBA test and by trained panel sensory evaluation.

Objective 3: Determine the effectiveness of dried WM as an inhibitor of rancidity in Summer Sausage (a nitrite-cured, pre-cooked sausage). Rancidity will be measured by the TBA test and by trained panel sensory evaluation.

Objective 4: Determine the optimum use levels and economic viability of using dried WM as an antioxidant in processed meats (fresh pork sausage, Italian sausage, summer sausage).
Objective 5: It appears likely that the insoluble calcium phosphate particles in WM bind iron released from meat pigments during cooking, preventing iron catalyzed lipid oxidation. To test this hypothesis, it is proposed to directly measure soluble ionic iron levels in fresh pork sausage, Italian sausage, and summer sausage before and after cooking and at various intervals during storage in samples with or without added WM. Whey mineral will be added at the optimum levels determined in objective 4 above.

Objective 6: Compare the effectiveness of WM to other known antioxidants (Rosemary, BHT, sodium nitrite) in a cooked ground beef model system.

Project Summary: (Suitable for inclusion in Center documents released to the public)
1. Significant Progress against Objectives:

Objective 1. Fresh Pork Sausage. Control (uncooked) pork sausage, with or without milk mineral (MM), had low rancidity, as measured by sensory evaluation and TBA values. TBA values for all uncooked sausages were less than 0.6 during 15 days storage at 2°C.

Objective 2. Italian Sausage (cooked but not cured). Milk mineral (1.5%) and sodium tripolyphosphate (STP; 0.5%) were excellent inhibitors of rancidity of these cooked pork sausages, with TBA values less than 0.5 during 15 days refrigerated storage after cooking.

Objective 3. Summer Sausage (cooked and nitrite-cured). The objective of this study was to determine the antioxidant activity of 1.5% milk mineral (MM) added to beef sausages, alone or in combination with 20 or 40 ppm sodium nitrite (nitrite-cured). All treatments were also formulated with 1.5% salt and 10% added water. Sausages were cooked in a hot water bath to an internal temperature of 71°C. Products were held at 2°C. Thiobarbituric acid (TBA) values were determined at 1, 8 and 15 days storage. Sausages with 1.5% MM alone or 1.5% MM and 20 ppm sodium nitrite had low TBA values of 0.8 – 0.9 after 1 or 15 days storage. Sausages with 1.5% MM and 40 ppm sodium nitrite had TBA values of 0.6 – 0.7 after 1 or 15 days storage. Thus, MM at 1.5% with or without nitrite was effective in maintaining low TBA values (< 1.0) of cooked beef sausages, compared to control samples without MM.

Objective 4. The optimum use level for MM in Italian sausage (cooked, uncured) was 1.5% of meat weight. The 1.5% level was significantly more effective that the 1.0% level, but not different from the 2% level. Meat balls (cooked, uncured) were cooked in a boiling water bath to an internal temperature of 85°C. Control meatballs had TBA values ranging from 2.2 on day 1 to 6.8 on day 15. Meatballs formulated with 1.5% MM had lower TBA values ranging from 0.7-0.9 on storage days 1 and 15, respectively. Thus, cooked meat balls formulated with 1.5% milk mineral were not rancid after 15 days storage at 2°C (TBA values < 1.0), while control meat balls without milk mineral were highly rancid.

Objective 5. MM antioxidant mechanism. 1.5% MM was effective in maintaining low TBA values in cooked pork products. In contrast, the controls without MM developed high TBA values (> 5.0) during 15 days refrigerated storage, accompanied by increases in nonheme iron level and decreased heme iron level. So, lipid oxidation was associated with heme degradation. Rancidity and heme degradation were inhibited by 1.5% MM.
STP was also an effective antioxidant. However, Rosemary or butylated hydroxytoluene (BHT) were not effective antioxidants at recommended levels of 0.2% of meat weight or 0.01% of fat content, respectively. Additional work in our lab showed that higher levels (0.4% Rosemary powder or 0.01% BHT as % of meat weight, respectively) were needed for antioxidant activity in a cooked meat system.

Objective 6. Comparison of MM to other antioxidants

1.5% MM and 0.5% STP were both highly effective in prevention of rancidity in cooked ground pork stored for 15 days at 2°C. Sodium nitrite (156 ppm) was intermediate in prevention of rancidity, and Rosemary oil extract (0.2% of meat weight) or BHT (0.1% of meat fat content) were not effective antioxidants. Increasing the levels of Rosemary or BHT increased their effectiveness, but would also increase costs. Rosemary powder (0.4%) and BHT (0.1% of total meat weight) were effective antioxidants. However, use of BHT at 0.1% of meat weight would not be permitted by USDA regulations.

Comparison of Type 1 and Type 2 antioxidant effectiveness in cooked ground pork during refrigerated storage.

Preetha Jayasingh, Charles E. Carpenter, and D. P. Cornforth
(Presentation at the 2003 meeting of the Institute of Food Technologists, Chicago, IL)

Introduction

Type 1 antioxidants such as vitamin E, Rosemary extract, and butylated hydroxytoluene (BHT) are electron donors capable of slowing the propagation step of lipid oxidation. Type 2 antioxidants such as phytate, sodium tripolyphosphate, or sodium nitrite bind iron, preventing iron catalysis of lipid oxidation.

Objective

The objective of this study was to compare antioxidant effectiveness of BHT and Rosemary extract (Type 1 antioxidants) with sodium tripolyphosphate (STP), milk mineral (MM; a natural phosphate source) and sodium nitrite in cooked ground pork during storage.

Methods

Antioxidants were added to raw ground pork at recommended levels (0.01% of fat content for BHT, 0.2% of meat weight for Rosemary extract, 0.5% of meat weight for STP, 1.5% MM, and 156 ppm sodium nitrite. Samples (100g) were mixed thoroughly with antioxidant, cooked at 163°C for 15 min, then stored at 2°C for 1-12 days. Thiobarbituric acid (TBA) values and heme iron values were measured periodically during storage.

Results

TBA values increased significantly and heme iron levels significantly decreased during storage of cooked controls and samples with type one antioxidants, but not for samples
containing phosphates or sodium nitrite. For example, TBA values of BHT and Rosemary samples reached mean TBA values of 7.4 and 8.2, respectively, compared to TBA values of 0.4, 1.1, and 0.26 for STP, MM, and sodium nitrite treated samples. Heme iron values of control, BHT and Rosemary treated samples decreased from 5.9, 6.3, and 5.3 ppm iron after 1 day storage to 3.7, 3.0, and 2.8 ppm at 12 days storage. Heme iron levels of samples treated with STP, MM, or sodium nitrite remained relatively constant during storage.

Significance
Type 2 antioxidants (STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork that the type 1 antioxidants (BHT or Rosemary extract).

2. Significant Conclusions:
Type 2 antioxidants (STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork that the type 1 antioxidants (BHT or Rosemary extract).

2. Anticipated Problems/Delays: None

Publications:


Dissertation:
Published Abstracts:


Presentations:


Jayasingh, P., Carpenter, C. E. and Cornforth, D. P. 2003. Comparison of Type 1 and Type 2 antioxidant effectiveness in cooked ground pork during refrigerated storage. Institute of Food Technologist’s meeting, Chicago, IL.

Addendum

**Patent/Invention Disclosures:** Dried Milk Mineral Fraction as an Antioxidant. U. S. patent application No. 09/604,622.

### Technology Transfer Activities
For information on licensing contact: Russell Price, Office of Technol. Management & Commercialization, USU, 570 North Research Parkway, Suite 1101, North Logan, UT 84341

**Visitors Hosted:** Dr. Oddvin Sorheim, Norwegian Food Science Institute (Matforsk), Oslo, Norway October-November, 2002.

**Invention Disclosures: (Title, Date)**


**Patents: (Title, Date, #)**

**Discoveries:**

Milk mineral (1.5%) and sodium tripolyphosphate (STP; 0.5%) were excellent inhibitors of rancidity of cooked pork sausages, with TBA values less than 0.5 during 15 days refrigerated storage after cooking.

At currently accepted use levels, Type 2 antioxidants (Iron binding agents STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork that the type 1 antioxidants (BHT or Rosemary extract; electron donors).
Objective 1: Determine suitable bulk suspended growth conditions for mushroom mycelia grown on whey permeate including: concentration of permeate in the media to accelerate growth while avoiding inhibition, temperature, pH, mixing, and O2 requirements.

[Hypothesis: there is an optimum level at which all variables (i.e. concentration of whey permeate, temperature, pH, mixing, and O2) must be maintained in order to promote maximum growth of the mycelia.]

Objective 2: Perform fermentations to determine if other cheese/whey/lactose byproducts can also be used to grow mycelia.

[Hypothesis: whey byproduct streams other than whey permeate can be used as a growth medium for mycelia.]

Objective 3: Complete experiments to indicate nutrient supplementation requirements for whey permeate and delactosed whey permeate (mother liquor) when used as the media for growing mycelia.
Objective 4: Develop a procedure for separating mycelia from spent media.

[Hypothesis: centrifugation will effectively separate mycelia from the spent media.]

Objective 5: Determine residual chemical oxygen demand, solids, macronutrients N & P, and odor that remain in spent media after mushroom mycelia has been harvested to reveal how much the potential pollution problem of cheese byproduct is solved using this process.

[Hypothesis: chemical oxygen demand (COD) of the spent media will be less than 100 mg/L and other discharge parameters will be low enough to dispose of the spent byproduct media into municipal sewers without surcharge.]

Project Summary: (Suitable for inclusion in Center documents released to the public)

Mycelia, which is one phase of the life cycle of edible mushrooms, is a health food considered to have outstanding medicinal qualities, including anti tumor activity and the ability to lower cholesterol. Mycelia are presently grown in bioreactors that use a relatively expensive carbon source. This project will grow mycelia in bioreactors using byproduct of little or even negative value from cheese making. The byproducts to be considered include whey, whey permeate, and spent solution from lactose manufacture. The cost of using byproduct such as whey permeate is less than 1/100 the cost of presently used commercially prepared media.

Progress: Objective 1

Determine suitable bulk suspended growth conditions for mushroom mycelia grown on whey permeate including: concentration of permeate in the media to accelerate growth while avoiding inhibition, temperature, pH, mixing, and O2 requirements.

Introduction

Edible mushroom mycelia are fastidious by nature and tend to grow much more slowly when they are not in optimal conditions. In order to facilitate the bulk processing of substrate (whey permeate in this case), it is therefore desirable to determine the conditions that will foster the most rapid growth and development of the mycelia. The purpose of this objective was to determine the optimal growing conditions for the five species of edible mushrooms selected for the project.

Materials and Methods
The five mushroom species under consideration were grown using solid state petri dish fermentation with various commercial growth media. Each species was then grown in the same manner using various concentrations of whey permeate as the growth medium. A Central Composite Design (CCD) was then utilized to create a set of testing conditions that would allow the optimum temperature, pH, and concentration of whey permeate to be determined. The initial CCD design consisted of 8 axial and five center points for each species. Each point was run in triplicate, and the results were averaged. Data was gathered by innoculating a petri dish from the CCD design with mushroom mycelium and then allowing it to incubate for one week. Every 24 hours, the growth radius of the mycelia was measured so that radial growth rate could be determined. After the data was collected, a statistical analysis software package known as E-Chip was used to analyze the results. E-Chip employs a method known as Response Surface Methodology (RSM) to create a graph showing the region containing optimal conditions.

Results and Discussion

The growth rate of the mushroom mycelium when grown on various commercial medium is shown in table I. A comparison of the growth rate of the mushroom mycelia when grown on whey permeate, whey, and the commercial media is shown in table 2. These tables show that the mycelia can be grown as quickly or more quickly using inexpensive whey permeate as they can be grown using expensive commercial media.

When using RSM technology as a method of analysis, a preliminary step analyzes the initial CCD data gathered, and then the researchers refine the original CCD and use E-Chip again to more accurately pinpoint the area of optimum growth. The initial results for this study were very promising and indicated that very little refinement of the initial CCD design would be needed in order to obtain accurate information about the optimum growth conditions. One species that appeared to yield definitive results in the original analysis was *Ganoderma lucidum*. For this mycelium, the optimum conditions were initially determined to be about 30°C, 4.3 pH, and 31 g lactose per L. These conditions correspond closely with those of another published study done to determine optimum growth conditions for biomass production when growing *Ganoderma lucidum* using whey as a substrate. The correlation between the values found for radial growth rate in our study, and those found for biomass development in the other study are promising because they suggest that it may be possible to obtain maximal growth rate and biomass production of the mycelium at the same time.

Another species that seemed to yield definitive results for its optimal growth conditions was *Lentinus edodes*, known commonly as the “shiitake” mushroom. Analysis showed that the data collected on the growth of this species could be predicted very well with our modeling equation, but that the results were not yet statistically significant. This led us to believe that the research was conducted correctly, and that the data simply needed to be modeled with a higher order equation to obtain statistical significance. After sequentially fitting the data into equations from linear to partial cubic, we were able to determine that a modified partial cubic equation was able to accurately predict and model the growth of *Lentinus edodes* on whey permeate. Analysis with this equation determined that the optimal growth conditions for this mushroom when grown on whey permeate in a petri dish were: temperature 23.6°C, pH 4.97, and substrate concentration 40g/l whey permeate. These results can now be used in small-scale bioreactors to help determine optimal mixing and oxygen content.
Table 1. Radial Extension Rate (mm/d)

<table>
<thead>
<tr>
<th>Media</th>
<th>G. lucidum</th>
<th>L. edodes</th>
<th>P. ostreatus</th>
<th>P. linteus</th>
<th>A. bisporus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose peptone yeast</td>
<td>8.7</td>
<td>2.9</td>
<td>3.7</td>
<td>1.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Yeast malt</td>
<td>7.2</td>
<td>3.8</td>
<td>3.9</td>
<td>2.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Czapek Dox</td>
<td>4.3</td>
<td>3.1</td>
<td>1.8</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Glucose ammonium chloride</td>
<td>3.8</td>
<td>3.0</td>
<td>1.6</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Malt</td>
<td>8.4</td>
<td>2.0</td>
<td>2.6</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Potato dextrose agar</td>
<td>8.8</td>
<td>4.5</td>
<td>4.0</td>
<td>2.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Table 2. Whey permeate and commercial media growth rate comparison

<table>
<thead>
<tr>
<th>Species</th>
<th>Growth rate (mm/d) on whey permeate medium</th>
<th>Percent Growth Rate Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. lucidum</td>
<td>8.4</td>
<td>95-221</td>
</tr>
<tr>
<td>L. edodes</td>
<td>4.4</td>
<td>98-220</td>
</tr>
<tr>
<td>P. ostreatus</td>
<td>1.6</td>
<td>40-100</td>
</tr>
<tr>
<td>P. linteus</td>
<td>1.9</td>
<td>38-238</td>
</tr>
<tr>
<td>A. bisporus</td>
<td>9.3</td>
<td>186-1163</td>
</tr>
</tbody>
</table>

Conclusions

Whey permeate is a suitable growth medium for edible mushroom mycelium. In most cases the mycelia will grow even more quickly on whey permeate than on commercial media. With careful control of the growth parameters, it could be possible to achieve maximal growth rate and biomass production simultaneously. The parameters that yielded the fastest growth for the species *Lentinus edodes* were: temperature 23.6°C, pH 4.97, and substrate concentration 40g/l whey permeate. These parameters may now be tested in a bio-reactor setting to determine the overall effect that may be expected when growing mushroom mycelia using whey permeate as the growth medium.
Anticipated Problems/ Delays

Facilities that are equipped and authorized to handle the fermentation experiments with the mushroom species chosen for study are not available at Utah State University. These fermentation experiments will have to be conducted with the help of our colleges at Postech University in South Korea.

Progress: Objective 2

Perform fermentations to determine if other cheese/whey/lactose byproducts can also be used to grow mycelia.

Materials and Methods

The species *Lentinus edodes* was chosen as the best candidate for investigating the ability of alternative whey byproducts to support growth of mushroom mycelia. This was done because of the excellent pharmaceutical uses currently ascribed to this species. The byproduct investigated in this research was delactosed whey, or mother liquor. Using solid state petri dish fermentation, *L. edodes* was grown using various concentrations (100% v/v, 80% v/v, 60% v/v, 40% v/v, 20% v/v and 10% v/v) of delactosed whey as the growth medium. *L. edodes* was placed in six different plates at each concentration, and the plates were then incubated at 30°C. The mycelia colonies were then measured every 24 hours in order to determine rate of growth.

Results and Discussion

The growth rate of *L. edodes* when grown on delactosed whey, or mother liquor, is shown in figure 1. Visual evidence of the growth of *L. edodes* in 100% delactosed whey, and 20% delactosed whey is shown in figures 2 and 3, respectively.

From these figures, we can readily come to two conclusions. The first is that delactosed whey, or mother liquor, can be utilized as a growth substrate for the cultivation of *L. edodes*. Figure 1 shows that *L. edodes* will grow at an acceptable rate in delactosed whey, and figure 3 shows a colony of *L. edodes* mycelia that is obviously alive and growing. The second conclusion that can be obtained from this research is that delactosed whey will not support the growth of *L. edodes* at high concentrations. High concentrations yield absolutely no growth. Figure 3 shows that high concentrations of delactosed whey don’t simply inhibit the growth of mycelia, they actually kill off the original mycelia inoculum.
Figure 1 Maximum mycelial growth rate of *L. edodes* when grown on delactosed whey. Low concentrations of delactosed whey allow vigorous growth of the mycelia whereas high concentrations exhibit no growth.

Figure 2 Mycelia grown in 100% delactosed whey. The mycelia on the original inoculation pellet have been killed off and the plate exhibits no mycelial growth.
Figure 3 Mycelia grown in 20% delactosed whey. These mycelia exhibit a viable colony that is constantly growing and expanding. The only source of nutrients for the mycelia is the delactosed whey substrate, yet they are finding adequate nutrition for growth.

Conclusions

Delactosed whey may be utilized to cultivate the mushroom species *Lentinus edodes*. Since this species is not considered to thrive on special nutrients that other species cannot utilize, these results indicate that delactosed whey would most likely serve as a good growth substrate for other species of mushrooms. Also, high concentrations of delactosed whey appear to exhibit lethal effects on the mushroom mycelia. This is most likely due to extreme concentrations of certain elements in the media. However, further research into the question could possibly yield a natural product that could find application as a fungicide.

Progress: Objective 3

Complete experiments to indicate nutrient supplementation requirements for whey permeate and delactosed whey permeate (mother liquor) when used as the media for growing mycelia.

Results and Discussion.

The nature of this objective was such that if the original hypothesis was correct (i.e. no
Western Dairy Center
Final Report
Reporting Period January 1, 2003-June 30, 2004

Principal Investigators: Gulhan U. Yuksel, University of Idaho
                       Joe R. Powers, Washington State University

Co-Investigators: NA

Project Title: Development of an efficient gene transfer system for Lactobacillus helveticus WSU-19

Institution's Project #: BKK 941 (University of Idaho)

National Research Plan:

Modifications to Project/Budget:

In addition to the pepN gene that we proposed to clone in our original proposal, we cloned and characterized the pepE, pepO, and pepO2 genes from Lb. helveticus WSU-19. Moreover, we confirmed the roles of the pepN, PepE, and PepO2 enzymes in degradation of two bitter peptides, \(-CN f193-209\) and \(-CN f1-23\).

Project Objectives:

Proteolysis of casein is important for the development of flavor and texture of many cheeses. Protein degrading enzymes in cheese include proteinases from added coagulant, the proteinase-peptidase system of starter lactic acid bacteria, the proteinase-peptidase system of non-starter lactic acid bacteria, and indigenous proteinase of milk. Aging of cheese increases value of the product but the process is expensive due to storage and inventory costs and defects can develop during the ripening process. For example, a common problem is the accumulation of certain peptides resulting in excessive bitterness. Adjunct cultures are often added to cheese to enhance aging and to reduce bitterness. While the enzymology of adjunct cultures has been a active area of research, the selection of adjunct strains remains largely empirical. Thus, more fully understanding of the enzymology of adjunct strains that have been successfully used in the production of Cheddar type cheeses will allow a more systematic development of improved cultures by the dairy industry and a more reliable economic return to the cheese enterprise.

Lactobacillus helveticus WSU-19 is successfully used as an adjunct in Cougar Gold cheese, a cheddar type cheese that is non-bitter even after extensive aging process. Unfortunately, we have a limited understanding of the proteolytic and peptidolytic enzyme system from this organism due in part to the lack of an efficient gene transfer system. Lb. helveticus WSU-19 is believed to have a unique enzyme system and/or enzymatic breakdown pattern that prevents the accumulation of bitter peptides while allowing production of appropriate positive flavors during ripening of cheese. Our long-range goal is to fully characterize Lb. helveticus WSU-19 in terms of the biochemistry and genetics of these enzymes.
of the strain and determine the contribution of casein degrading enzymes of the strain to the production of a high quality, non-bitter Cheddar type cheese. The objective of this research proposal, which is the next step toward attaining our long-range goal, is to develop an efficient gene transfer system (i.e., electrotansformation) for \textit{Lb. helveticus} WSU-18. The central hypothesis of the proposed research is that an efficient gene transfer system can be developed for \textit{Lb. helveticus} WSU-19, allowing us to manipulate this organism at the molecular level and enhancing our understanding of its contribution to cheese flavor development. We expect to test our central hypotheses and to achieve the objective of this research by pursuing the following specific aims:

1. Develop/optimze an electrotansformation procedure for \textit{Lb. helveticus} WSU-19
2. Confirm the effectiveness of the electrotansformation procedure by constructing a peptidase-deficient mutant of \textit{Lb. helveticus} WSU-19

\textbf{Project Summary:} (Suitable for inclusion in Center documents released to the public) Develop/optimze an electrotansformation procedure for \textit{Lb. helveticus} WSU-19; It has been reported that lactobacilli can be transformed with vectors by electrotansformation of \textit{Lb. acidophilus}, \textit{Lb. bavaricus}, \textit{Lb. casei}, \textit{Lb. curvatus}, \textit{Lb. fermentum}, \textit{Lb. helveticus}, \textit{Lb. reuteri}, \textit{Lb. sake} and others. Although electrotansformation has been successfully applied in many \textit{Lactobacillus} strains, transformation efficiencies and optimal electrotansformation parameters vary greatly from strain to strain, especially in the case of \textit{Lb. helveticus}. In the proposed study, the electrotansformation of \textit{Lb. helveticus} WSU-19 will be carried out using selected procedures established for other \textit{Lactobacillus} strains. The efficiency of transformation will be determined in each case. The vectors of interest will include pSA3 and pGK12. PSA3 is a temperature-sensitive integration vector that we plan to use for the construction of isogenic \textit{Lb. helveticus} WSU-19 strains that are deficient in selected peptidase enzymatic acitivities. PGK12 is a vector that is commonly used to develop/optimze electrotansformation procedures for \textit{Lactobacillus}. The most efficient electrotansformation procedure will be subject to an optimization process in which the effects of several parameters on transformation frequency will be studied. These parameters will include electroporation buffers (type, pH, ionic strength), growth media (MRS, APT), cell-wall weakening agents, and field strength (4000-12,500 V/cm). Our goal is to achieve an electrotansformation efficiency of 10 to the 4th to 10 to the 5th transformants per _g of pGK12.

Confirm the effectiveness of the electrotansformation procedure by construction of a peptidase-deficient mutant of \textit{Lb. helveticus} WSU-19: To confirm the effectiveness of the newly established procedure, a pSA3-based integration plasmid that contains a deleted version of the Aminopeptidase N gene (\textit{pepN}) will be constructed and introduced into \textit{Lb. helveticus} WSU-19. An efficient method for gene replacement using the temperature-sensitive vector pSA3 will be applied to replace the wild-type chromosomal \textit{pepN} gene with the deleted version of the gene. The PepN-deficient derivative of \textit{Lb. helveticus} WSU-19 will be confirmed genotypically and phenotypically. To accomplish our long-range goal, future research will deal with Cheddar-type cheese trials using the PepN-deficient strain and other peptidase-deficient strains as adjuncts. \textit{A Lactococcus}
3. Significant Progress against Objectives:

Specific Aim 1: Develop / optimize an electrotransformation procedure for *Lb. helveticus* WSU-19

Prior to developing an electrotransformation procedure for *Lb. helveticus* WSU-19, we confirmed the species designation of this organism using a 16S rRNA analysis (MIDI Labs; Newark, DE).

Previously, an efficient electroporation protocol has been developed for *Lb. helveticus* CNRZ32 by Bhowmik and Steele (1993). To obtain a good understanding of the efficient electroporation protocol, *Lb. helveticus* CNRZ32 was obtained from Dr. Jim Steele of the University of Wisconsin-Madison. A preliminary electroporation experiment was performed where *Lb. helveticus* CNRZ32 competent cells were prepared using MRS Broth, MRS Broth containing Glycine (1%), and MRS Broth containing DL-Threonine (40 mM). We decided not to use MRS Broth containing L-Lysine (40 mM) since *Lb. helveticus* CNRZ32 did not grow well in this particular medium. The CNRZ32 competent cells were electroporated with PGK12 DNA (1.5 g). The efficiency of transformation was as follows: MRS, 195 transformants/1.5 g of pGK12; MRS with Glycine, 526 transformants/1.5 g of pGK12; MRS with DL-Threonine, 107 transformants/1.5 g of pGK12.

With the goal of applying the *Lb. helveticus* CNRZ32 electroporation protocol to *Lb. helveticus* WSU-19, *Lb. helveticus* WSU-19 was grown in MRS broth and MRS broth containing different cell-wall weakening agents. *Lb. helveticus* CNRZ32 was used as the positive control. *Lb. helveticus* WSU-19 did not grow in MRS Broth containing L-Lysine (40 mM) and grew poorly in MRS Broth containing Glycine (1%). However, it could grow well in MRS containing DL-Threonine (40 mM). Therefore, we have decided to concentrate our efforts on DL-Threonine as the cell-wall weakening agent.

When no cell-wall weakening agents was used or when DL-Threonine was used as the cell-wall weakening agent, early log cultures (A₆₀₀ of ~0.25-0.3) of *Lb. helveticus* WSU-19 did not pellet well using centrifugation (Beckman Coulter Avanti J-25; 4°C, 7500 rpm, 10 minutes) after washes with ice-cold electroporation buffer. Increasing the centrifugal force (13,000 rpm) and centrifugation time (60 minutes) did not appear to work due to the excessive time spent and the amount of cells lost during washes. Therefore, we decided to use a mid log (A₆₀₀ of ~ 0.5-0.75) or a late log culture of *Lb. helveticus* WSU-19 for future electroporation experiments.

Electroporation experiments were conducted to determine the effects of field strength and antibiotic type on the electroporation efficiency. Various erythromycin (Em; 2.5-20 μg/ml) and chloramphenicol (Cm; 2.5-20 μg/ml) concentrations were tried to determine the appropriate concentrations of Em and
Cm for use in MRS agar plates containing 5 mM CaCl₂. The Em and Cm concentrations of 2.5 \( _\text{g}/\text{ml} \) and 3.5 \( _\text{g}/\text{ml} \), respectively, were selected for future electroporation experiments.

Electroporation of \textit{Lb. helveticus} WSU-19 with pGK12 DNA was more efficient when the electroporated cells were grown in MRS agar medium containing Cm than Em. The transformation efficiency of mid log culture (\( A_{600} \) of ~0.5-0.75) of \textit{Lb. helveticus} WSU-19 (field strength = 6250 kV/cm) was as follows: MRS agar containing Cm, 945 transformants/1.5 \( _\text{g} \) of pGK12; MRS agar containing Em, 21 transformants/1.5 \( _\text{g} \) of pGK12.

Increasing the field strength decreased transformation efficiency. Mid log culture of \textit{Lb. helveticus} WSU-19 electroporated with pGK12 DNA and plated on MRS agar medium containing 3.5 \( _\text{g}/\text{ml} \) Cm and 5 mM CaCl₂ had the following transformation efficiencies: field strength of 4000 V/cm: 294 transformants/1.5 \( _\text{g} \) of pGK12; field strength of 6250 V/cm: 191 transformants/1.5 \( _\text{g} \) of pGK12; field strength of 9000 V/cm: 98 transformants/1.5 \( _\text{g} \) of pGK12; field strength of 12500 V/cm: 9 transformants/1.5 \( _\text{g} \) of pGK12.

Based on the results from the experiments outlined above, an electroporation protocol that utilizes \textit{Lb. helveticus} WSU-19 cells grown until mid-log phase (\( A_{600} \) of 0.5-0.75) in MRS broth containing 40 mM DL-Threonine, electroporated using a field strength of 4000 V/cm, and plated upon electroporation on MRS agar medium containing 3.5 \( _\text{g}/\text{ml} \) Cm and 5mM CaCl₂ has been established.

Specific Aim 2: Confirm the effectiveness of the electrotransformation procedure by constructing a peptidase-deficient mutant of \textit{Lb. helveticus} WSU-19


2.1.1. Probe synthesis

Four sets of primers were designed to synthesize the \textit{pepN} (2.5 kb), \textit{pepE} (1.3 kb), \textit{pepO} (1.9 kb), and \textit{pepO2} (1.9 kb) probes using the coding sequence of the peptidase genes from \textit{Lb. helveticus} CNRZ32 (Table 1).

Table 1. Forward and reverse primers for probe synthesis

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>PepN forward</td>
<td>5'-TGTCAGTTAAACGTTCCTTCCTC-3'</td>
</tr>
<tr>
<td>PepN reverse</td>
<td>5'-ATGAATGCTTTAGCAACTG-3'</td>
</tr>
<tr>
<td>PepE forward</td>
<td>5'-ATGGCTCATAATGCGGTATAACG-3'</td>
</tr>
<tr>
<td>PepE reverse</td>
<td>5'-TTAAGCAAGTGAATCCAGCAT-3'</td>
</tr>
<tr>
<td>PepO forward</td>
<td>5'-AGAAGATATTTAGCTGTACG-3'</td>
</tr>
<tr>
<td>PepO reverse</td>
<td>5'-TAATACCTATATTAGCTGTACG-3'</td>
</tr>
<tr>
<td>PepO2 forward</td>
<td>5'-TGAAATGCAAAAAATCCGT-3'</td>
</tr>
<tr>
<td>PepO2 reverse</td>
<td>5'-ACCACATGACTACCGCGTTA-3'</td>
</tr>
</tbody>
</table>
The following plasmids containing the previously cloned peptidase genes from *Lb. helveticus* CNRZ32 were obtained from Dr. Steele of the University of Wisconsin-Madison and used as DNA templates: pTRKH2::pepN, pTRKL2::pepE, pTRKL2::pepO, and pJDC9::pepO2. The probes were labeled with non-radioactive digoxigenin-dUTP (DIG).

2.1.2. Southern hybridizations

Genomic DNA was isolated from *Lb. helveticus* WSU-19 and CNRZ32, digested with a number of restriction enzymes located in the multiple cloning site of the vector pJDC9, and electrophoresed in 0.8% agarose gel. Southern hybridizations were performed under high stringency conditions using the previously synthesized probes. The putative pepN, pepE, pepO, and pepO2 genes from *Lb. helveticus* WSU-19 were successfully identified using Southern hybridizations. Restriction enzymes for cloning of the pepN, pepE, pepO, and pepO2 genes were determined to be *XbaI* and *SalI*, *PstI* and *XbaI*, *HindIII* and *SalI*, and *XbaI* and *SphI*, respectively, based on the results from Southern hybridizations.

2.1.3. Cloning of the pepN, pepE, pepO, and pepO2 genes

Genomic DNA from *Lb. helveticus* WSU-19 was digested with the appropriate restriction enzymes and separated by agarose gel electrophoresis. Hybridization-positive genomic DNA fragments were gel extracted and ligated to the pJDC9 vector DNA digested with the same enzymes. At least five ligation mixtures containing different ratios of the genomic insert and vector DNA were prepared and introduced into electrocompetent *E. coli* DH5_- (ElectroMAX™ DH5_—ETM) cells. Antibiotic-resistant transformants were screened using blue/white screening and colony hybridization. The high stringency colony hybridizations were performed using the probes that were originally prepared for Southern hybridizations.

Two hybridization positive colonies were obtained for the cloning of the putative pepE gene. Plasmid DNA was extracted from the hybridization-positive colonies and subjected to restriction enzyme analyses and DNA sequencing. Restriction enzyme analyses of the plasmids from the hybridization positive colonies revealed an expected restriction pattern. With the goal of genotypic confirmation of the pepE gene from *Lb. helveticus* WSU-19, complete sequencing of one of the genomic inserts was conducted at the Nucleic Acid and Protein Facility at University of Wisconsin Biotechnology Center. The nucleic acid as well as the amino acid BLAST analyses of the genomic insert confirmed the successful cloning of the putative pepE gene. Complete sequencing of the 2.7-kb *PstI-Xbal* genomic insert revealed 2 open reading frames (ORFs), ORF1 and ORF2, of 444 bp and 1314 bp, respectively. The ORF1 was determined to contain a conserved domain of small heat shock proteins. Based on the BLAST analyses, the ORF2 was determined to encode for the endopeptidase E (PepE) enzyme, a protein of 438 amino acids with a calculated molecular weight of 50 kDa. The ORF2 was also determined to contain a conserved domain of cysteine protease family aminopeptidases. The putative promoters, a ribosomal binding site (RBS), and a rho-independent terminator for the putative pepE gene were identified. Nucleic acid BLAST analyses revealed that the pepE gene from *Lb. helveticus*
WSU-19 shares 98% homology with nucleotides 1 to 1469 of the 1680 bp pepE gene from *Lb. helveticus* CNRZ32. Results from the amino acid BLAST analyses revealed that the deduced amino acid sequence from the putative pepE gene shares 99% homology with PepE from *Lb. helveticus* CNRZ32, but only a 52% homology with endopeptidase E2 (PepE2) from *Lb. helveticus* CNRZ32.

A single colony hybridization-positive colony was obtained for the cloning of the putative pepO2 gene. Complete sequencing of the 5.7-kb *Xbal* and *SphI* genomic insert revealed an open reading frame (ORF) of 1944 bp. Based on the BLAST analyses, the 1944-bp ORF was determined to encode for the endopeptidase O2 (PepO2) enzyme, a protein of 648 amino acids with a calculated molecular weight of 74 kDa. This ORF contained a conserved domain of metalloendopeptidases. The putative promoters, ribosomal binding sites (RBS), and rho-independent transcriptional terminators of the putative pepO2 gene were identified. Nucleic acid BLAST analyses revealed that the pepO2 gene from *Lb. helveticus* WSU-19 shares a 96% homology with the nucleotides 275 to 2395 of the 2395 bp-pepO2 gene from *Lb. helveticus* CNRZ32. Results from the amino acid BLAST analyses revealed that the deduced amino acid sequence from the pepO2 gene shares a high homology (98%) with PepO2 from *Lb. helveticus* CNRZ32, but only a 62% and 56% homology with endopeptidase O3 (PepO3) and endopeptidase O (PepO) from *Lb. helveticus* CNRZ32, respectively. In addition, the deduced PepO2 sequence from *Lb. helveticus* WSU-19 shares 41% homology with neutral endopeptidase O from *Lactococcus lactis* subsp. lactis I11403 and *Lc. lactis* subsp. cremoris P8-2-47.

Fourteen hybridization-positive colonies were obtained for the cloning of the pepO gene. Phenotypic and genotypic analyses are currently in progress in order to identify a pepO clone for future work.

The approach of using the pepN probe from *Lb. helveticus* CNRZ32 was unsuccessful in cloning the pepN gene from *Lb. helveticus* WSU-19. Therefore the following approach was utilized instead.

### 2.1.4. Cloning of the pepN structural gene

A set of primers was designed based on the sequence of the pepN genes and PepN enzymes from *Lb. helveticus* CNRZ32, *Lb. helveticus* 53/7, *Lb. gasseri*, *Lb. plantarum* WCFS1, and *Lb. delbrueckii* ssp. *lactis* DSM7290. The sequences of the forward and reverse degenerate primers were 5'-GAGCTCACWTTYCAHCCAGWCAYTAYRAHTHN-3' and 5'-GCTCTAGARTCCATCTRAYTCCGVBTHAR-3', respectively. The *SacI* and *XbaI* sites were added to the forward and reverse degenerate primers, respectively. The structural pepN gene from *Lb. helveticus* WSU-19 was amplified using the degenerate primers and genomic DNA from *Lb. helveticus* WSU-19 as the template. The amplicon (2.3 kb) was ligated to the pJDC9 vector DNA digested with *SacI* and *XbaI*, and introduced into electrocompetent *E. coli* DH5_ cells. Antibiotic resistant transformants were screened by blue/white screening and restriction enzyme analyses. Three clones containing the pepN structural gene from *Lb. helveticus* WSU-19 were obtained. Nucleic acid BLAST analyses based on 5'- and 3'-end DNA sequencing revealed a 98% homology with the nucleotide regions 161-975.
and 1791-2587 of the pepN gene from *Lb. helveticus* 53/7, respectively. Additionally, the pepN structural gene from *L. helveticus* WSU-19 was determined to have a 98% homology with the nucleotide regions 905-1719 and 2535-3331 of the pepN gene from *Lb. helveticus* CNRZ32.

### 2.1.5. Cloning of the entire pepN gene

The pepN gene primers were designed based on the sequence of the pepN structural gene from *Lb. helveticus* WSU-19. The pJDC9::pepN construct was used as the template for the synthesis of DIG-labeled pepN probe (2.3 kb). High stringency Southern hybridization was performed using this newly synthesized probe to identify the pepN gene from *Lb. helveticus* WSU-19. Based on the results from the Southern hybridization, Sacl and Sphl were determined to be the restriction enzymes for cloning of pepN gene from *Lb. helveticus* WSU-19. Using this new probe, seven hybridization-positive colonies were obtained. All of the colonies were determined to have activities on Lys-pNA substrate. The 5’- and 3’-end DNA sequencing of the genomic inserts from the plasmid DNA obtained from three of the seven colonies confirmed that the pepN gene from *Lb. helveticus* WSU-19 was successfully cloned. The sequencing of a 5-kb genomic insert from one of the clones is currently in progress.

### 2.1.6. Additional phenotypic confirmation of the peptidase clones

We confirmed the peptidase activities of the pepN, pepE, and pepO2 clones from *Lb. helveticus* WSU-19 on _-CN f193-209 and _-CN f1-23 substrates. The _-CN and _-casein fragments were hydrolyzed under simulated cheese conditions (pH 5.2 and 4% sodium chloride) by the cell free extracts (CFEs) from *E. coli* DH5_ derivatives expressing the peptidase genes from WSU-19. The degradation patterns of the _-CN and _-casein fragments by CFEs were determined by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

### 4. Significant Conclusions:

We have successfully cloned the pepN, pepE, pepO, and pepO2 genes, fully sequenced the pepE and pepO2 genes, and developed a gene transfer system for *Lb. helveticus* WSU-19. We are currently in the process of the cloning of the pepO3 gene and fully sequencing of the pepO and pepN genes.

We have confirmed that PepN, PepE, and PepO2 from *Lb. helveticus* WSU-19 are involved in the degradation of bitter peptides _-CN f193-209 and _-CN f1-23 under simulated cheese ripening conditions.

Finally, we have initiated our efforts on the inactivation of the pepN, pepE, pepO, and pepO2 genes. These efforts include the generation of in-frame and out-of-frame deletions in the peptidase genes of interest, cloning of the deleted versions of the peptidase genes into pSA3 (a temperature-sensitive integration vector), and gene replacement using the pSA3-based integration vectors.
5. Anticipated Problems/Delays:

We have yet to obtain a PepN-deficient *Lb. helveticus* WSU-19 derivative. However, we have successfully cloned/characterized three peptidase genes that we did not include in our original proposal.

The inactivation of the *pepN*, *pepE*, *pepO*, and *pepO2* genes from *Lb. helveticus* WSU-19, the characterization of the resulting isogenic mutants in selected growth media, and the use of the mutants in Cheddar-type cheesemaking trials are expected to contribute greatly to our understanding of the role of these enzymes in bacterial growth, cheese flavor development, and prevention of bitterness.

Publications:


Theses: NA

Published Abstract:


Presentations:


Patent/Invention Disclosures: NA

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted: NA
Western Dairy Center  
Project Report  
Reporting Period January 1, 2003-June 30, 2004

Principal Investigators: Jeffrey R. Broadbent  
Co-Investigators: Dr. Dennis L Welker, Dr. Craig J Oberg

Project Title: Analysis of Capsule Production in Streptococcus Thermophilus by Comparative Genomics

Institution's Project #: 03136

Project Completing Date: Dec. 31, 2004

Modifications to Project/Budget: none

Project Objectives:
Objective 1. Use comparative genomics to identify genes that may be required for capsule attachment in Streptococcus thermophilus MR-2C.  
Objective 2. Determine the physiological significance of those genes in capsule attachment.

Project Summary:
Our group has shown that encapsulated, but not ropy, exopolysaccharide-producing (EPS) *S. Thermophilus* strains can significantly increase cheese yield and improve functionality without deleteriously affecting cheese quality, whey viscosity, or UF concentration time. We have also identified genes that may be involved in capsule attachment through genetic comparisons of EPS genes in very closely related encapsulated and ropy *S. thermophilus* strains. In this project, we will create isogenic derivatives of these strains to investigate the role of these genes in cell capsule attachment.

1. Significant Progress against Objectives:

   Nucleotide sequence and structural organization of the group of genes required for exopolysaccharide synthesis (*cps* gene cluster) in the capsule-producing strain MR-2C are almost identical to *cps* gene clusters of three ropy, non-encapsulated strains (MTC360, MTC330, and Sfi6). As a result, it is our hypothesis that comparative genomic analysis of *cps* clusters in these four strains will allow us to identify genes encoding proteins that attach the exopolysaccharides to the cell surface. In order to test this hypothesis, we first extended DNA sequence data from the genomic regions flanking known portions of the *cps* gene clusters of MR-2C, MTC360 and MTC330, to ensure that the entire *cps* gene cluster of each strain has been identified. BLASTx protein homology searches against the nucleotide sequence data confirmed that we had identified all of the genes associated with exopolysaccharide synthesis in these three strains.

   Comparative genomic analysis of MR-2C, MTC360, MTC330, and Sfi6 sequence data identified two conserved mutations in ropy strains that were not present in the capsule-producing strain MR-2C; one in the *cpsA* gene and the other in the *cpsM* gene.
The \textit{cpsA} gene is thought to be involved in regulation of polysaccharide synthesis, but the function of the \textit{cpsM} gene remains to be established. In addition, a putative nonsense mutation was detected in the \textit{cpsG} gene of strain MTC330, and compositional studies have indicated that the polysaccharide produced by this strain contained different sugar linkages and types than those of MR-2C and MTC360 (which had a similar composition). Gene replacement and complementation studies are now underway to investigate the relationship between the genetic polymorphisms we have detected and exopolysaccharide type and composition in these strains.

At present, we have constructed plasmid-based complements that express the MR-2C \textit{epsA}, \textit{epsD}, \textit{epsG}, and \textit{epsM} genes. The \textit{epsG} complement has been transformed into strain MTC330 and work is underway to characterize the effect of this construct on EPS composition of MTC330. Although we were able to isolate a large amount of polysaccharide from the mutant, our initial efforts to characterize EPS structure by NMR were confounded by contaminating sugar in the growth medium (M17). To overcome this limitation, we tested our strains for the ability to grow in a variety of chemically defined media that lack ingredients containing complex sugars (i.e., yeast or beef extracts). After considerable work, we were successful in identifying a defined medium that supports the growth of our strains, and we have been using this medium to prepare sufficient EPS for a 2nd round of NMR work. In the coming year, we expect to complete our collection of complement and null mutants, and determine the effect of our target genes on EPS production by \textit{S. thermophilus}.

2. Significant Conclusions:

see progress, above

3. Anticipated Problems/Delays:

none

Publications:


Theses:

none

Published Abstract:

none

Presentations:


Paten/Invention Disclosures:  
none

Technology Transfer Activities
For information on licensing contact:
Dr. Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center  
Project Report  
Reporting Period: January 1, 2002—June 30, 2004

Principal Investigators: Jeffrey R. Broadbent  
Co-Investigators: Carl Brothersen

Project Title: Effect of Oxidation-Reduction Potential on Growth of Lactic Acid Bacteria

Institution’s Project #: 02132

Project Completion Date: 12/31/03

National Research Plan (1997): Priority: Goal: Tactic:

Modifications to Project/Budget: none

Project Objectives: (Include any revisions to objectives)

Objective 1: To determine the effect of oxidation-reduction potential (Eh) on the growth rate of selected starter and nonstarter lactic acid bacteria (NSLAB).

Objective 2: To determine if Eh can be used to preferentially control the growth of starter and NSLAB at the species or strain level.

Objective 3: To determine the correlation between manufacturing protocols, cheese Eh, and NSLAB populations in Cheddar-type cheese.

Project Summary:

All bacterial-ripened cheeses contain nonstarter lactic acid bacteria (NSLAB) that enter cheese through milk or processing equipment and grow to high numbers during ripening. NSLAB can have a significant effect on flavor development, but little is known about factors that influence the growth and composition of the nonstarter biota. As a result, the types and numbers of NSLAB in cheese, and their impact on flavor, is still largely a matter of chance. This project will define influence of oxidation-reduction potential on growth of NSLAB and determine whether this property is a key factor in strain dominance.

1. Significant Progress against Objectives:

Research to date continues to focus on objectives 1 and 3. Redox experiments with different cheeses have worked well, and we have set up a fermentation system to study the relationship between growth of starter and NSLAB and redox. As reported previously, data from cheese trials performed under objective 3 support our hypothesis that Eh may be influenced by the cheese manufacturing process and by NSLAB populations. Colby cheese is a
washed curd variety of Cheddar cheese, and has a higher moisture content and lower acidity than Cheddar because lactose and lactate are rinsed from the curd during washing. Previous workers have reported that the initial decrease in cheese Eh is due to oxidative fermentation of residual lactose by starter bacteria, so Colby cheese would be expected to show a higher initial Eh and more gradual Eh drop than milled or stirred curd Cheddar. These trends have been observed in our trials, but the Eh of Colby cheese ultimately reached values more negative than those measured in milled or stirred curd Cheddar cheese. The latter observation was unexpected, but enumeration of NSLAB in each cheese revealed populations in Colby cheese were approximately two orders of magnitude higher than those of the milled or stirred curd Cheddar at 1 mo ($10^5$ versus $10^3$ cfu/g, respectively). Starter numbers in all three cheeses were similar. Characterization of the NSLAB species in each cheese type was performed by API-50 tests and by partial 16S rRNA sequence analysis, and showed both cheese types had NSLAB populations that were dominated by *Lactobacillus (Lb.) casei* and *Lb. curvatus*.

Research to study the relationship between NSLAB growth and redox has thus far been limited to investigation of the effect of starter and NSLAB isolates on redox. Results from that work have demonstrated that Eh goes up with lactose fermentation by some strains, but goes down with others. This strain-dependent phenomenon has been noted with strains of *Lactococcus lactis* starter bacteria as well as non-starter and adjunct strains of *Lb. casei*. In our next series of experiments, we plan to hold pH constant at 5.2 and determine the effect of starter and NSLAB growth on redox. After that, we will hold redox constant and measure the specific growth rates of our strains to see if Eh can be used to preferentially control the growth of starter and NSLAB at the species or strain level (objective 2).

2. Significant Conclusions:
see progress, above

3. Anticipated Problems/Delays:
Efforts to set up the fermentation system for growth and redox trials with NSLAB isolates proved more problematic than anticipated. We believe these difficulties have since been overcome, but more trouble shooting may still be required.

Publications:

Theses:
none

Published Abstract:
none
Presentations:

Patent/Invention Disclosures:
none

Technology Transfer Activities
For information on licensing contact:
Dr. Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center  
Project Report  
Reporting Period January 1, 2003-June 30, 2004

Principal Investigators: Jeffery R. Broadbent  
Co-Investigators: Dennis Welker

Project Title: Compilation of a Whole Genome Sequence for *Lactobacillus casei* ATCC 334

Institution’s Project #: 03142

Modifications to Project/Budget: none

Project Objectives:  
1. To obtain a finished genome sequence for *Lactobacillus casei* ATCC 334

**Project Summary:** Flavorful cheese has premium value as food or a food ingredient, and flavor development requires lactic acid bacteria (LAB). While starter LAB have the greatest influence on flavor, adventitious (non-starter) LAB (which are primarily *Lactobacillus casei*) can also modify and accelerate cheese flavor development. As a result, the cheese industry now uses a variety of lactobacilli, including *Lb. casei*, as flavor enhancing adjunct cultures to intensify and accelerate cheese flavor development. Our group is involved in a project with the U.S. Department of Energy’s Joint Genome Institute to determine a draft-quality (gapped) genome sequence for *Lb. casei* ATCC334. This project will facilitate completion (gap closure) of the *Lb. casei* genome sequence and provide industry and academia with fundamental information required to predictably enhance or intensity flavor development in a variety of bacterial-ripened cheeses.

1. **Significant Progress against Objectives:**  
   As noted above, strains of *Lb. casei* are now commonly used to intensify and modulate cheese flavor, and this species also dominates populations of adventitious bacteria in ripening cheese. Additionally, *Lb. casei* is also amenable to genetic manipulation. Our group is involved in a project with the U.S. Department of Energy’s Joint Genome Institute to determine a draft-quality (gapped) genome sequence for *Lb. casei* ATCC334. Subsequently, JGI and the PI developed a contractual arrangement with Fidelity Systems, Inc. (Gaithersburg, MD) to finish (close all sequence gaps and polish all areas of low quality sequence coverage) the *Lb. casei* genome sequence for a flat fee of $15,000. Fidelity Systems has finished all of the sequence runs and provided the PI with a single contig assembly for the *Lb. casei* ATCC334 genome sequence. That sequence was subsequently provided to bioinformatics specialists at the Department of Energy’s Oak Ridge National Laboratory, who ran it through the DOE’s computer-based annotation program and provided the PI with a password-protected web-based output file. That file has now been provided to Nimblegen Systems, Inc., who are currently using it to construct whole genome DNA microarrays for transcriptional studies with *Lb. casei*. As a whole, the
results of this project will allow the PI to perform research that will provide industry with the fundamental information required to predictably enhance or intensify flavor development in a variety of bacterial-ripened cheeses.

2. Significant Conclusions:
see progress, above

3. Anticipated Problems/Delays:
none

Publications:
none

Theses:
none

Published Abstract:
none

Presentations:


Patent/Invention Disclosures:
none

Technology Transfer Activities
For information on licensing contact:
Dr. Jeff Broadbent

Visitors Hosted:
none
Western Dairy Center
Reporting Period January 1, 2003 — June 30, 2004

Principal Investigators: Bart Weimer
Co-Investigators: 
Project Title: Genome finishing of Brevibacterium linens

Institution’s Project #: 03145

Project Completion Date: 12/31/03

Modifications to Project/Budget: NONE

Project Objectives: (Include any revisions to objectives)
Objective 1: Fill gaps in the genome of Brevibacterium linens ATCC9174.

Project Summary: (Suitable for inclusion in Center documents released to the public)

The high quality draft sequence of Brevibacterium linens ATCC9174, DNA, and the sequencing library held by the Joint Genome Institute was transferred to Fidelity Systems (Rockville, MD). The company is under contract via a special agreement with the LABGC to produce gap-free, polished genome sequences for all 11 genomes. Once completed the genome will be reannotated, published, and used to design arrays to discover unique gene expression patterns associated with fermented dairy products. Dr. Weimer and his group will provide the needed materials and work with Fidelity Systems to assist in gap filling and annotation. Once the gaps are filled and annotation is done, the data will be returned to Dr. Weimer for further curation of the annotation and for use in experiments directly related to cheese safety and flavor. This sequence will be used for experiments to understand the genetics, physiology, and role of this organism in producing safe and flavorful products.

Significant Conclusions: The genome was finished into a single contig by Fidelity Systems. Once finished, the sequence was sent to Oak Ridge National Labs (ORNL) to check the assembly, gap finishing, and automated annotation.

The genome is 4.4 Mb with 3925 candidate ORF’s. The genome contains many unusual transporters and has a very diverse metabolic potential. The circular map indicates that the genome has a very evenly distributed GC content around the genome of 62.7%. Of the candidate genes, 445 (~11%) did not match any known gene. Additional analysis is proceeding to verify the annotation and
reconstruct the metabolic potential of this organism with Pathway tools.

A gene expression array is being designed for future studies on the metabolism and physiology of this organism with the aim of determining how to improve flavor development of fermented dairy products.

**Publications:**
Two in progress

**Theses:**
NONE

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**
NONE

**Technology Transfer Activities**
For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures:** (Title, Date)

**Patents:** (Title, Date, #)

**Licensing Activities:**

**Discoveries:**
Western Dairy Center

Reporting Period January 1, 2003 —June 30, 2004

Principal Investigators: Bart Weimer
Co-Investigators: Eric Bastian
Project Title: Antimicrobial potential of enzyme modified peptides

Institution’s Project #: 03146

Project Completion Date: 6/30/04

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)
Objective 1: Screen whey samples for antimicrobial activity.

Project Summary: (Suitable for inclusion in Center documents released to the public).

We hypothesize that the specific enzyme treatments used making whey produces specific compounds that are antimicrobial (bacteriostatic or bactericidal) or inhibits microbe binding to host tissue. The antimicrobial effect will be determined.

Methods:
In this project we used mass spectroscopy and disc inhibition assays to determine the antimicrobial potential and activity, respectively, in hydrolyzed whey protein preparations. Multiple bacterial strains and types were used to determine the inhibitory action.

Significant Conclusions:
The preparation investigated was a commercial whey protein hydrolyzate (WPH). Mass spectral analysis determined that the preparation contained many different peptides of various sizes ranging from intact β-lactoglobulin (19,800 D) to short peptides. Without fractionation of the sample, it was tested for antibacterial activity at multiple protein concentrations after dissolving the sample in water. None of the concentrations produced inhibition. Further analytical characterization with MS and tryptic digestion, it was determined that the sample contained known antibacterial peptides. However, these peptides were unavailable for inhibition. Combining the WPH with an antibacterial rhamnolipid produced by Pseudomonas spp. resulted in the abolishment of the antibacterial activity. These data indicate that the commercial preparation contained the potential for inhibition, but the hydrolysis was not sufficient to release these peptides. Further, the WP masked the inhibition of known
antibacterial compounds that are likely to be present in aging milk preparations.

Publications:
None

Theses:
Prerak Desai – in progress

Published Abstract:
None

Presentations:
None

Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:
Western Dairy Center
Project Report
Reporting Period: January 1, 2002 — May 31, 2004
Final Report
Principal Investigator: Ann W. Sorenson
Co-Investigators: Roxane Pfister, Karl Smith (Student Research Assistant) Gary Straquadine, Judith Hallfrisch (emeritus),
Project Title: Identify the Role of Milk and Milk products on Nutrition and Health Issues of Importance for adults fifty Years of Age and Older
Institution’s Project #: 01131
Project Completion Date: 6/30/04
National Research Plan (1997): Priority: Goal:  Tactic:
Modifications to Project/Budget: None
Project Objectives: (Include any revisions to objectives)
1. Establish the dietary patterns of people over 50 years of age living in Utah by nutrients, foods and food groups.
2. Determine how nutrients and nutrachemicals found in dairy products add to the nutritional adequacy of the diets of older people in Utah.
3. Test for associations of milk and milk products with outcomes variables representing good health and of disease and disability of old age while controlling for co-variants and confounding effects.
4. Determine the factors such as cost, convenience, ethnicity and prestige that determine the food choices of older people in order to develop new foods made from dairy products that will appeal to this age group.
Project Summary: Almost all older adults in Utah consume dairy products with yellow cheeses, ice cream and milk heading the list of most popular milk products. Dairy products are a good source of nutrients, namely calcium, vitamin A, vitamin D, riboflavin, minerals and protein and they are a cost effective way to achieve the daily recommendations of many nutrients. Dairy Products also have health benefits. In general, they, especially the higher fat
products, are protective for cancers and heart disease and osteoporosis but have little or no impact on diabetes. Calcium appears to be protective against heart, and cancers in men and overweight in women.

**Methodology:** The data management and data linkages with statistical packages (SAS and SPSS) are complete. Original software for analyzing foods, food groups, nutrients, nutraceuticals and dietary patterns has been written and installed. It is linked to the online National Nutrient Data Base (Release 11, 2004) maintained by USDA. Ann Sorenson’s research assistant and student, Karl Smith, wrote the computerized diet analysis program used in this research. This original computer program can analyze more variables and is much more flexible than the popular commercial packages that are available to the public.

**Questionnaire:** The 41 page validated questionnaire is a compilation of the questions that best reflect successful aging, the major causes of mortality and morbidity and their risk modifiers. The questions address specifically demographics, lifestyle and health habits, drug and supplement use, social support networks, nutritional habits and an assessment of food intake. The questionnaire is based on data in the literature, National Health and Nutrition Examination Surveys; information collected by government programs for older people and standardized questionnaires used in other aging studies.

The Food Frequency Questionnaire was developed and validated specifically for older people in Utah. We took the data from two pilot projects in which we collected 24-hour recalls from a random sample of people 50 years of age and older living in Utah to determine the staple foods and foods that contributed key nutrients to their diets. This study provided a list of 153 foods and food combinations, which provided the basis for the food frequency.

The age range of the participants in the next round of data collection will be increased to include people 35 years of age and older. The Questionnaire has been revised and is currently under going review by other professionals and pilot testing by people in the 35 to 49 year age group. The questionnaire was adjusted for people 35 to 49 years according to the NHANES III questionnaires for the same age group. The original questionnaire will be used for the older groups (ages 50 through 79). The Food Frequency Questionnaire has been revised for all age groups to reflect the foods that have entered the diet over the past 5 years and the foods removed that are no longer consumed. The food groupings were also revised to better capture the individual foods people consume. We plan on having the new questionnaires printed by July 1 and the questionnaires sent out by the end of the summer of 2004.
**Data collection:**
The sample for the study was obtained from the latest updates of Utah driver's license roles, which were supplied by the Utah Division of Motor Vehicles. A random sample of persons was drawn from the files, which included all persons with driver's licenses or identification cards. The sample was stratified by gender, age and urban/rural place of residence.

A pilot of the Utah survey of older people was begun in 1999. During 1999–2000, 550 usable questionnaires were and coded and used in the first analysis of the study.

Two waves of data were collected during 2002. In the spring, 184 usable questionnaires were collected from people 50 to 64 years of age. During the fall, 278 participants returned questionnaires ages 50 to 79 years for a total of 462. Some additional questionnaires were returned after being sent a reminder card. The final number of complete and usable questionnaires was collected from 485 men and 522 women with a population distribution shown in Table 1.

**Table 1**
**Distribution (%) Of People Living In Utah 50 Yrs Of Age And Older 1998 And 2002 Samples, By Gender And Year**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age class</th>
<th>1998 n(%)</th>
<th>2002 n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>50 - 64 yrs</td>
<td>73(25.2)</td>
<td>143(73.3)</td>
</tr>
<tr>
<td></td>
<td>65 - 79 yrs</td>
<td>139(47.9)</td>
<td>45(23.2)</td>
</tr>
<tr>
<td></td>
<td>≥ 80 yrs</td>
<td>78(26.9)</td>
<td>7(3.6)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>290(100)</td>
<td>195(100)</td>
</tr>
<tr>
<td>Women</td>
<td>50 - 64 yrs</td>
<td>92(35.4)</td>
<td>202(77.1)</td>
</tr>
<tr>
<td></td>
<td>65 - 79 yrs</td>
<td>103(39.6)</td>
<td>49(18.7)</td>
</tr>
<tr>
<td>≥ 80 yrs (a)</td>
<td>65(25.0)</td>
<td>11(4.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>260(100)</td>
<td>262(100)</td>
</tr>
</tbody>
</table>

(a) 80 age group was dropped in 2000, because Medicare files were no longer available for our sampling pool.

All data results have been age and gender adjusted according to the year 2000 Utah census. People of all races and ethnicities are included in the survey. However, due to the small size of Utah's minority population (< 11% of the state population, which are predominantly working age adults and children), it is unlikely that definitive information on Utah's minorities in the general population of older people will be obtained from this survey. The participation rate averages slightly over 50 percent, which is consistent with other similar diet and/or health surveys.

**Data Analysis:** The questionnaires collected in 1999-2003 have been coded
and the data analyzed for several studies that compared aspects the diets of the Utah cohort to the National survey NHANES III and to a population of the same age in Geneva Switzerland.

In order to justify combining the data from the 1999 and 2002 rounds of the survey, the same variables were compared between the 1999-2000 (550 people) and the 2002 (462 people) cohorts to determine any significant changes in the dependent variables. This comparison was made because factors that may confound or work synergistically with the variables of interest (in this case, dairy products) must be controlled for in the analysis to eliminate their effects on the outcomes (the relationship to diseases). Of particular concern were the risk modifiers including physical activity, alcohol use, smoking, use of red meat, age and gender. There were no differences in these variables between the 1999 and 2002 cohorts except men smokers (ages 65-79; \( p = 0.01 \)) decreased and the number of Women who use alcohol increased (ages 50-64; \( p = 0.04 \))

In addition, the intake of fourteen dairy foods was compared between the 1999 and 2000 cohorts. The intake of only three of the foods had changed significantly in the 2002 group; men ate more yogurt (\( p = 0.03 \)), women ate less cream soups (\( p = 0.01 \)), and both men and women indicated that they were drinking more of all types of milk rather than limiting their intake to mainly one type (\( p < 0.000 \)).

The survey data was combined for a total sample of 1007 people: 522 men and 485 women over 50 years of age.

**Statistical Analysis:** The analyses fall into 3 main categories. First, there were many cross-tabulations of health, nutrition, and life-style variables with each of, and all of, the stratification variables, and with each other. All this information was organized and stored in a web site. Because the distribution of nutrient intake is always skewed, all nutrient data were transformed, using a log linear transformation and other adjustments as appropriate before the data is analyzed.

Analysis also included exploratory regression, logistic regression, and other regression tests for the purpose of identifying predictors and correlates with health and nutrition outcomes, and with successful (robust) aging. Most of the data analysis is conducted with SPSS or SAS. Karl Smith, our programmer and research assistant, is responsible for the design and construction of the web site for information distribution among the research team.

**Significant Progress against Objectives: 1998 – 2004**

**OBJECTIVE 1: Establish the dietary patterns of people over 50 years of age living in Utah by nutrients, foods and food groups.**
This preliminary report was based on analysis of the 550 questionnaires collected in 1999-2000 and coded during 2001. For purposes of this report, 9 foods and 5 types of milk were classified as dairy products. Table 1 is the rank order of dairy products consumed by men and women in Utah over 50 years of age.

### Table 1
**Rank Order of Top Ten Dairy Products by Gender**

<table>
<thead>
<tr>
<th>MEN</th>
<th>WOMEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard cheese</td>
<td>Hard cheese</td>
</tr>
<tr>
<td>Milk (all types)</td>
<td>Milk (all types)</td>
</tr>
<tr>
<td>Ice cream</td>
<td>Cottage cheese</td>
</tr>
<tr>
<td>Cottage cheese</td>
<td>Ice cream</td>
</tr>
<tr>
<td>Butter</td>
<td>Yogurt</td>
</tr>
<tr>
<td>Puddings</td>
<td>Butter</td>
</tr>
<tr>
<td>Yogurt</td>
<td>Puddings</td>
</tr>
<tr>
<td>Hot chocolate</td>
<td>Cream cheese</td>
</tr>
<tr>
<td>Frozen Yogurt/Ice milk</td>
<td>Hot chocolate</td>
</tr>
</tbody>
</table>

Table 1

The lists are similar with only two foods, yogurt and cream cheese, more than one rank apart in the lists for men and women. Milk is the sum of the types (whole, 2%, 1%, and skim) available at most retail outlets.

The older people in Utah are a population of dairy food users. Only 3 percent of the male participants did not eat any dairy products and all women reported eating some type of milk-based product. The following table (2) shows the percent of participants who consumed dairy products.

### Table 2
**PERCENT OF PARTICIPANTS CONSUMING 6 MAJOR CATEGORIES OF DAIRY FOODS**

<table>
<thead>
<tr>
<th>Dairy products groups</th>
<th>Men (N=485)</th>
<th>Women (N=522)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n(%)</td>
<td>n(%)</td>
</tr>
<tr>
<td>Hard cheese</td>
<td>485(88.0)</td>
<td>461(88.3)</td>
</tr>
<tr>
<td>Ice cream</td>
<td>409(84.3)</td>
<td>444(85.0)</td>
</tr>
<tr>
<td>Low fat milk</td>
<td>432(89.1)</td>
<td>462(88.5)</td>
</tr>
<tr>
<td>High fat milk</td>
<td>354(72.0)</td>
<td>392(75.1)</td>
</tr>
<tr>
<td>Butter</td>
<td>309(63.7)</td>
<td>396(75.9)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>253(52.2)</td>
<td>378(72.4)</td>
</tr>
</tbody>
</table>

| Total dairy products  | 482(99)   | 522(100)      |
|                       | *age adjusted |
The table shows that all women and all but 0.04 percent of the men in the age-adjusted sample used one or more dairy products. However not all of the people consumed all dairy products. The intake of dairy products was similar for both men and women except that 52 percent of the men ate yogurt compared to 72 percent of the women.

Figure 1 is an age-adjusted comparison of the percent of men and women over 50 years of age using dairy foods. Over 90 percent report using hard cheeses and more than 80 percent drink milk. Even cream cheese, the dairy product eaten least is consumed by an average of 40 percent of the older population.

Figure 2 shows that older people than middle age people use 2% milk and women drink more of the reduced fat milks. Less than 10 percent of the older people in Utah select whole or use all types.
OBJECTIVE 2: Determine how nutrients and nutraceuticals found in dairy products add to the nutritional adequacy of the diets of older people in Utah

Dairy products contribute many nutrients to the diet. They provide almost one-third of the Vitamin A and three quarters of the calcium but only about one-quarter of the total calories in the of the diet. Hard cheeses, milk, ice cream and butter are the main dairy sources of Vitamin A, while hard cheeses, milk, yogurt, ice cream and hot chocolate are the main sources of calcium. Table 3 shows the rank of dairy products compared to the top 10 of all foods as sources of Vitamin A, Calcium and energy (Kcals).

<table>
<thead>
<tr>
<th>Age Categories</th>
<th>Vitamin A (rank)</th>
<th>Calcium (rank)</th>
<th>Energy (Kcal) (rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALES 50-64</td>
<td>Hard cheese (5)</td>
<td>Hard cheese (1)</td>
<td>Ice cream (9)</td>
</tr>
<tr>
<td></td>
<td>2% milk (10)</td>
<td>2% milk (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skim milk (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1% milk (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yogurt (5)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Dairy Food rankings from Top 10 Sources of All Foods For Selected Nutrients
It is clear that dairy products are the overwhelming sources of dietary calcium. No other foods rank in the top ten with the exception of liquid supplements and instant breakfast. Instant breakfast was not considered a dairy product but the powder is usually mixed with milk. Hard cheese is the number one contributor to total calories, not because it is higher in fat but because so many people eat cheese several times a week. The proportion of fat in natural hard cheeses is as high as 75 percent of total calories (about 78 fat Kcal per ounce) but the low fat processed cheddar or Swiss cheeses range from 25 to 50 percent fat calories (13 to 35 fat Kcal per ounce).

A word should be said about the influence of liquid supplements (like Ensure) on the diets of older people in Utah. Although 57 of the respondents reported use of liquid supplements, those that used them used them in many cases as meal replacements. Figure 3 illustrates that use of liquid supplements increases with age and over 20 percent of the oldest group used them. Of those who use liquid supplements, 55 percent are in the oldest age group.
To show the impact of liquid supplements on the diet of the total cohort, Table 4 shows the rank of liquid supplements as a source of Vitamin A, calcium and energy. Note that liquid supplements ranks as the number one source of vitamin A for people 65 years of age and older.

**Table 4**

**Liquid Supplements as a Source of Selected Nutrients**

<table>
<thead>
<tr>
<th>Age Categories</th>
<th>Vitamin A (rank)</th>
<th>Calcium (rank)</th>
<th>Energy (Kcal) (rank)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MALES</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-64</td>
<td>2</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>65-79</td>
<td>1</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>80+</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-64</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>65-79</td>
<td>2</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>80+</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

In addition, dietary supplements must be considered when determining the total nutrient intake of Utahans.

In the Utah survey and NHANES III, multiple vitamins or mineral preparations were used most widely. See Figure 4. In both surveys data were also collected on use of single vitamin and mineral supplements. The NHANES estimated that close to 16 percent of the older population used single vitamin supplements. The most frequently used were Vitamin C and Vitamin E. Approximately 2 percent used single mineral supplements, mainly Calcium and Iron. Figure 4 shows that the comparable Utah population used markedly more of each of these single supplements.
In addition to the supplements listed in Figure 4, the Utah survey also collected data on Beta-carotene, Vitamin B6, Vitamin B12, Vitamin D, Folic Acid, Zinc Magnesium, Iodine and copper. The intake results are shown in Figure 5.

**Figure 5**

Additional supplements used by older people in Utah

Age range 50–99 years of age, Mean Age: 72.5 +/- 12.9; Median age: 70.5

Dairy products are good sources Calcium, Vitamin A, Vitamin B12, Vitamin D, Zinc and Magnesium. Since dairy products are consumed in quantities great enough to meet the needs of older people in Utah, most people don’t need these supplements. The recommended amounts of only four nutrients are not obtained from food sources; Folate(67%), Vitamin E (48%), Calcium (90%), magnesium (95%)

**OBJECTIVE 3:** Test for associations of milk and milk products with outcome variables representing good health, disease and disability of old age while controlling for covariants and confounding effects.
Background  Health patterns of older adults living in Utah are somewhat different than the national average. As in the rest of the United States, adiposity is common in Utah with 68 percent of the men and 72 percent of the women either overweight or obese. However, the prevalence of heart disease, cancer and osteoporosis are lower than the National average while diabetes rates are higher.

Mortality Rates
The following graphs; Figures 6a, 6b and 6c; from the CDC Behavioral Risk Factor Surveillance System illustrate the difference in trends of mortality rates for heart disease, cancers and Type II diabetes.

Figure 6a
Coronary Heart Disease Deaths Utah & U.S., 1980-2001

Figure 6b

Figure 6c
According to the latest figures, Utah has the lowest mortality rate for most cancers and heart disease but the diabetes related mortality continues to escalate.

Morbidity Rates
The prevalence of common age adjusted health problems are displayed in the table below.

<table>
<thead>
<tr>
<th>Risk factor or disease</th>
<th>Men (N=485)</th>
<th>Women (N=522)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(%)*</td>
<td>n(%)*</td>
</tr>
<tr>
<td>Overweight (a)</td>
<td>211(43.3)</td>
<td>165(36.8)</td>
</tr>
<tr>
<td>Obesity (b)</td>
<td>122(25.1)</td>
<td>158(35.3)</td>
</tr>
<tr>
<td>Heart disease</td>
<td>87(17.9)</td>
<td>65(14.5)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>56(11.5)</td>
<td>41(9.2)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>11(2.3)</td>
<td>19(4.2)</td>
</tr>
<tr>
<td>Cancer (b)</td>
<td>101(21.4)</td>
<td>101(20.1)</td>
</tr>
</tbody>
</table>

* age adjusted
(a) Overweight : BMI ≥ 25 kg/m²
(b) Obesity : BMI ≥ 30 kg/m²
(b) All types of cancer

Our calculations agreed with the national estimates for adiposity in Utah, as well as the relation of prevalence of heart disease, cancer, osteoporosis and diabetes as compared to the National average. The National averages were calculated by the Centers for Disease Control.

In addition, we calculated the correlation of the cluster of diseases categorized as “Syndrome X” in the Utah population of adults 50 years of age and older. Syndrome X is the constellation of athrosclorotic risk factors including insulin resistance, hyperinsulinemia, dyslipidemia, essential hypertension, and abdominal obesity.

Indicators of Syndrome X used in Utah study were:
• Diabetes
• Heart Disease
• Hypertension
• Stroke
• Obesity

We found that, unlike most populations, these diseases were not correlated in the Utah population of older people. Obesity and diabetes were strongly correlated but they were not significantly associated with heart disease, stroke or...
hypertension. However, as expected, the incidence of heart disease, and stroke were highly correlated.

Past studies indicate that up 60% of the risk for chronic diseases of old age is associated with diet. Because older people consume more dairy products than the national average, we investigated the impact of dairy products on the health outcomes of this population. Specifically we investigated the association between dairy products and several chronic diseases of public health importance: Heart disease, Cancer, Osteoporosis, Diabetes, and Obesity.

The combined sample of 522 men and 485 women was large enough to determine the association between dairy products and pathology and public health problems of concern to the older population is Utah. The lifetime prevalence of risk factors or disease, the distribution of non-users for each dairy product group and the amount of each dairy product consumed were age adjusted. The analyses were performed with Stata 7.0.

The analysis revealed no significant associations between diabetes nor osteoporosis and dairy products. Though dairy products as the major source of calcium have been shown to be protective in other studies, the sample of cases in this study was too small for inferential analysis.

The sample was divided into 2 age groups by gender. The 15 dairy products examined were combined into 7 groups: high fat milk (whole milk, hot cocoa, cream based soup), low fat milk (milk skim, milk 1% or 2%, cottage or ricotta cheese), butter (butter, cream cheese), yogurt (yogurt, ice milk, frozen yogurt, non-fat ice cream, sorbet), hard cheese (cheddar, jack, mozzarella or Swiss), ice cream (ice cream, pudding) and all-dairy products (sum of the 6 groups). Daily intakes of the 6 dairy products groups were transformed using a square root. The all-dairy products group was log transformed. For each dairy group, we calculated the percent of users and non-users. In addition to the Dairy products, calcium was also investigated and a risk modifier because of its protective association in others studies against cancer and obesity. Dairy products are the primary source of calcium in the diet.

The associations between consumers and non-consumers of the dairy product groups and diseases were analysed using logistic regression models. The associations between daily intakes of dairy products of users, the amount of calcium and their relation to diseases were analysed by linear regression models. Calcium is defined as the sum of calcium from the diet and calcium supplements, which was calculated for each individual participant. All analyses were done separately for men and women and for each age group: 50-64 years of age and 65-79 years of age. All regressions were adjusted by age alcohol use, physical activity, smoking, and red meat consumption and with and without total kilocalorie adjustment. A 5% significance level was used for all tests.

Table 6 below shows that all women and all but 0.04 percent of the men in the age adjusted sample used one or more dairy products. However not all of the people consumed all dairy products. The intake of dairy products was similar
for both men and women except that 32 percent of the men ate yogurt compared to 24 percent of the women.

Table 6

<table>
<thead>
<tr>
<th>Dairy products groups</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=485)</td>
<td>(N=522)</td>
</tr>
<tr>
<td></td>
<td>n(%)*</td>
<td>n(%)*</td>
</tr>
<tr>
<td>Hard cheese</td>
<td>58(8.0)</td>
<td>61(10.2)</td>
</tr>
<tr>
<td>Ice cream</td>
<td>76(10.5)</td>
<td>78(13.0)</td>
</tr>
<tr>
<td>Low fat milk</td>
<td>53(7.3)</td>
<td>60(10.0)</td>
</tr>
<tr>
<td>High fat milk</td>
<td>131(18.0)</td>
<td>130(21.7)</td>
</tr>
<tr>
<td>Butter</td>
<td>176(24.2)</td>
<td>126(21.0)</td>
</tr>
<tr>
<td>Yogurt</td>
<td>232(32.0)</td>
<td>144(24.0)</td>
</tr>
<tr>
<td>Total dairy products</td>
<td>3(0.4)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

*age adjusted

Significant associations between users and non-users of dairy products: A protective effect was found for butter against heart disease (p = 0.036) in men 50 to 64 years of age. Of those with disease, 36.9 percent used butter but 95.2 percent without disease used butter. Butter was also protective against hip fracture (osteoporosis) and hypertension (p = .05) Because margarine is the most common alternative spread and baking fat to butter we determined its relationship to health outcomes. It is of note that Margarine is the major source of trans fatty acids, which are thought to have adverse health effects. However, we found that margarine was protective for heart disease and for diabetes along with vegetable oil at greater that the p = .05 level. Both hard cheeses (p = 0.001) and low fat milk (p = 0.028) were inversely associated with cancer in women. Before Calorie adjustment, a weak positive association (p = 0.044) was noted between butter and overweight women.

Significant associations between users of dairy products: There were no significant associations, either positive or negative, were found between diabetes and dairy products. However, there were a number of significant associations found with other diseases and conditions. In general, trends of lower intakes of dairy products were associated with heart disease, cancer, and obesity. Several significant associations were found with specific dairy product groups and disease. These associations are summarized in the table below.
Table 7

Mean daily consumption (in grams) for each dairy product group for selected health conditions by gender Utah 1998-2002.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Dairy products groups</th>
<th>Heart disease</th>
<th>Overweight</th>
<th>Obesity</th>
<th>Cancer</th>
<th>Osteoporosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (a)</td>
<td>Yes (a)</td>
<td>No (a)</td>
<td>Yes (a)</td>
<td>No (a)</td>
<td>Yes (a)</td>
</tr>
<tr>
<td>Men</td>
<td>Hard cheese (a)</td>
<td>27.1*</td>
<td>17.3*</td>
<td>23.3</td>
<td>27.7</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td>Ice cream (a)</td>
<td>30.1</td>
<td>30.2</td>
<td>35.1</td>
<td>29.0</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>Low fat milk (a)</td>
<td>136.9</td>
<td>104.1</td>
<td>102.2</td>
<td>171.4</td>
<td>102.4</td>
</tr>
<tr>
<td></td>
<td>High fat milk (a)</td>
<td>100.9</td>
<td>154.4</td>
<td>108.6</td>
<td>96.7</td>
<td>109.4</td>
</tr>
<tr>
<td></td>
<td>Butter (a)</td>
<td>4.8</td>
<td>3.5</td>
<td>5.6</td>
<td>3.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Yogurt (a)</td>
<td>41.1</td>
<td>28.3</td>
<td>45.5</td>
<td>52.6</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>Total dairy products (b)</td>
<td>251.4</td>
<td>239.3</td>
<td>244.6</td>
<td>291.7</td>
<td>250.2</td>
</tr>
<tr>
<td></td>
<td>Calcium (mg) (b,c)</td>
<td>1482.9</td>
<td>1100.1</td>
<td>1210.8</td>
<td>1692.8</td>
<td>1202.0</td>
</tr>
<tr>
<td>Women</td>
<td>Hard cheese (a)</td>
<td>17.1</td>
<td>16.6</td>
<td>17.8</td>
<td>13.9</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Ice cream (a)</td>
<td>25.7</td>
<td>22.6</td>
<td>21.1</td>
<td>29.0</td>
<td>22.4</td>
</tr>
<tr>
<td></td>
<td>Low fat milk (a)</td>
<td>181.1*</td>
<td>120.5*</td>
<td>191.3</td>
<td>167.0</td>
<td>177.4</td>
</tr>
<tr>
<td></td>
<td>High fat milk (a)</td>
<td>118.2*</td>
<td>73.7*</td>
<td>123.3</td>
<td>104.9</td>
<td>116.3</td>
</tr>
<tr>
<td></td>
<td>Butter (a)</td>
<td>4.8</td>
<td>2.8</td>
<td>4.6</td>
<td>3.9</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Yogurt (a)</td>
<td>36.7**</td>
<td>71.9**</td>
<td>44.4</td>
<td>49.7</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td>Total dairy products (b)</td>
<td>252.7</td>
<td>240.1</td>
<td>322.0</td>
<td>248.2</td>
<td>283.1</td>
</tr>
<tr>
<td></td>
<td>Calcium (mg) (b,c)</td>
<td>1699.8</td>
<td>1437.1</td>
<td>1928.0</td>
<td>1435.3</td>
<td>1818.0</td>
</tr>
</tbody>
</table>

* p<0.05  
** p<0.01

(a) variables with a square root transformation
(b) variables with a log transformation
(c) calcium = calcium + calcium supplement
(d) mean daily amount (in grams) adjusted for age, gender, alcohol use, physical activity, total energy, red meat consumption and smoking
With the exception of yogurt intake and heart disease in women \( (p = 0.008) \) and osteoporosis in men, and calcium and overweight in men \( (p = 0.035) \), all of the significant associations between dairy products and health conditions were protective.

Calcium is strongly and inversely related to overweight in women \( (p = 0.002) \) but a weaker risk factor for overweight \( (p = 0.035) \) for men. The most robust protective associations are between higher fat dairy products groups (hard cheese, high fat milk and butter) and diseases. Riboflavin, which is found to the greatest extent in milk products, had no significant association with any health outcome. In other studies it has been associated with heart disease prevention.

Three highly significant age-specific associations were noted. High fat milks appears to be protective against heart disease for women in the 50 to 64 year age group \( (p = 0.006; \) women with disease consumed 39 grams per day and those without disease drank 137 grams per day) Calcium was particularly protective for heart disease in the 50 to 64 year old women \( (p = 0.006; \) 744 mg calcium for those women with heart disease and 1533 mg calcium for those without). The total of all dairy products was associated with overweight \( (p = 0.002) \) in men 65 +, with the overweight group consuming 434 grams of dairy products compared to an intake of 225 grams per day of those who were not overweight. The association with overweight in this group is probably due in part to the excesses intake of calories, and lower activity levels, which exceeds their energy requirement.

We also used the same analytical strategy to investigate the associations of meats to the same health outcomes as those investigated in the dairy product study. The results from meats and dairy products were then combined to determine their combined effect on health. Higher consumption of plain beef products were not associated with any increased risks of chronic disease, while consumption of mixed foods which include beef (such as stew) were associated with a decline in the risks of heart disease \( (p=0.04) \). In a model that contained both meat and dairy categories, both butter \( (p=0.017) \) and mixed beef \( (p=0.04) \) were protective for heart disease in both men and women. Moderate intakes of higher fat beef arrears to reduce risk for obesity \( (\text{BMI} \geq 30), (p=0.014) \) while consumption of dairy products are inversely associated with hip fracture in women only.

**OBJECTIVE 4:** Determine the factors such as cost, convenience, ethnicity and prestige that determine the food choices of older people in order to develop new foods made from dairy products that will appeal to this age group.

According to Food and Drink Europe market reports, the present worldwide dairy market is large and dynamic. Given current trends the dairy industry is likely to increase in volume sales growth over the next few years. Milk and cheese remain staples in most parts of the world. The North and South American market is strongly driven by US sales, which is twice is twice the size
of the Asia-Pacific market. Although Utah is not one of the major US dairy product producers, the dairying and cattle ranching are important industries in this state.

In our study, we investigated health benefits of dairy products. However, are dairy products affordable and convenient sources of many key nutrients? To answer this question, we selected 39 foods available in local supermarkets that are good sources of vitamins and minerals found in dairy products and 10 dairy products sold in volume in retail outlets. In November 2003, my research assistant and I visited three supermarkets in Logan and surrounding areas to collect data on cost, serving size and availability of these foods. The results are found in the Table below:

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Household Units</th>
<th>Cost per Cup</th>
<th>Cost cmp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy Milk</td>
<td>Cup</td>
<td>$0.16</td>
<td>100%</td>
</tr>
<tr>
<td>2%</td>
<td>Cup</td>
<td>$0.17</td>
<td>108%</td>
</tr>
<tr>
<td>Skim</td>
<td>Cup</td>
<td>$0.14</td>
<td>87%</td>
</tr>
<tr>
<td>Cheese Cheddar</td>
<td>1.5 oz.</td>
<td>$0.29</td>
<td>183%</td>
</tr>
<tr>
<td>Cottage</td>
<td>1/2 Cup</td>
<td>$0.88</td>
<td>560%</td>
</tr>
<tr>
<td>Cream</td>
<td>1 Tbl</td>
<td>$0.11</td>
<td>71%</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>1 Tbl</td>
<td>$0.06</td>
<td>38%</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>Cup</td>
<td>$0.37</td>
<td>234%</td>
</tr>
<tr>
<td>Yogurt</td>
<td>Cup</td>
<td>$0.57</td>
<td>360%</td>
</tr>
<tr>
<td>Butter</td>
<td>1 Tbl</td>
<td>$0.06</td>
<td>37%</td>
</tr>
<tr>
<td>Produce Apricots Canned/Water</td>
<td>Cup</td>
<td>$0.75</td>
<td>474%</td>
</tr>
<tr>
<td>Dried</td>
<td>10 halves</td>
<td>$0.54</td>
<td>344%</td>
</tr>
<tr>
<td>Peaches Canned/Water</td>
<td>Cup</td>
<td>$0.52</td>
<td>331%</td>
</tr>
<tr>
<td>Greens Turnip</td>
<td>1/2 Cup</td>
<td>$0.38</td>
<td>242%</td>
</tr>
<tr>
<td>Spinach Fresh cooked</td>
<td>1/2 Cup</td>
<td>$0.36</td>
<td>231%</td>
</tr>
<tr>
<td>Bag</td>
<td>1/2 Cup</td>
<td>$0.21</td>
<td>132%</td>
</tr>
<tr>
<td>Broccoli cooked</td>
<td>1/2 Cup</td>
<td>$0.28</td>
<td>176%</td>
</tr>
<tr>
<td>Dry Beans Lima, cooked</td>
<td>1/2 Cup</td>
<td>$0.26</td>
<td>164%</td>
</tr>
<tr>
<td>Asparagus</td>
<td>1/2 Cup</td>
<td>$0.68</td>
<td>431%</td>
</tr>
<tr>
<td>Carrots</td>
<td>1/2 cups</td>
<td>$0.35</td>
<td>225%</td>
</tr>
<tr>
<td>Green Peppers</td>
<td>1 pepper</td>
<td>$0.26</td>
<td>166%</td>
</tr>
<tr>
<td>Pumpkin Canned/Water</td>
<td>1/2 up</td>
<td>$0.30</td>
<td>190%</td>
</tr>
<tr>
<td>Squash Acorn/Butternut</td>
<td>1/2 Cup</td>
<td>$0.20</td>
<td>129%</td>
</tr>
<tr>
<td>Sweet Potatoes Baked</td>
<td>1 med potato</td>
<td>$0.33</td>
<td>209%</td>
</tr>
<tr>
<td>Tomatoes Raw</td>
<td>1 (1.5&quot; diam)</td>
<td>$0.40</td>
<td>254%</td>
</tr>
<tr>
<td>Mushrooms Raw</td>
<td>1/2 cup</td>
<td>$0.25</td>
<td>162%</td>
</tr>
<tr>
<td>Meat Eggs</td>
<td>1 egg</td>
<td>$0.13</td>
<td>82%</td>
</tr>
<tr>
<td>Beef Rump roast</td>
<td>4 oz.</td>
<td>$0.92</td>
<td>586%</td>
</tr>
<tr>
<td>Rib Roast</td>
<td>4 oz.</td>
<td>$0.70</td>
<td>442%</td>
</tr>
<tr>
<td>Hamburger</td>
<td>4 oz.</td>
<td>$0.60</td>
<td>383%</td>
</tr>
<tr>
<td>Liver</td>
<td>4 oz.</td>
<td>$0.35</td>
<td>223%</td>
</tr>
<tr>
<td>Lamb Chop</td>
<td>4 oz.</td>
<td>$1.78</td>
<td>1131%</td>
</tr>
<tr>
<td>Pork Roast</td>
<td>4 oz.</td>
<td>$0.48</td>
<td>303%</td>
</tr>
</tbody>
</table>
It is readily apparent that milk is inexpensive when compared on a serving size basis with the other foods. Using whole milk as the reference food, we determined the ratio of serving size prices of other foods to milk (last column) and found that only a serving of eggs, whole wheat bread and peanuts were less expensive than milk.

Although dairy products are inexpensive on a per serving basis, are they delivering nutrients in quantities comparable to other foods per serving? Calcium, Protein, Vitamin A, Riboflavin, Selenium, Phosphorus, Potassium and energy (Calories) were used to test this hypothesis. The results of Calcium, Protein, Vitamin A, Selenium, and Calories are presented here but the entire database is available upon request.

Table 9a

<table>
<thead>
<tr>
<th>Food Category</th>
<th>Calcium</th>
<th>RDA/DRI</th>
<th>Nutrients per Serving</th>
<th>Compared to Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Requirement</td>
<td>% per Serving</td>
<td>Nutrients</td>
</tr>
<tr>
<td><strong>Dairy</strong></td>
<td>Milk</td>
<td>1200</td>
<td>24%</td>
<td>285.6000</td>
</tr>
<tr>
<td></td>
<td>Whole</td>
<td>1200</td>
<td>24%</td>
<td>292.8000</td>
</tr>
<tr>
<td></td>
<td>2% 1%</td>
<td>1200</td>
<td>25%</td>
<td>295.2000</td>
</tr>
<tr>
<td></td>
<td>Skim</td>
<td>1200</td>
<td>11%</td>
<td>302.8200</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>135.6000</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>16.8000</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>16.2400</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>184.8000</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>296.4500</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>3.3600</td>
</tr>
<tr>
<td></td>
<td>1% 1%</td>
<td>1200</td>
<td>1%</td>
<td>1.7500</td>
</tr>
<tr>
<td><strong>Produce</strong></td>
<td>Min</td>
<td>1200</td>
<td>16%</td>
<td>197.2800</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>1200</td>
<td>3%</td>
<td>41.4738</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1200</td>
<td>0%</td>
<td>4.1000</td>
</tr>
<tr>
<td><strong>Meats</strong></td>
<td>Min</td>
<td>1200</td>
<td>31%</td>
<td>367.0800</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>1200</td>
<td>4%</td>
<td>46.3325</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>1200</td>
<td>2%</td>
<td>24.6400</td>
</tr>
</tbody>
</table>

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The table above presents data on all the dairy products and, in the interest of brevity, the maximum, minimum and average of the produce, meat, grain and the miscellaneous groups. The requirement, the RDA or the DRI, for the nutrient and the percent of the requirement per serving is given in the RDA/DRI columns. The next column is the amount of the nutrient per serving size. In the case of Calcium, the units are mg. The next two columns are the comparisons of the percent of the requirement per serving and the number of servings it would take to match the amount of nutrient in whole milk, the reference food. The last column gives the dollar amount that the food would cost to match the nutrient content in a serving of whole milk. It is clear that dairy products equal or exceed the amount of calcium per serving compared to most other foods and at a less expensive price. For example, almonds (last food in the list) has only 19% of the calcium in a serving compared to a serving of milk and one would have to consume 5.26 servings and pay $1.34 to consume the amount of calcium in one serving of whole milk.

The table for protein has the same format as the table above for calcium.

<table>
<thead>
<tr>
<th>Food Category</th>
<th>RDA Requirement</th>
<th>Nutrients per Serving</th>
<th>Compared to Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dairy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk Whole</td>
<td>56 14%</td>
<td>7.8960</td>
<td>100% 1.00 $0.16</td>
</tr>
<tr>
<td>Skim</td>
<td>56 15%</td>
<td>8.1840</td>
<td>104% 0.96 $0.13</td>
</tr>
<tr>
<td>Cheddar</td>
<td>56 19%</td>
<td>10.4580</td>
<td>132% 0.76 $0.22</td>
</tr>
<tr>
<td>Cottage</td>
<td>56 50%</td>
<td>28.2274</td>
<td>357% 0.28 $0.25</td>
</tr>
<tr>
<td>Cream</td>
<td>56 3%</td>
<td>1.5900</td>
<td>20% 4.97 $0.56</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>56 1%</td>
<td>0.4424</td>
<td>6% 17.85 $1.07</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>56 9%</td>
<td>5.0540</td>
<td>64% 1.56 $0.58</td>
</tr>
<tr>
<td>Yogurt</td>
<td>56 15%</td>
<td>8.5015</td>
<td>108% 0.93 $0.53</td>
</tr>
<tr>
<td>Butter</td>
<td>56 0%</td>
<td>0.1190</td>
<td>2% 66.35 $3.83</td>
</tr>
<tr>
<td><strong>Produce Min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>56 1%</td>
<td>0.4588</td>
<td>6% 1.23 $0.32</td>
</tr>
<tr>
<td>Max</td>
<td>56 11%</td>
<td>6.4014</td>
<td>81% 17.21 $4.49</td>
</tr>
<tr>
<td>Average</td>
<td>56 3%</td>
<td>1.7464</td>
<td>22% 6.55 $2.29</td>
</tr>
<tr>
<td><strong>Meats</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>56 11%</td>
<td>6.0700</td>
<td>77% 0.19 $0.06</td>
</tr>
<tr>
<td>Max</td>
<td>56 73%</td>
<td>41.0480</td>
<td>520% 1.30 $0.76</td>
</tr>
<tr>
<td>Average</td>
<td>56 47%</td>
<td>26.2164</td>
<td>332% 0.36 $0.25</td>
</tr>
<tr>
<td><strong>Baked</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>56 4%</td>
<td>2.4360</td>
<td>31% 3.24 $0.30</td>
</tr>
<tr>
<td>Max</td>
<td>56 13%</td>
<td>7.4984</td>
<td>27% 3.66 $0.61</td>
</tr>
<tr>
<td>Average</td>
<td>56 12%</td>
<td>6.8180</td>
<td>95% 1.05 $0.15</td>
</tr>
<tr>
<td><strong>Misc</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td>56 4%</td>
<td>2.1560</td>
<td>86% 1.16 $0.24</td>
</tr>
<tr>
<td>Nuts</td>
<td>56 13%</td>
<td>7.4984</td>
<td>67% 1.48 $0.38</td>
</tr>
</tbody>
</table>

Table 9b
It is clear that dairy products are competing sources of complete protein in the food supply with meat and eggs as the other good sources. The price per serving also compares well. Note that a serving of cottage cheese provides the 50% of the daily requirement for protein.

The Table below shows that Vitamin A

<table>
<thead>
<tr>
<th>Vitamin A</th>
<th>R.D.A.</th>
<th>Nutrient per Serving</th>
<th>R.E. Compared to Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food Category</td>
<td>Requirement</td>
<td>% per Serving</td>
<td>Nutrients</td>
</tr>
<tr>
<td>Dairy</td>
<td>Milk Whole</td>
<td>900</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>2%</td>
<td>900</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Skim</td>
<td>900</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Cheese Cheddar</td>
<td>900</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>Cottage</td>
<td>900</td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td>Cream</td>
<td>900</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>Sour Cream</td>
<td>900</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>Ice Cream</td>
<td>900</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Yogurt</td>
<td>900</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Butter</td>
<td>900</td>
<td>12%</td>
</tr>
<tr>
<td>Produce</td>
<td>Min</td>
<td>900</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>900</td>
<td>301%</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>900</td>
<td>68%</td>
</tr>
<tr>
<td>Meats</td>
<td>Min</td>
<td>900</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>900</td>
<td>1347%</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>900</td>
<td>87%</td>
</tr>
<tr>
<td>Baked Bread Whole Grain</td>
<td>900</td>
<td>0%</td>
<td>0.000</td>
</tr>
<tr>
<td>Misc Chocolate Milk</td>
<td>900</td>
<td>0%</td>
<td>3.080</td>
</tr>
<tr>
<td></td>
<td>Nuts Peanuts</td>
<td>900</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>Walnuts</td>
<td>900</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>Almonds</td>
<td>900</td>
<td>0%</td>
</tr>
</tbody>
</table>

Dairy products are good sources of Vitamin A as are many vegetables and fruits (produce), but the best source is beef liver, which is reflected in the maximum values in the meat group. Since Vitamin A is found in relatively few foods in significant amounts, dairy products and produce become the major sources of Vitamin A.
Selenium deficiency has been associated with cancer risk. The following shows the best sources of selenium and compared the nutrient contribution to the diet compared to dairy products.

**Table 9d**

Meats are the best sources of selenium with an average of 60% of the Selenium.

<table>
<thead>
<tr>
<th>Food Category</th>
<th>RDA/DRI Requirement % per Serving</th>
<th>Nutrients per Serving ug</th>
<th>Compared to Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>Whole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>55 16%</td>
<td>8.8800</td>
<td>100% 1.00 $0.16</td>
</tr>
<tr>
<td>Skim</td>
<td>55 10%</td>
<td>6.0000</td>
<td>68% 1.48 $0.25</td>
</tr>
<tr>
<td>Cheese</td>
<td>Cheddar</td>
<td>5.2800</td>
<td>59% 1.68 $0.23</td>
</tr>
<tr>
<td>Cottage</td>
<td>55 11%</td>
<td>5.8380</td>
<td>66% 1.52 $0.44</td>
</tr>
<tr>
<td>Cream</td>
<td>55 37%</td>
<td>20.3400</td>
<td>229% 0.44 $0.38</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>55 1%</td>
<td>0.3600</td>
<td>4% 24.67 $2.77</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>55 1%</td>
<td>0.5740</td>
<td>6% 15.47 $0.93</td>
</tr>
<tr>
<td>Yogurt</td>
<td>55 10%</td>
<td>5.9300</td>
<td>28% 3.52 $1.30</td>
</tr>
<tr>
<td>Butter</td>
<td>55 0%</td>
<td>0.1400</td>
<td>61% 1.65 $0.93</td>
</tr>
<tr>
<td>Produce</td>
<td>Min</td>
<td>0.2220</td>
<td>2% 63.43 $3.66</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>3.1150</td>
<td>3% 2.85 $0.73</td>
</tr>
<tr>
<td>Meats</td>
<td>Min</td>
<td>0.9817</td>
<td>35% 40.00 $27.13</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>6.5360</td>
<td>11% 16.76 $6.40</td>
</tr>
<tr>
<td>Baked</td>
<td>Min</td>
<td>55 12%</td>
<td>74% 0.16 $0.06</td>
</tr>
<tr>
<td></td>
<td>Max</td>
<td>55 101%</td>
<td>624% 1.36 $1.75</td>
</tr>
<tr>
<td>Misc</td>
<td>Min</td>
<td>55 0%</td>
<td>369% 0.39 $0.31</td>
</tr>
<tr>
<td>Chocolate</td>
<td>Milk</td>
<td>55 60%</td>
<td>32.7535 $40.00</td>
</tr>
<tr>
<td>Nuts</td>
<td>Peanuts</td>
<td>8.2600</td>
<td>93% 1.08 $0.10</td>
</tr>
<tr>
<td>Walnuts</td>
<td>55 4%</td>
<td>2.1000</td>
<td>24% 4.23 $0.59</td>
</tr>
<tr>
<td>Almonds</td>
<td>55 9%</td>
<td>4.7600</td>
<td>54% 1.87 $0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.7840</td>
<td>9% 11.33 $2.89</td>
</tr>
</tbody>
</table>

The last Table gives an overview of the energy supplied by the food examined.

**Table 9e**

<table>
<thead>
<tr>
<th>Calories</th>
<th>RDA/DRI Requirement % per Serving</th>
<th>Nutrients per Serving Kcal</th>
<th>Compared to Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>Whole</td>
<td>2000 7%</td>
<td>146.4000</td>
</tr>
<tr>
<td>2%</td>
<td>2000 6%</td>
<td>120.0000</td>
<td>100% 1.00 $0.16</td>
</tr>
<tr>
<td>Skim</td>
<td>2000 4%</td>
<td>84.0000</td>
<td>82% 1.22 $0.21</td>
</tr>
<tr>
<td>Cheese</td>
<td>Cheddar</td>
<td>2000 8%</td>
<td>169.2600</td>
</tr>
<tr>
<td>Cottage</td>
<td>2000 12%</td>
<td>232.7800</td>
<td>57% 1.74 $0.24</td>
</tr>
<tr>
<td>Cream</td>
<td>2000 2%</td>
<td>34.6500</td>
<td>116% 0.86 $0.25</td>
</tr>
<tr>
<td>Sour Cream</td>
<td>2000 1%</td>
<td>29.9600</td>
<td>159% 0.63 $0.55</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>2000 14%</td>
<td>282.8000</td>
<td>24% 4.23 $0.47</td>
</tr>
<tr>
<td>Yogurt</td>
<td>2000 7%</td>
<td>149.4500</td>
<td>20% 4.89 $0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>193% 0.52 $0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>102% 0.98 $0.55</td>
</tr>
</tbody>
</table>
Dairy products provide an average number of calories to the diet but these foods have a high nutrient density. Ice cream is high in calories relative to other foods but is an ecumenical ($0.19 per serving) and a nutrient rich food which, eaten in moderation, provides pleasure for many.

2. Significant Conclusions:
Dairy products are a good as well as an economical source of several nutrients of public health importance: calcium, protein, vitamin A, riboflavin, selenium, phosphorus, potassium and energy (Calories). Milk and hard yellow cheeses are eaten by more older people in Utah than any other single foods. In general, dairy products, especially the higher fat products, are protective for cancers and heart disease but have little or no impact on diabetes. Calcium also shows strong protective qualities against heart disease and cancers, which confirms earlier studies in Utah and elsewhere of calcium as a mitigating factor in colon cancer. Calcium appears to decrease the rate of overweight in women but had a direct but weaker relation to overweight in men. Therefore we conclude dairy products provide important nutrients and have an effect in reducing the expression of several chronic diseases of major public health importance and should have a place in the composition of healthy diets in the older population residing in Utah.

3. Anticipated Problems/Delays:
The application was submitted in 1999 but was not funded until January of 2002. The principal investigator was on sabbatical during 2000. The first wave of questionnaires sent out in March 2002 included people ages 50-65. A second wave of questionnaires was to be sent out as soon as Medicare files were available to us as a pool for selecting a representative sample of people 65 years and older. Unfortunately, the Health and Human Services Center for Medicare and Medicaid Services has adopted a new policy and will no longer allow researchers to use Medicare files. The sample for the second wave of questionnaires was adjusted to increase the upper age limit of people selected from driver’s license files. However, driver’s license files will limit the response from the oldest group because the number of people registered with the Utah Department of Motor Vehicles decrease with age. The last adjusted sample was sent out early in September 2003. No father delays were encountered

Plans for the future:
If funding allows, we plan on pursuing the following dairy product studies:

- Determine age-specific trends in the associations between dairy products and specific health endpoints including obesity, diabetes, heart disease, osteoporosis, and hypertension and stroke.
- Further characterize the patterns of dairy product consumption in various subgroups by education, employment, urban-rural residence, physical and mental functions and determine the associations of dairy products with health in these subgroups.
- Determine the contribution of dairy products to total dietary intake and their role as risk modifiers in health outcomes.
- Consider the commodity, milk, as the carrier of beneficial nutrachemical additives, accidental contaminants, purposely added toxins or dietary supplements by tracking the array of all foods containing milk through the food supply and summing the total milk additive intake for individuals and groups in the population.

Publications:

Peer Reviewed Publications:


  **Abstract**

  Health and nutrition surveys representative of national, regional or local populations provide data that cannot be derived in any other way. However, existing national surveys cannot evaluate many of the sub-populations or geographic regions, leaving gaps in data needed by state, county or local health and government data users. Data collection protocols must be adjusted for each sub-population while maintaining a standardized methodology.

Theses:

None

**Published Abstracts:**

- **Vitamin A Intakes in Two Populations.** Sorenson, Ph.D., R. Cutler, Ph.D., A. Morabia, M.D., Ph.D., C. Delhumeau, M.P.H., M. Bernstein, M.D., M. Constanza, Ph.D., 1Utah State University, 2Division of Clinical Epidemiology Geneva University Hospitals, Vitamin A Intake of Two Populations, Abstracts of the Society of Experimental Biologists (EB), April 20-24, 2002, New Orleans, Louisiana, Program, Session on Surveillance of Diet and Nutritional Status, No. 492.2.

  **Abstract**

  Using questionnaires derived from the Third National Health and Nutrition Examination Survey, we assessed the vitamin A intake of adults in populations in Utah and Geneva Switzerland. During 1999, we found the mean intake for Utah women was 3969 _RE_ /day and 2880 _RE_ /day for men. However, in Utah, the contribution of liquid supplements (like Ensure), which increases
with age, is substantial; 784 Rg/day for women and 347 Rg/day for men. The mean intake from food sources alone is 2113 Rg/day (246% RDA) for Utah women compared to 953 Rg/day (119%) for women in Geneva. For Utah men, the vitamin a intake is 2001 Rg/day (200% RDA) verses 1018 Rg/day (102%) for men in Geneva. (US means for women ~ 1170 Rg/day, men ~ 1419 Rg/day).

The food sources of vitamin A also differed between the two populations. In Utah, the food sources contributing the most dietary Vitamin A (with the percentage of persons consuming the food in parentheses) were: liquid supplement (11%), carrots (72%), cold cereal (83%), liver (12%), hard cheeses (85%) and mixed vegetables (71%). For Geneva, the most important sources of Vitamin A were: liver (27%), carrots (88%), fruit tarts (71%), lettuce salad (97%), cheese (89%) and berries (65%).

Supported in part by USDA/Utah State University Experiment Station and the Western Dairy Center

- Title: Relating the Intake of Animal Products with 6 Chronic Diseases in the Aging Population in Utah
  Experimental Biology Meetings – April 18, 2004
  Author’s names: Roxane Pfister1, Ann Sorenson2, Karl Smith2; 1 Utah State University, UMC 2905, Logan, UT 84322, 2 Utah State University, UMC 8700, Logan, UT 84322,

  Abstract

In a population based survey of approximately 1000 people aged 50 and above living in Utah between 1998 and 2002 respondents were questioned as to their intake of meat and dairy products (including wild game, fish and poultry) as well as the their incidence of several chronic conditions such as diabetes, stroke, heart disease, hypertension, cancer and hip fracture in order to identify animal products that are significantly associated with these chronic conditions. In logistic regression models controlled for by age, gender, smoking, BMI, and energy intake; higher consumption of plain beef products were not associated with any increased risks of chronic disease, while consumption of mixed foods which include beef (such as stew) were associated with a decline in the risks of heart disease (p=0.04). In a model that contained both meat and dairy categories, both butter (p=0.017) and mixed beef (p=0.04) were protective for heart disease in both men and women. Total dairy products were protective for cancer (p=0.000) for all people over 50 years while butter was negatively associated with cancer in men only (p=0.01, 1st quartile). Moderate intakes of higher fat beef arrests to reduce risk for obesity (BMI >30), (p=0.014) while consumption of dairy products are inversely associated with hip fracture in women only.

Supported by Utah State Univ. Experiment Station and the Western Dairy Center

- Title: Surveillance of chemical composition of, or contaminants in, consumed foods
  Experimental Biology Meetings – April 18, 2004
  Smith, K1, Sorenson, AW1, Halfrisch, JW2; 1 Utah State University, UT 84322, 2 Emeritus, USDA Human Nutrition Research Center, Beltsville, MD

  Abstract

We developed a program in cooperation with USDA, which allows chemicals contained in, or added to food commodities to be tracked through all processing steps the food undergoes before consumption. This allows final chemical composition of a given food to be calculated, as well as complete information on the intake of individuals to be estimated. The program is further capable of aggregating data on any level, allowing complete meal or total diet composition to be calculated, or general information about a group of people to be assessed. Output data is flexible as amount consumed per day, per kilogram, per calorie, or any other comparative unit available. The program also tracks multiple survey databases, and is capable of incorporating data from any surveys, or comparing information between surveys. For instance, apples on the tree contain 5.7mg of Vitamin C per 100mg of apple. In an apple pie, this raw apple is washed, peeled, then baked; changing the Vitamin C by 100% retained
(washed), 70% retained (peeled), 42% retained (baked); for a total of 1.7mg of Vitamin C per 100mg of apple in apple pie. Using a hypothetical Chlorinated Hydrocarbon (hCH) (pesticide) with initial residue of .03mg, and retention factors of 20%, 4%, and 109% for the above processes, we have a final residue of 0.26μg of hCH per 100mg of apple in apple pie. Similar algorithms are used in breaking down and combining data throughout the program. Supported by Utah State Univ. Experiment Station and the Western Dairy Center

- Title: Identifying the Role of Milk and milk products on Nutritional and Health Issues for adults fifty Years of Age and Older

Diary Product – Poster Session – EB – April 20, 2004
Author’s names: Ann Sorenson¹, Cecile Delhumeau², Karl Smith¹, Roxane Pfister³; ¹Utah State University, UMC 8700, Logan, UT 84322, ²University of Geneva, Switzerland Medical School, ³Utah State University, UMC 2905, Logan, UT 84322,

Abstract
We used data from approx. 1000 participants in a representative state-wide survey on the health, nutrition and lifestyle of older people in Utah to investigate the impact of dairy products on chronic diseases common in older people: heart disease, cancer, diabetes, hypertension, osteoporosis and obesity. The respondents were asked independently about their intake of 15 different dairy products. Less than one percent of participants did not consume any dairy products. Logistic regression models controlled for by age, gender, smoking, BMI, and energy intake yielded some strong food disease associations. Overall, dairy products were protective for heart disease (p=0.036) and cancer (p=0.044) in men, and negatively associated with overweight in women (p=0.002). Individual foods were independently associated with the disease outcomes, especially the higher fat dairy products. For example, whole and 2% milk were more protective for heart disease in women (p=0.006) than the lower or no fat milks (p=0.028). However, high fat dairy products were associated with overweight, but not obesity, in men over age 65 (p=0.002). Many of the other dairy foods showed the same protective trends but did not reach a level of significance. No negative associations were found in this study other than yogurt in men (p=0.008).
This study was supported by the Utah State University Experiment Station and the Western Dairy Center.


Presentations:
- Sorenson, AW, R Cutler, A. Morabia, C. Delhumeau, M Bernstein, M, Constanza, 2002: Vitamin A Intakes in Two Populations, Poster session,
Sorenson, AW, B Athas, What Utah Seniors are currently eating and how to make better dietary choices, one-hour breakout session, Utah Geontological Society annual meeting, October 29-30, 2002, Park City, Utah


Patent/Invention Disclosures: NA

<table>
<thead>
<tr>
<th>Technology Transfer Activities</th>
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</tr>
</tbody>
</table>

Visitors Hosted:
Judith Halfrisch, Ph.D., Senior Scientist, Human Nutrition Research Center,
Cecile Delhumeau, Ph.D., Post Doctoral Student, University of Grenoble, France, Biostatistics
Western Dairy Center
Reporting Period January 1, 2003 —June 30, 2004

Principal Investigators: Antonio J. Torres
Co-Investigators: Michael Qian, Anna B. Marin, Vivek Savant
Project Title: Pressure processing to improve milk freshness and refrigerated shelf-life

Modifications to Project/Budget:


Project Objectives: (Include any revisions to objectives)

1. Objective 1: Operational parameters for the mild heating and high pressure processing (HPP) capable of extending the shelf life of fresh milk

A strategic two-step search was used to find a combination of mild heating and high pressure processing (HPP) extending the shelf life of fresh milk. We identified three possible processes based on obtained data from microbial deactivation, enzyme activity, viscosity and color of whole and 1% fresh milk. The efficacy of the selected processes has been confirmed and we found that it is possible to extend the shelf life of whole and 1% fresh milk for at least 45 days under refrigeration using the following milk treatments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
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<tr>
<td>Pressure, MPa</td>
<td>586</td>
<td>586</td>
<td>586</td>
</tr>
<tr>
<td>Time, min</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Temperature, C°</td>
<td>60°</td>
<td>60°</td>
<td>55°</td>
</tr>
</tbody>
</table>

Objective 2: Demonstration by chemistry, sensory analysis and consumer testing of the freshness and extended shelf life possible by mild heating and high pressure processing (HPP)

Two techniques have been tested for the volatiles extraction of milk: Purge and Trap (PT), and Solid Phase Micro Extraction (SPME). These techniques do not involve the use of high temperature (45°C) or solvent, therefore minimizing artifact caused by extraction steps and leading to a real headspace analysis of the volatiles. SPME showed a better
sensitivity and ability to extract more compound that PT, so it was chosen and the more suitable technique for this project. Triplicate samples of 3% milk from the same batch were heated 15 sec at 78°C and 15 min at 98°C. A High Pressure (HPP) treatment of 85 kPsi at 55°C for 5 min was also performed, and a sample of 3% raw milk was also analyzed as a reference. The SPME fibers were exposed for 30 min to the headspace of 25 g of milk sample in a 40 ml vial under stirring. The fibers were then desorbed in a Gas Chromatograph equipped with a Mass Analyzer (GC/MS) and in a Gas Chromatograph equipped with a Pulsed Flame Photometric Detector (GC/PFPD) for the identification of sulfur compounds.

Eleven compounds were identified in the milk samples heated at 98°C using GC/MS: dimethyl sulfide, 2-propanone, 2-butanone, hexanal, 2-heptanone, heptanal, octanal, limonene, 2-nonanone, nonanal, 2-undecanone. In the sample heated at 75°C the compounds detected were dimethyl sulfide, 2-propanone, 2-butanone, octanal and limonene. In the raw milk samples, as well as in the HPP ones only 2-butanone, 2-propanone and hexanal were detected. Methyl ketones of higher molecular weight are directly correlated to the severity of the heat treatment, and 2-heptanone is one of the best markers. These are formed during the heat treatment via two pathways: either β-oxidation of saturated fatty acids followed by decarboxilation, or decarboxilation of β-ketoacids naturally present in milk. Aldehyde formation can derive from the autooxidation of unsaturated fatty acids promoted by the heat treatment. Dimethyl sulfide has been reported to occur naturally in raw milk.

The sulfur compounds identified using the GC/PFPD for the headspace volatiles of raw milk were dimethyl sulfide, methionol and very small concentrations of ethanethiol and methanethiol. The chromatogram of the milk sample heated at 78°C also showed the peak for dimethyl sulfide and there was a large increase in the peak area for the ethanethiol, methanethiol and methionol, and dimethyl disulfide was also detected. In the milk sample heated to 98°C, an increase in peak area was observed for dimethyl sulfide, ethanethiol, methanethiol and diethyl disulfide peaks compared to those for milk heated at 78°C. It also showed the peak for hydrogen sulfide. The chromatogram for the HPP milk sample showed only the peaks for dimethyl sulfide, ethanethiol, methanethiol and methionol. It has been reported that cabbagy flavor (cooked flavor) correlates well with total volatile sulfur compounds. Dimethyl disulfide is formed during the heat treatment via the oxidation of methanethiol, and the formation of the sulfur compounds can be due to the degradation of the sulfur-containing amino acids from milk.

The results obtained so far indicate that none of the heat treatment volatile indicators were found in the HPP milk, and the sulfur compounds
responsible of the cooked flavor in heated milk were present in very low concentrations and some of them were not detected when compared to those for heated milk. Furthermore, the volatile profiles of HPP and raw milk were very similar. Further work in progress will allow the quantification of the compounds of interest in milk treated under the conditions selected in Objective 1.

Objective 3: Determine by chemistry analysis “cooked” and “fresh” flavors in milk

Hypothesis/Task: HPP treatments under 300s combined with mild heating can retain the flavor of fresh milk. This is an opportunity that we are uniquely qualified to evaluate at OSU because of our experience in pressure processing, strength in dairy flavor chemistry and recently enhance instrumentation for flavor research.

Characterization of pressure-treated milk: Fresh, UHT, and pressure treated milk (up to three most promising treatment among those studied in Objective 2) will be subjected to flavor analysis immediately after processing and during storage for up to 60 days. This sensory-directed instrumental analysis will be used to study the flavor chemistry of aroma-active components responsible for the freshness and cooked notes as well as deducing the off-flavor formation mechanism(s) involved.

Objective 4: Conduct consumer evaluation of HPP-treated milk produced by a semi-continuous.

Hypothesis/Task: OSU will collaborate with an industrial partner to evaluate semi-continuous HPP treatments developed on the basis of our batch treatment findings. Pressure treated whole and 1% milk will be subjected to consumer sensory analysis immediately after processing and during refrigerated (6.1C) storage.

Characterization of pressure-treated milk: Consumer tests of pressure treated whole and 1% milk (two most promising treatments among those studied in Objective 3) will compare fresh and 15-day old commercial milk samples with treated samples at time 0, 15, 30, 4 and 60 days. This will help identify combination of pressure and mild heating for a semi-continuous process meeting consumer demand for fresh flavor and industry need for longer shelf life.

Objective 5: Disseminate to peers and industry our findings and recommendations for the production of HPP-treated milk.

Hypothesis/Task: Dissemination of high-pressure milk processing results to peers and industry will favor commercial implementation.
Communication strategies: In addition to research publication and presentations at professional meetings we have cameras and non-linear video editing equipment to prepare an industry-targeted video in various formats including a compressed version on CD. The video will describe research findings including cost estimations for the equipment and process, manufacturing of high-pressure equipment, equipment maintenance and cleaning recommendations and a commercial high pressure processing facility.

**Project Summary:** (Suitable for inclusion in Center documents released to the public)
The consolidation trend in the dairy industry will continue for the foreseeable future as processors seek to improve their competitive position in the market. As distribution chains become longer, more and more U.S. companies will need ways to extend shelf life to meet consumer expectations for freshness and safety. Considering this consolidation trend and consumer demand characteristics, a longer shelf life, particularly of fresh milk, is critical to the future success of American dairy producers. This project will assess the combination of high pressure and moderate heating to meet these consumer and industry requirements.

**Methods:**

- **Significant Conclusions:**

**Publications:**

**Theses:**

**Published Abstract:**

**Presentations:**

**Patent/Invention Disclosures:**

**Technology Transfer Activities**
For information on licensing contact:

**Visitors Hosted:**

**Invention Disclosures:** (Title, Date)
Western Dairy Center
Reporting Period January 1, 2003—June 30, 2004

Principal Investigators: Carl Brothersen

Co-Investigators: Bart Weimer
Tim Taylor
Don McMahon

Project Title: Mathematical model of diffusion in Cheddar cheese

Institution's Project #: 

Project Completion Date:


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)
Develop a mathematical model that describes the diffusion of molecules through the Cheddar cheese matrix. The model will include the effect of molecular weight, storage temperature, cheese moisture, and molecular charge on diffusion.

Project Summary: (Suitable for inclusion in Center documents released to the public)

In a previous project we developed a technique utilizing epifluorescence to monitor the movement of fluorescein through Cheddar cheese. The purpose of this project is to use this technique to develop a mathematical model for the diffusion of small molecules, such as lactose, through Cheddar cheese. The model will include effects of time, concentration, storage temperature, and cheese moisture. Tim Taylor (Biological Engineering) has been added to our group to provide the expertise in mathematical modeling. This information will be useful in predicting the rate of movement, and distribution of substances such as vitamins, flavors, and enzymes, which may be added to the finished cheese.

Significant Conclusions:

Models for two different methods of adding substances to cheese will be developed. One method involves placing the cheese in a solution containing the substance of interest, and allowing the substance to diffuse into the cheese. This is similar to brining cheese. In this method, the concentration of the diffusing molecule in the brine remains constant on the surface of the cheese as the substance diffuses into the cheese. The second method involves injecting a solution containing the substance of interest in to the cheese. In this method there
is a finite amount of the available for diffusion, and the amount at the injection point decreases with time.

Cheese was made containing 32, 37, and 42 percent moisture, pressed into 20 pound blocks and stored at 4°C overnight. The cheese was then cut into 2 X 6 X 10 cm or 2 X 2 X 10 cm pieces. The larger pieces were placed in to brine containing fluorescein, and the smaller pieces injected with the fluorescein brine. The samples were randomly sorted into three groups. and stored at 4°C, 12°C or 20°C. One sample from each treatment and storage temperature was selected daily over a 72 day storage period, and the diffusion of the fluorescein was detected by epifluorescence. The digital images of the micrographs were converted to grayscale, posterized, and the number of pixels of each shade of gray counted using PhotoShop. A calibration curve was generated to determine the concentration of fluorescein at each shade of gray and the diffusion distance calculated. The data will then be used to generate the model.

Images of all moisture and storage temperature treatments over the 72 day storage time have been generated. The images are now being processed to determine the extent of diffusion. The mathematical model will then be generated.

Publications:

Theses:

Published Abstract:

Presentations:

Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:
Western Dairy Center
Reporting Period January 1, 2003 — June 30, 2004

Principal Investigators: Donald J. McMahon
Co-Investigators: Craig J. Oberg

Project Title: An objective test for measuring stretch properties of mozzarella cheese.

Institution’s Project #: 89093

Project Completion Date: December 31, 2003


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)
Objective 1: Determine the optimum conditions for measuring the functional properties of melted cheese using the USU Stretch test.
Objective 2: Correlate the melt and stretch parameters of cheese measured using the Stretch test with the fork stretch test used by the pizza industry.

Project Summary: (Suitable for inclusion in Center documents released to the public)

The performance of cheese on a pizza depends on how readily the cheese melts and how well it can be stretched. Suppliers of cheese to the pizza industry, subjectively measure this performance using a fork test. A test has been developed at Utah State University that can be used to objectively measure cheese performance when heated. To determine if the “Stretch” test was a suitable replacement for the fork test, a comparison between the two tests was performed. The cheeses was tested in the range of 65°C to 85°C using the USU Stretch Test and then compared to evaluations of the same cheese by an industrial partner using the fork test. An advantage of the USU Stretch test is that it measures a number of different characteristics during the stretching process which can be used in combination to provide a better description of the melting and stretching properties of the cheese.

Significant Conclusions:

The USU stretch test provides data that can be used to objectively analyze the functional properties of mozzarella cheese. A variety of data can be attained, which gives an indication of not only how far the cheese will stretch, but also provides a measure of
elasticity and viscosity. Determination of the optimum conditions for analysis of pizza cheese by the USU stretch test showed that the test had a high degree of repeatability when 50 g of shredded cheese was tempered for 45 minutes and stretched using a three pronged hook (25 mm in diameter) as a probe. Some correlation was found between data collected from the USU stretch test and the pizza fork test. The greatest correlation was found when the cheese tested by the USU stretch test was tempered at higher temperatures (80 and 85°C). At 80°C the highest individual correlation between the pizza fork test and the USU stretch test was seen when SL_{10-15} (R^2 = 0.74), SL_{20-30} (R^2 = 0.75), or SE_F (R^2 = 0.70) were used. Multiple linear regression studies show that at 80°C a combination of SE_F and SL_{22} (R^2 = 0.85, Adj. R^2 = 0.80) or a combination of four parameters (F_M, slope from 10 to 20 cm, SE_{0.1}, and SL_{22}, R^2 = 0.97, Adj. R^2 = 0.93) provides an increased correlation to the pizza fork test. At 85°C, two values were used in a multiple linear regression study, SE_F and SE_{0.05}, to provide a high correlation to the pizza fork test (R^2 = 0.90, Adj. R^2 = 0.85).

Values of R^2 for different parameters measured by the USU stretch test at different temperatures with the single value of stretch provided by the pizza fork test.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>F_M</th>
<th>SL_{10-15}</th>
<th>SL_{20-30}</th>
<th>Slope from 10-15 cm</th>
<th>SE_{0.1}</th>
<th>SE_F</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C</td>
<td>0.538</td>
<td>0.521</td>
<td>0.344</td>
<td>0.429</td>
<td>0.000</td>
<td>0.083</td>
</tr>
<tr>
<td>70°C</td>
<td>0.532</td>
<td>0.410</td>
<td>0.499</td>
<td>0.207</td>
<td>0.271</td>
<td>0.715</td>
</tr>
<tr>
<td>75°C</td>
<td>0.371</td>
<td>0.351</td>
<td>0.417</td>
<td>0.168</td>
<td>0.300</td>
<td>0.288</td>
</tr>
<tr>
<td>80°C</td>
<td>0.377</td>
<td>0.735</td>
<td>0.745</td>
<td>0.463</td>
<td>0.590</td>
<td>0.701</td>
</tr>
<tr>
<td>85°C</td>
<td>0.497</td>
<td>0.606</td>
<td>0.483</td>
<td>0.640</td>
<td>0.690</td>
<td>0.130</td>
</tr>
</tbody>
</table>

An aging study was useful in demonstrating how the parameters of the USU stretch test, generally F_M, SE_F, and SE_{0.1}, could be used to characterize the functional properties of a pizza cheese. It was seen that not only is the USU stretch test much more objective than the pizza fork test, but the different parameters of the USU stretch test give a greater understanding of how the pizza cheese stretches than does the single value of stretch supplied by the pizza fork test.

Multiple linear regression models using parameters measured by the USU stretch test that showed high correlation to the pizza fork test.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Variables used in model</th>
<th>R^2</th>
<th>Adjusted R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70°C</td>
<td>75°C</td>
<td>80°C</td>
</tr>
<tr>
<td>------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>( F_M, \text{SL}<em>{5-10}, SE</em>{0.5} )</td>
<td>0.847</td>
<td></td>
<td>0.853</td>
</tr>
<tr>
<td>( F_M^2, \text{SL}<em>{5-10}^2, SE</em>{0.5}^2 )</td>
<td>0.886</td>
<td></td>
<td>0.965</td>
</tr>
<tr>
<td>( SE_{p}, SE_{0.1} )</td>
<td></td>
<td>0.732</td>
<td></td>
</tr>
<tr>
<td>( SE_{p}, \text{SL}<em>{20}, SE</em>{0.1} )</td>
<td></td>
<td>0.767</td>
<td></td>
</tr>
<tr>
<td>( SL_{20-30}, SL_{30} )</td>
<td></td>
<td>0.793</td>
<td></td>
</tr>
<tr>
<td>( SL_{10-15}, SL_{20-30}, SL_{30} )</td>
<td></td>
<td>0.883</td>
<td>0.965</td>
</tr>
</tbody>
</table>

"FM", "SL", "SE", and "p" refer to various parameters, with "p" indicating a probability or proportion, and "SE" typically representing standard error.
An example of a typical stretch profile, indicating points where measurements of Melt Strength ($F_M$), Stretch Extension at $F_M$ ($SE_F$), Stretch Load at 5 cm ($SL_5$), and Stretch Extension at 0.4 N ($SE_{0.4}$) are taken.

In the future, some further modifications could be made to the USU stretch test in order to improve its correlation to the pizza fork test. Conducting the USU stretch test using a texture profile analyzer capable of raising the probe at a faster rate could provide increased correlation with the pizza fork test by distinguishing between cheeses in the "none" stretch category and those in the remaining four categories. Another recommendation would be to use sample cups that are shallower and have a larger diameter. The melted cheese in these sample cups would more closely resemble the melted cheese spread atop a pizza crust than the cheese melted in the sample cups that were used in this study. Also, since it was seen in this study that increasing the sample size from 30 to 50 g resulted in increased repeatability and a reduction in slippage, another increase in sample size, to 100 g, could result in a higher degree of repeatability and a greater reduction in slippage. If the sample size is increased, the size of the sample cup, as well as the size of the hook should be examined and possibly enlarged as well.
Publications:

Theses:
Moyes, B. L., 2003 Correlation between the USU stretch test and the pizza fork test, M.S.

Published Abstract:

Presentations:

Patent/Invention Disclosures:

<table>
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</table>

Visitors Hosted:

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:
Western Dairy Center
Project Report
Reporting Period February 1, 2001 — June 01, 2004

Principal Investigators: Don McMahon, Utah State University
Co-Investigators:

Project Title: Rehydration and structure of reconstituted casein micelles.

Institution’s Project #: 00129

Project Completion Date: February 1, 2004


Modifications to Project/Budget:
This is a non-DMI funded project.

Project Objectives: (Include any revisions to objectives)

1. Characterization of the structure of casein micelles reconstituted from dry powders in comparison to the structure of native casein micelles in milk.
1. Investigate any differences in coagulation properties of milk containing reconstituted casein micelles.

Project Summary: (Suitable for inclusion in Center documents released to the public)

The changes in quaternary structure of casein supramolecules with various physical and chemical treatments were studied using transmission electron microscopy, and a model to account for the changes was put forth. The effects of casein structure on coagulation properties were also studied. The sample preparation for transmission electron microscopy involved physical methods of fixation and flash freeing to preserve the structure of caseins in the sample.

The structure of caseins in sodium and calcium caseinate varied with sodium caseinate not exhibiting any spherical structure as opposed to the spherical structure seen in calcium caseinate, non-fat dried milk and native milk. This difference in structure was carried over to rennet coagulum made from those sources of casein. Addition of calcium and phosphate to sodium and calcium caseinate, respectively, improved their coagulation properties. Hydration parameters such as time and shear of hydration affected the extent of
hydration. High shear or approximately 10 hr of hydration was required to disperse and hydrate the dried milk protein powders.

Acidification and treatment with excess EDTA resulted in dissociation of casein supramolecules into various sizes and shapes. Heat treatment of milk in the presence of ethanol also resulted in its dissociation. High heat treatment of milk at various pH levels induced different types of whey protein casein interactions.

All these changes can be explained using an irregular supramolecular structure of caseins based on a node and strand network of proteins and calcium phosphate nanoclusters. Such a filigree sponge-like appearance is seen in native bovine milk and in milk of other species.

2. Methods:

Microstructure of caseins in sodium caseinate (NaCN), calcium caseinate (CaCN), and non-fat dried milk (NFDM) were studied using transmission electron microscopy. Solutions of all the dried products were made to a casein concentration of 2.4% and the pH of the solution adjusted to 6.65±0.05. The powders were hydrated at 40 °C, and allowed to stabilize for 18 h with moderate mechanical stirring. Samples were drawn at 4, 10, and 18 h of hydration. In another experiment the powders were hydrated at high shear for 5 min using a hand held high speed blender (Omni 5000) and subsequently stabilized for 1h with moderate mechanical stirring.

Casein solutions were diluted 100 times and the casein micelles were adsorbed on to parlodion coated copper grids. Parlodion coated copper grids were coated with poly-L-lysine to improve the adsorption of protein on to the parlodion film. These grids were stained using uranyl acetate and oxalic acid and washed to remove excess stain. The stained grids were quick frozen in liquid nitrogen cooled liquefied Freon 22, and freeze dried so that casein micelles or particles in a form as close to their native state was imaged. Images were photographed at 30,000×, 85,000× and 140,000× at 80 kV using a Zeiss 902 transmission electron microscope.

Coagulation properties such as rennet coagulation time and curd firmness of skim milk fortified with NaCN or CaCN or NFDM or ASM to a protein concentration of 2.99%, 3.17% and 3.35% were measured using a Formagraph. Rennet coagulation time (RCT) and curd firmness at 60 min (A60) after rennet addition were calculated At each level of protein concentration, the sodium caseinate treatments were added with different levels of calcium chloride and calcium caseinate treatments with potassium-dihydrogen-phosphate so as to bring the coagulation properties within the range of the control skim milk. Protein solutions for fortification were prepared by high-shear mixing the powder in water so as to make a final concentration of 12% protein. These were allowed to stabilize for 8 h with moderate stirring. Skim milk was added with 1, 3 or 5 %
of the above 12% protein solutions. This fortification increased the protein content in skim from 2.91% to 2.99%, 3.17% and 3.35% respectively. In this report, when names of protein powders are preceded by 1, 3, and 5 it represents the level of fortification of milk with that 12% protein powder solution. The supplemented milks were allowed to stabilize for 1h before preparing it for Formagaph testing. As higher amounts of CaCN and NaCN reflected undesirable coagulation properties (such as longer RCT), phosphate in the form of KH₂PO₄ was added to CaCN and calcium in the form of CaCl₂ was added to NaCN 30 min prior to rennet addition in the Formagaph. Calcium and phosphate was added up to a concentration of 2.4 and 72 mM of Ca and PO₄ respectively. Ten milliliters of these milks were brought to 35 °C and allowed to stand for 30 min at that temperature when 100 µl of double strength rennet diluted 1:100 was added and allowed to set in a Formagaph to get time-firmness curves.

3. Significant Conclusions

Physical methods of fixation, uranyl staining, flash freezing, and freeze drying in sample preparation can be successfully used to image the quaternary structure of caseins using TEM. Such a method of sample preparation yields images without altering the native conformation of the proteins.

Dispersion and solubilization of dried milk proteins require longer hydration times and/or high shear. Microstructure of sodium and calcium caseinate differ significantly from native casein found in milk. Sodium caseinate lacks the spherical colloidal structure while calcium caseinate forms compact and highly electron dense spherical aggregates. Fortification of milk with dried milk proteins destabilizes the coagulation properties and microstructure of rennet gels depending on the nature of the protein. These coagulation properties can be brought back to that of original milk by addition of relevant salts. In case of sodium caseinate and calcium caseinate, addition of calcium chloride and potassium phosphate respectively reduced their adverse effect on coagulation properties.

During acidification at 40 °C there was progressive partial breakup of the casein supramolecule from outside to inside and was visualized as tendrils extending from the periphery of casein supramolecule. These polymers formed small (<50 nm) loosely entangled aggregates outside the supramolecule until pH 5.9. Further reduction in pH to the isoelectric points of caseins might have contributed to the changes in charge associated with the various aggregates resulting in their aggregation and reassociation with casein supramolecules. Calcium chelation using EDTA at 5 °C resulted in disruption of the casein supramolecules resulting in linear polymers and their cross-links. The disrupted system also formed particles with long tendrils and loosely entangled proteins. At 40 °C dissociation of the supramolecule was not as extensive as at 5 °C. The resultant proteins aggregates had similar appearance of native supramolecule with multiple ring-like structures.

The changes in structure of casein supramolecule during change in temperature was monitored using transmission electron microscopy. Renneting of milk at 5 °C
caused no aggregation of casein supramolecules until 30 min. At 35 °C, aggregated particles were seen at 15 min. By 30 min of renneting, the individual supramolecules that formed the aggregate started to fuse together. When milk was heated at lower pH, there was more whey protein association on the casein supramolecule surface. At pH 7.3, larger aggregates of whey proteins were formed in the serum outside the casein supramolecule surface. Heat treatment of a mixture of milk and ethanol dissociated the casein supramolecules into smaller particles of which some were interconnected.

These studies provide evidence to support an irregular supramolecular structure for the colloidal casein particles in milk. This irregular structure, supports an open structure in which different caseins can attach to calcium phosphate nanoclusters preventing calcium phosphate crystallization in the mammary gland. Chains of proteins can then grow until they encounter a chain terminating protein or bond with another chain. Also, different dissociation and aggregation behavior of casein supramolecules may be explained using this model. Overall, this study has put forth a molecular model for the casein supramolecule that satisfies the principles of self aggregation, interdependence, and diversity that are often observed in nature. Synthesis of casein supramolecules in the mammary gland rely on a controlled synergy between two concomitant aggregation processes. Calcium phosphate is formed into clusters because of its low solubility, and caseins are simultaneously undergoing polymerization because of their calcium sensitivity and hydrophobic nature. Precipitation of calcium phosphate is limited to formation of nanoclusters by binding of caseins via their phosphoserine side chains, and the polymerization of the caseins is limited to colloidal size by the chain-terminating influence of κ-casein.

Schematic model of the casein supramolecule (a) based on interconnected protein strands between calcium phosphate nanoclusters (b). Grey-colored shapes indicate particles off the cross-sectional plane.
Publications: None

Theses:

Published Abstract:

Presentations: