2002 Annual Report

Various Authors
Western Dairy Center
Annual Report
2002

Western Dairy Center
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Western Dairy Center
Activities Summary
2002

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, 2002 through December 31, 2002.

Of the technologies completed in 2002, several will be highlighted through Dairy Management Inc. (DMI) over the 2003 year. These projects include the Accelerating shreddability of Cheddar cheese by moderate hydrostatic pressure by A. Torres at Oregon State. Dr. Torres’ research showed that milled and stirred curd cheddar could be shredded and packaged immediately after high-pressure treatment, therefore eliminating the traditional storage time for curd knitting. The properties of pressure treated and control samples were practically identical after 15 days of refrigerated storage with respect to consumer acceptance and textural properties.

Dr. Daren Cornforth’s research on the use of dried milk minerals as an antioxidant in crumbled meat products has shown that milk mineral (MM) is equivalent in antioxidant activity to Rosemary and sodium tripolyphosphate in cooked ground beef, pork, and turkey, and is much more effective than BHT. DMI is also in the process of planning a symposium to summarize the last 10 year’s of research into the metabolic pathways of cheese cultures and adjuncts for both improved cheese flavor and functionality. Our WDC researchers involved in this area include Drs. Jeff Broadbent, Bart Weimer and Donald McMahon.

The color and flavor injected cheese technology developed by Carl Brothersen was featured by DMI at the Institute of Food Technologists meeting in August, 2002. DMI presented grape, green apple, and blueberry flavored cheese to children in the Chicago area. A video of the children's response to the cheese as well as cheese samples were available at the IFT meeting.

The Center conducted the 17th Annual Cheese Making Short Course was held February 4 to 8 2002, at Utah State University with 12 attendees. 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 16th Biennial cheese Industry conference in Sun Valley Idaho in August 2004. The success of last year’s conference is due to the excellent speakers, the teaming with the Idaho Milk Processors Association Annual Meeting, and our sponsors, Glanbia Foods, Scherping systems, DSM Food Specialties, The Cheese
Western Dairy Center

We are soliciting topic areas of interest and sponsors for the next meeting. Please contact the center for speaker and topic suggestions.

In 2002, the number of competitive grants awarded by Dairy Management for 2003 was 4 and this resulted in $231,112 research dollars. The Western Dairy Center funded 4 seed proposals for a total of $39,000. We also help support the Fall 2002 meeting of the Lactic Acid Bacterial Genome Consortium. Project progress reports of all research projects active in 2002 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 2002

Research projects funded by DMI

Effect of oxidation-reduction potential on growth of lactic acid bacteria
PI: Jeff Broadbent .................................................................$42,123

New starter systems for accelerated ripened Cheddar cheese
PI: Jeff Broadbent .................................................................$54,267

Production of intensely flavored Cheddar-type cheeses by adjunct cultures
PI: Jeff Broadbent .................................................................$16,748

Development of vitamin fortified cheese using high pressure injection technology
PI: Carl Brothersen ...............................................................$8,975

Dried whey minerals as an antioxidant in processed meats
PI: Daren Cornforth ..............................................................$35,900

Controlling chemical composition and functionality of cheese
PI: Conly Hansen .................................................................$27,930

Nonthermal attenuation of Lactobacilli to accelerate cheese ripening
PI: Barry Swanson .................................................................$32,000

Accelerating shredding suitability of Cheddar cheese my moderate hydrostatic pressure
PI: Antonio Torres .................................................................$54,741

Importance of glutamic acid and alpha-keto acids in cheese flavor development
PI: Bart Weimer .................................................................$29,975

Rapid detection of Listeria in dairy products
PI: Bart Weimer .................................................................$34,790

Research projected funded by the Western Dairy Center

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

Chemical and sensory properties of CLA enriched milk
PI: Tilak Dhiman .................................................................$16,700
Western Dairy Center

Project Report
Reporting Period: January 1, 2002—December 31, 2002

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

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 has been focused on objectives 1 and 3. Redox experiments with different cheeses are working well, but efforts to set up the fermentation system for growth and redox trials with NSLAB isolates has not yet proved successful. Our first experiments in the latter area were performed using a benchtop system that had been used successfully in previous trials, but we found...
that it did not provide us with reproducible baseline data. As a result, we have shifted our attention toward a New Brunswick 2.5 L. fermentation system available to us through the USU Biotechnology and Genomics Center. Redox probes suitable for use with that system have been acquired, and we hope to complete the NSLAB growth and redox trials before the end of summer.

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 (10^8 versus 10^6 cfu/g, respectively). Starter numbers in all three cheeses were similar. Further work is now underway to characterize the NSLAB species in each cheese type.

2. Significant Conclusions:
Data from cheese trials support our hypothesis that Eh may be influenced by the cheese manufacturing process and by NSLAB populations. If strain dominance among NSLAB is closely related to Eh, then processors may be able to promote a specific and desirable NSLAB biota in ripening cheese using adjuncts that help establish a particular Eh.

3. Anticipated Problems/Delays:
Efforts to set up the fermentation system for growth and redox trials with NSLAB isolates has proved more problematic than anticipated. We anticipate these difficulties will be overcome in the near future, but more trouble shooting may be required before that part of the project is working well.

Publications:

Presentations:
Technology Transfer Activities
For information on licensing contact:
Dr. Jeff Broadbent
Western Dairy Center
Project Report
Reporting Period January 1, 2000 — December 31, 2002

Principal Investigators: Jeff Broadbent
Co-Investigators: Dr. Charlotte Brennand, Utah State University
Dr. James Steele and Mark Johnson, University of Wisconsin-Madison

Project Title: New starter systems for accelerated ripened Cheddar cheese

Institution’s Project #: 00114

Project Completion Date: 6/30/03


Modifications to Project/Budget:
none

Project Objectives: (Include any revisions to objectives)
1. Determine bitter taste thresholds for casein derived peptides in a cheese model system.
2. Define the contribution of Lactobacillus helveticus CNRZ32 peptidases to the hydrolysis of casein derived bitter peptides.
3. Construct food-grade Lactococcus lactis S2 derivatives with enhanced activity of peptidases demonstrated to be important in hydrolysis of bitter peptides.
4. Develop a food-grade, genetic system for proteinase gene exchange in industrial strain of Lactococcus lactis.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Bitterness is a significant concern in accelerated ripened cheese. It is our hypothesis that the most effective strategy to control bitterness and over-ripening by proteolysis in accelerated ripened cheese is to develop a starter system that combines a low propensity for the production bitter peptides with high debittering peptidase activity. A key advantage to this approach is that is will not only help to retard over-ripening by proteolysis (via control over bitterness), it will also boost (via the production of free amino acids) levels of cheese flavor precursors in the curd matrix. Our group has shown that the starter proteinase is a key determinant in the production of bitter peptides, and that we can increase the activity of enzymes that degrade bitter peptides up to 1000-fold using a starter-based enzyme delivery system. This project will provide industry with information and technology transfer tools to develop food grade starter systems that control bitterness and over ripening in accelerated ripened cheese.
1. Significant Progress against Objectives:

Research at Utah State University is addressing objectives 1 and 4, while objectives 2 and 3 are being pursued at the University of Wisconsin-Madison. In objective 1, we have synthesized and purified several peptides at large scale for sensory analysis with our model cheese system. The "model cheese" for this work is made using a proteinase-negative and autolysis-resistant starter culture, then the cheese is cut into 1 lb blocks and stored at -80°C until needed. Dr. Brennand has assembled an expert bitterness sensory panel, and used that panel for sensory studies of peptide-spiked cheese samples.

For objective 4, we determined the complete nucleotide sequence of \textit{L. lactis} genes for the group h (bitter) and b (nonbitter) cell envelope proteinases (CEP). Those sequences were then utilized to design unique PCR primers that can discriminate between each of these paralogous genes, and to clone a region of the group b \textit{prtP} gene into a temperature-sensitive plasmid for gene replacement experiments. We determined optimal transformation parameters for 2 industrial strains of \textit{L. lactis} that contain the group h enzyme, then transformed both strains with the group b \textit{prtP} clone. Selection and PCR characterization confirmed that we had successfully replaced the group h \textit{prtP} regions encoding substrate binding sites with the corresponding group b fragment, and that the cell envelope proteinase in these strains remained functional. Further characterization these strains showed no change in the rate of acidification for 1 strain, but a slightly slowed rate in the second. Experiments to confirm our industrial isolates have an altered CEP specificity toward _\textit{sl}-casein f1-23 will be completed before the end of summer.

Work on objective 3 at the UW-Madison has led to the identification of a new \textit{Lb. helveticus} post-prolyl endopeptidase, designated PepO2, that appears to have a major role in hydrolysis of the bitter peptide _\textit{CN} (f193-209). Because proline is a common constituent of bitter peptides, we believe PepO2 may be one of the most important de-bittering enzymes in \textit{Lb. helveticus}. It was not been possible to construct an isogenic derivative of \textit{Lb. helveticus} CNRZ32 lacking the post-proline endopeptidase (PepO2) by gene replacement. The most likely explanation for this observation is that PepO2 has an essential function for the growth of CNRZ32. Therefore, we have constructed food-grade strains of \textit{Lc. lactis} that over-express PepO2 and PepN. Initial assays have confirmed that cell-free extracts from these derivatives hydrolyze \textit{\beta-CN}(f193-209) more rapidly than the unmodified (wild-type) \textit{Lc. lactis} parental strain. In addition, we have identified 3 other endopeptidase genes in CNRZ32, and have shown 2 of these enzymes are functional and active against \textit{\beta-CN}(f193-209). Efforts are now underway to establish that these enzymes are active against \textit{\beta-CN}(f193-209) in cheese, and to show that degradation of these peptides reduces the bitter flavor intensity of peptide solutions.

Food-grade vectors based on dominant and complementation markers have been constructed. Complementation markers are dependent on a mutation in the host strain and can be developed only for a specific vector-host combination. Dominant markers on the other hand are widely applicable. A food-grade vector based on the \textit{a-galactosidase}, a dominant marker, from \textit{Bifidobacterium longum} has been successfully used in the construction of a food-grade vector utilizing a theta replicon from lactococci. Selection for this vector is
based upon the vector encoding the ability of lactococcal strains expressing the α-galactosidase to grow on melibiose.

2. Significant Conclusions:
see progress, above

3. Anticipated Problems/Delays:
The loss of Dr. Marie Strickland, our expert on peptide analysis in cheese, to sudden illness continues to slow our progress on the project.

Publications:


Theses:


Presentations:


Steele, J.L. 2000. Role of lactic acid bacteria in cheese flavor development –Part II. Invited oral presentation for special ADSA pre-meeting workshop on the basics of flavor development in cheese. Sponsored by the American Dairy Science Association and Rhodia, Inc. July 24, Baltimore, MD.


Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:
Dr. Jeff Broadbent
Western Dairy Center
Project Report
Reporting Period April 1, 2000 — December 31, 2002

Principal Investigators: Jeff Broadbent
Co-Investigators: Drs. James Steele and Bill Wendorff, University of Wisconsin-Madison

Project Title: Production of intensely flavored Cheddar-type cheeses by adjunct cultures.

Institution’s Project #: 00116

Project Completion Date: 6/30/03

National Research Plan Priority: cheese Goal: 3.3

Modifications to Project/Budget: none

Project Objectives: (Include any revisions to objectives)
1. The construction of strains of Lactobacillus casei which produce elevated levels of diacetyl.
2. Construction of strains of Lactobacillus casei which over-express a bacterial lipase known to enhance cheese flavor.
3. Manufacture processed cheese from Cheddar cheese having significantly elevated levels of free fatty acids or furanones and pyrazines.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Use of cheese as an ingredient is in part dependent on the impact of the cheese on final product flavor. Process cheese is a significant cheese group and an excellent model system to study carry through of specific flavor compounds into natural cheese-derived products. This project seeks to utilize flavor adjunct lactic acid bacteria to produce elevated levels of specific flavor compounds in natural cheese and then determine the impact of those flavor compounds in processed cheese.

1. Significant Progress against Objectives:
   Dr. Broadbent’s part of this project is focused on Objective 1, and entails construction of Lb. casei mutants which lack the ability to produce pyruvate formate lyase (Pfl). To accomplish this, we cloned the pfl gene from Lb. casei using degenerate PCR primers designed from consensus regions of pfl genes in other microorganisms. This approach allowed us to isolate an internal fragment of the Lb. casei pfl gene, then upstream and downstream flanking regions were isolated and sequenced using inverse PCR. The Lb. casei pfl gene and some flanking DNA was cloned in E. coli, and this construct was used to derive a pfl
deletion derivative that lacked the coding sequence for the enzyme's catalytic center. Unfortunately, efforts to use this construct to create a *Lb. casei* mutant that lacks *pfl* activity by gene replacement have not been successful, primarily known because temperature-sensitive plasmid vectors have not been functional in *L. casei*. Subsequent efforts to utilize a gene inactivation system for this species using single crossover with a suicide vector (insertional inactivation) has been successful in one strain of *L. casei* using a different gene, but not with the *pfl* construct. More recently, we have learned of an alternative gene replacement strategy for this species, and will pursue that option with our *pfl* construct under a newly funded USDA proposal that is aimed at the role of *L. casei* in cheese flavor development.

Dr. Steele's laboratory has been working on objective 2, and they have successfully adapted a flood plate screen in *Escherichia coli* to identify lipase/esterase genes from *Lb. casei* Lila. This assay has allowed them to identify genes encoding four distinct lipase/esterses from this organism, and these enzymes have been characterized in significant detail. Unfortunately, none of these enzymes are active on milk triacylglycerides; therefore, they are unlikely to have a role in the formation of free fatty acids in cheese. Subsequently an enzyme with similarity to a clostridial lipase was observed in the genome sequence of *Lactobacillus helveticus* CNRZ32. This enzyme was characterized and was also determined to not have activity on milk triacylglycerides.

2. Significant Conclusions:
see progress, above

3. Anticipated Problems/Delays:
The difficulties in developing a gene replacement method for *Lb. casei* were unexpected and have delayed progress. However, we continue to work towards the development of this component of the project under a newly funded USDA project. Therefore, we will continue to address objectives 1 and 3 even though this project is scheduled for termination.

Publications:

Theses:

Presentations:

**Technology Transfer Activities**
For information on licensing contact:
Dr. Jeff Broadbent
Western Dairy Center

Project Report
Reporting Period January 1, 2001 — December 31, 2002

Principal Investigators: Carl Brothersen, Utah State University
Co-Investigators:

Project Title: Development of vitamin fortified cheese using high pressure injection technology
Institution’s Project #: 01128
Project Completion Date: December 31, 2001


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

High pressure injection technology can be used to improve the nutritional quality of cheese.

Objective 1: Data to show the effect of fortifying cheese with vitamins D, B<sub>6</sub>, E and folic acid on the flavor of the cheese
Objective 2: Data to show the stability of added vitamins D, B<sub>6</sub>, E and folic acid in cheese over time.
Objective 3: Data on how the addition of vitamins D, B<sub>6</sub>, E and folic acid effects the dynamics of starter and non-starter bacteria in the cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public) High pressure injection technology has been developed at the Western Center as a method of adding flavors, colors, enzymes, bacteria, nutrients and other liquid preparations into cheese. Use of this technology to fortify cheese with vitamins will obviate loss of fortified nutrient and subsequent whey contamination problems. In this project we will evaluate the fortification of cheese with vitamins to determine the suitability of using injection technology to produce a vitamin fortified cheese. Cheddar and Mozzarella cheese was fortified with vitamin D, B<sub>6</sub>, and folic acid, and aged at 4°C for up to 330 days. A trained taste panel did not detect any significant difference in flavor between the fortified and non fortified controls. Fortification did not alter the microflora of the cheese. Some degradation of the vitamins occurred after 330 days of storage.
1. Significant Progress against Objectives:
Methods:

One day old Cheddar cheese, obtained from the USU Dairy Products Laboratory, and one day old Mozzarella cheese, obtained from Glanbia Inc., was and cut in to 20 X 20 X 60 mm blocks. The blocks weighed approximately one ounce. The blocks were then injected with 250 µl of a vitamin preparation to fortify the cheese with 400 IU of vitamin D, 1.3 mg vitamin B₁₂, and 400 µg folic acid. These amounts are 100% of the RDI for the vitamins. Control cheese blocks were also prepared but not injected with vitamin. All cheese blocks were vacuum packaged. Half of the vitamin and control blocks were stored at 4°C, and half at -80°C.

Standard plate counts and enumeration of lactic acid bacteria were done in triplicate for each treatment at days 1, 30, 90 and 150 of age. Non starter lactic acid bacteria were done in triplicate for each treatment at 1 and 150 days of age.

Vitamin analysis was performed for each treatment at 1 and 150 days of age.

A trained taste panel evaluated each treatment at 1, 60, and 270 days of age. The flavor characteristics evaluated were bitter, acid, flat, fruity, cooked, rancid, oxidized, sulfur, unclean, whey, and other

2. Significant Conclusions:

Results of total plate count (TPC), starter (LAB), and non starter lactic acid bacteria (NSLAB) are shown in Table 1, and Table 2, for Cheddar and Mozzarella respectively. These results indicate that the vitamin treatment had no affect on the microflora of the cheese.
Table 1. Enumeration of microflora in Cheddar cheese fortified with vitamins D, B₁₂, and folic acid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
<th>TPC</th>
<th>LAB¹</th>
<th>NSLAB²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>2.6 X 1₀⁹</td>
<td>4.0 X 1₀⁹</td>
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<tr>
<td>Vitamin</td>
<td>1</td>
<td>2.3 X 1₀⁹</td>
<td>4.8 X 1₀⁹</td>
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<tr>
<td>Control</td>
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<tr>
<td>Vitamin</td>
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<td>3.6 X 1₀⁹</td>
<td></td>
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<tr>
<td>Control</td>
<td>90</td>
<td>1.3 X 1₀⁹</td>
<td>10 X 1₀⁹</td>
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<tr>
<td>Vitamin</td>
<td>90</td>
<td>1.3 X 1₀⁹</td>
<td>2.7 X 1₀⁹</td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>1.9 X 1₀⁹</td>
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<tr>
<td>Vitamin</td>
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<td>Vitamin</td>
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<td>6.2 X 1₀⁸</td>
<td>2.8 X 1₀⁹</td>
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</tbody>
</table>

¹Elliker's Agar  
²Rogosa Agar

Table 2. Enumeration of microflora in Mozzarella cheese fortified with vitamins D, B₁₂, and folic acid.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age (days)</th>
<th>TPC</th>
<th>LAB</th>
<th>NSLAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>3.8 X 1₀⁷</td>
<td>3.2 X 1₀⁷</td>
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<tr>
<td>Vitamin</td>
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<tr>
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<td>5.5 X 1₀⁷</td>
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<td>Vitamin</td>
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<td>Control</td>
<td>150</td>
<td>2.2 X 1₀⁷</td>
<td>9.9 X 1₀⁷</td>
<td></td>
</tr>
<tr>
<td>Vitamin</td>
<td>150</td>
<td>1.3 X 1₀⁷</td>
<td>7.3 X 1₀⁷</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>270</td>
<td>7.6 X 1₀⁷</td>
<td>1.7 X 1₀⁸</td>
<td>7.2 X 1₀⁷</td>
</tr>
<tr>
<td>Vitamin</td>
<td>270</td>
<td>6.0 X 1₀⁷</td>
<td>8.1 X 1₀⁷</td>
<td>6.6 X 1₀⁷</td>
</tr>
</tbody>
</table>

¹Elliker's Agar  
²Rogosa Agar
Retention of vitamin content of samples aged for 330 days at 4°C versus samples stored for 330 days at -80°C is shown in Figure 1. Some loss of vitamin activity occurred. The highest loss was 40% for folic acid in Mozzarella, and the least was 0% for vitamin D in Mozzarella.

Figure 1. Retention of vitamins after 330 days of storage.
Eight trained taste panelists evaluated the fortified and control cheese for 11 flavor characteristics. Evaluations were on a nine point hedonic scale. The results for Cheddar and Mozzarella cheeses are shown in Tables 3 and 4 respectively. There was no significant difference in flavor between the vitamin fortified cheese and non fortified controls.

Table 3. Mean scores and significance of flavor attributes of Cheddar cheese by trained taste panel.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Treatment</th>
<th>Mean Score</th>
<th>Duncan Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter</td>
<td>Vitamin</td>
<td>3.1042</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.9167</td>
<td>A</td>
</tr>
<tr>
<td>Acid</td>
<td>Vitamin</td>
<td>3.7708</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.6875</td>
<td>A</td>
</tr>
<tr>
<td>Flat</td>
<td>Vitamin</td>
<td>2.7708</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.7708</td>
<td>A</td>
</tr>
<tr>
<td>Fruity</td>
<td>Vitamin</td>
<td>1.8750</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.7292</td>
<td>A</td>
</tr>
<tr>
<td>Cooked</td>
<td>Vitamin</td>
<td>1.5417</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.4167</td>
<td>A</td>
</tr>
<tr>
<td>Oxidized</td>
<td>Vitamin</td>
<td>1.8750</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.6250</td>
<td>A</td>
</tr>
<tr>
<td>Rancid</td>
<td>Vitamin</td>
<td>1.4792</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.4583</td>
<td>A</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Vitamin</td>
<td>2.3125</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.1875</td>
<td>A</td>
</tr>
<tr>
<td>Unclean</td>
<td>Vitamin</td>
<td>1.9375</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.8542</td>
<td>A</td>
</tr>
<tr>
<td>Whey</td>
<td>Vitamin</td>
<td>1.6458</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.5208</td>
<td>A</td>
</tr>
<tr>
<td>Other</td>
<td>Vitamin</td>
<td>1.5417</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.5208</td>
<td>A</td>
</tr>
</tbody>
</table>
Table 4. Mean scores and significance of flavor attributes of Mozzarella cheese by trained taste panel.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Treatment</th>
<th>Mean Score</th>
<th>Duncan Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter</td>
<td>Vitamin</td>
<td>3.8750</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>3.6750</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>Vitamin</td>
<td>4.3500</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>3.9000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flat</td>
<td>Vitamin</td>
<td>2.5000</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>2.2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruity</td>
<td>Vitamin</td>
<td>1.6250</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.5000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked</td>
<td>Vitamin</td>
<td>1.3750</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.3250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidized</td>
<td>Vitamin</td>
<td>1.8000</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.6000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rancid</td>
<td>Vitamin</td>
<td>1.3750</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfur</td>
<td>Vitamin</td>
<td>1.7000</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.7000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unclean</td>
<td>Vitamin</td>
<td>2.0000</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
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<td></td>
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<tr>
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<td>Vitamin</td>
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<td>A</td>
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<tr>
<td>Control</td>
<td>1.2308</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>Vitamin</td>
<td>1.9750</td>
<td>A</td>
</tr>
<tr>
<td>Control</td>
<td>1.8500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Anticipated Problems/Delays:
The cooler containing the cheese samples malfunctioned resulting in the loss of all samples. New samples were prepared, delaying the completion of the project by six months. The vitamin E preparation was too viscous and could not be injected into the cheese. It was dropped from the study.
Western Dairy Center

Project Report
Reporting Period October 1, 1999 — December 31, 2001

Principal Investigators: Carl Brothersen, Utah State University
Co-Investigators: Bart Weimer, Utah State University

Project Title: Effect of size, hydrophobicity and temperature on the diffusion of molecules within the Cheddar cheese matrix.

Institution’s Project #: 99110

Project Completion Date: 4/1/2000

National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1

Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)
1. Determine the extent of diffusion for macro molecules in Cheddar cheese.
2. If significant diffusion is detected in objective 1, determine the effect of storage temperature on the migration of molecules in Cheddar cheese.
3. If significant diffusion is detected in objective 1, determine the effect of age at injection on the migration of molecules in Cheddar cheese.

Project Summary: (Suitable for inclusion in Center documents released to the public)
Cheese flavor development is the result of the breakdown of cheese protein and lipids into specific flavor compounds by a series of enzymatic reactions. The overall rate of production of these flavor compounds is a function of the activity of the individual enzymes involved and the rate of diffusion of the enzymes, substrates and products through the cheese matrix. The effect of diffusion on the activity of enzymes in aqueous solutions is generally small and can be ignored. However, within the cheese matrix the effect of diffusion becomes a limiting factor on the overall activity of the enzymes. This project will determine the diffusion of fluorescent markers through the Cheddar cheese matrix.

1. Significant Progress against Objectives:
Method:
Cheddar cheese manufactured at the USU Dairy Products Lab was removed from the press and cut into 20 X 20 x 60 mm blocks. Using a microsyringe, 50µl of a 4% fluorescein solution was injected in a line through the center of the block. The blocks were then wrapped in foil to retain moisture and protect from light. Samples were randomly sorted into three groups, and individual groups stored
at 4, 12, or 20°C. Each day, a block was randomly selected and a 1mm slice was cut from the block, perpendicular to the line of injection. The sample was examined by epifluorescence microscopy and the images recorded with a digital camera. The images were analyzed using PhotoShop by posterizing at level 3 and counting the pixels in each color band. The radius of the diffusing dye was then calculated. A sample of a posterized image is shown in Figure 1.

Figure 1. Posterized image of fluorescein in Cheddar cheese.
2. Significant Conclusions:

Diffusion rates were on the order of one millimeter in the first five days. Diffusion rates decreased for the next 20 days.

The effect of temperature on the rate of diffusion of fluorescein in cheese is shown in Figure 2. The rate of diffusion increases with increasing temperature. Regression lines for the model $y = m \ln x + b$ are shown.

![Diffusion of fluorescein in Cheddar cheese.](image)

Figure 2. Diffusion of fluorescein in Cheddar cheese.

A more accurate model of diffusion is being calculated.
Western Dairy Center
Project Report
Reporting Period January 1, 2001 — December 31, 2002

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 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. However, we have recently shown that ground beef stored in high (80%) oxygen to enhance redness has significantly elevated TBA values and rancidity after cooking. So, fresh pork sausage stored in high oxygen environment would probably have a similar rancidity problem, which may be alleviated by use of MM.

   **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).** Tests are currently underway on the combined effects of MM and sodium nitrite as antioxidants in nitrite-cured summer sausages. Sodium nitrite alone is a well known antioxidant in cured meats, but our recent work indicates that sodium nitrite at the USDA approved level of 156 ppm (1/4 ounce per 100 pounds meat) is not as effective an antioxidant as 1.5% MM (see abstract by Jayasingh and Cornforth, 2003, below). So, there is the possibility that addition of MM to cured meats would improve the storage stability of cooked, cured sausages.

   **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. The optimum MM level in cured meats will be determined in work conducted this summer (2003).

   **Objective 5.** MM antioxidant mechanism. In work to be presented at the 2003 IFT national meeting, we will report that 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.

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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.

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).

3. Anticipated Problems/Delays: None

Publications:

Published Abstract:

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.

**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 Technology 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.
Project Title: Consumer Acceptability Characteristics of Conjugated Linoleic Acid (CLA) Enriched Milk

Institutions Project #:

Project Completion Date:
National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1

Abstract
An experiment was conducted to study the consumer acceptability characteristics of conjugated linoleic acid (CLA) enriched milk containing 5.2, 16.2, or 16.9 mg CLA/g fat. An open panel of consumers evaluated the fluid milk for consumer acceptability on a hedonic scale and a trained panel of experts evaluated overall quality and flavor characteristics. Consumer acceptability characteristics (mouth-fill, color, flavor, and overall quality of milk) of low or high CLA milk were not different. Trained panel observed no significant trends in overall quality and flavor characteristics of CLA enriched milk that would be considered objectionable compared with low CLA milk. Results from the present study suggest that consumer acceptability characteristics, overall quality, color, and flavor characteristics of milk naturally enriched with CLA were the same as milk with low level of CLA, suggesting that milk with high CLA was acceptable to a majority of consumers.

Project Summary
In recent years, there has been a growing interest in increasing CLA content in food products due to its potential health benefits (Ip et al., 1991; Pariza, 1999). The CLA is the only fatty acid that has been shown unequivocally to inhibit carcinogenesis in experimental animals (Ip et al., 1991). Increasing its content in milk and milk products would, therefore, increase the nutritive and therapeutic value of these foods. About 92% of total CLA in milk fat is C18:2 c-9, t-11 isomer. The average CLA content of milk is 4.0 mg/g of fat (Dhiman et al., 1999). Under typical dietary conditions, this is not sufficient to meet the dose that has been shown to be effective in reducing the incidence of tumors or inhibiting the growth of tumors in laboratory animals (Ip et al., 1994). The CLA content of milk
can be enhanced through animal’s diet. Cows grazing pasture or fed oil seeds have CLA content of milk ranging from 12-22 mg/g of fat. An important question to be considered is whether the consumers will accept CLA enhanced milk and milk products. Our hypothesis is that CLA enriched milk would have similar consumer acceptability characteristics as that of milk with low level of CLA. The objective of this study was to evaluate the consumer acceptability (color, mouth-fill, flavor, and overall quality) of CLA enriched milk.

**Materials and methods**

Milk with different levels of CLA was produced by feeding cows either a diet containing 50% conserved forage and 50% grain (Low CLA), grazing cows on pasture (High CLA-1), or cows grazing on pasture supplemented with extruded full-fat soybeans (High CLA-2). Fatty acid profile of the milk in different treatments is given in Table 1. Milk was pasteurized and homogenized within 36 h after collection at the farm. Whole milk bought from the store with a self life of more than 3 wk was used as a positive control.

Within 72 h after pasteurization milk was offered to an open panel of judges comprising 62, 86, and 92 judges in three sessions each week apart. Approximately 5-ml of refrigerated milk was served in plastic cups. Random numbers were assigned to each milk sample. The panel was conducted in individual booths and water pitchers and spittoons were provided to rinse the mouth between samples. The testing area had artificial white light, fresh air circulation and was free from outside disturbances. Judges were asked to rate the samples for mouth-fill, flavor, color, and overall quality. A 9-point hedonic scale (where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely) was used.

A trained panel performed the study on flavor characteristics, color and overall quality of milk. Eight judges were selected from number of people who had been regularly exposed to training on dairy products, including fluid milk. For flavor characteristics the scale used was: 9 and 8 = extremely strong, 7 and 6 = very strong, 5 = moderately strong, 4 = normal for the specific characteristics in such products, 3 and 2 = slight and 1 or less none. For color and overall quality: 9 would indicate the evenness of color and highest overall quality; whereas 1 would indicate extremely poor color and quality. Reference samples for the specific attributes were provided for all flavor characteristics during sampling. Refrigerated milk samples (20-ml) were served in plastic cups with random code numbers assigned to each sample. This was repeated a second time after a 20-minute break. Sample position was changed every time the sample was offered to different judges in order to minimize the positional biasness. The booth and judging conditions were the same as described previously. The data were analyzed in SAS (SAS, 1999/2000) using proc GLM. Statistical significance was declared at $P < 0.05$ and trends were described at $P < 0.1$. 

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### Table 1. Fatty acid profile of low and high CLA containing milk.

<table>
<thead>
<tr>
<th>Fatty acid mg/g of fat</th>
<th>Low-CLA</th>
<th>HighCLA-1</th>
<th>High CLA-2</th>
<th>SEM</th>
<th>$P = $</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trt</td>
<td>Trt</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;8:0&lt;/sub&gt;</td>
<td>11.0</td>
<td>10.0</td>
<td>10.1</td>
<td>0.7</td>
<td>0.51</td>
</tr>
<tr>
<td>C&lt;sub&gt;10:0&lt;/sub&gt;</td>
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<td>3.5</td>
<td>3.4</td>
<td>0.3</td>
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<td>1.7</td>
<td>0.40</td>
</tr>
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<td>92.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>&lt;0.01</td>
</tr>
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<td>23.0</td>
<td>20.6</td>
<td>2.1</td>
<td>0.37</td>
</tr>
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<td>C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>12.6&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.02</td>
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<td>273.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>244.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4</td>
<td>&lt;0.01</td>
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<td>16.5</td>
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<td>1.1</td>
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<tr>
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<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
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<td>100.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>51.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.4</td>
<td>&lt;0.01</td>
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<td>59.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1 t-9&lt;/sub&gt;</td>
<td>21.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1 c-9&lt;/sub&gt;</td>
<td>238.3</td>
<td>261.2</td>
<td>274.2</td>
<td>11.0</td>
<td>0.13</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:1 c-11&lt;/sub&gt;</td>
<td>8.2</td>
<td>8.1</td>
<td>7.6</td>
<td>0.4</td>
<td>0.60</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:2&lt;/sub&gt;</td>
<td>40.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:3&lt;/sub&gt;</td>
<td>4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.7&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CLA C&lt;sub&gt;18:2 c-9, t-11&lt;/sub&gt;</td>
<td>5.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:2&lt;/sub&gt;</td>
<td>ND&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.6</td>
<td>0.5</td>
<td>0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:3&lt;/sub&gt;</td>
<td>ND&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.3</td>
<td>0.3</td>
<td>0.04</td>
<td>0.59</td>
</tr>
<tr>
<td>C&lt;sub&gt;20:4&lt;/sub&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;22:4&lt;/sub&gt;</td>
<td>1.5</td>
<td>1.2</td>
<td>1.3</td>
<td>0.1</td>
<td>0.10</td>
</tr>
<tr>
<td>SFA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>607.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>562.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>522.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.9</td>
<td>0.04</td>
</tr>
<tr>
<td>UFA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>392.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>437.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>477.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.9</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>Means with different superscripts in the same row are significantly different.

<sup>1</sup>Standard error of mean

<sup>2</sup>Not detected

<sup>3</sup>Saturated fatty acid

<sup>4</sup>Unsaturated fatty acids

**Results and discussion**

Milk used in the study contained 3.4, 3.6, and 3.6 % fat, 3.0, 2.9, and 3.0 % protein, and 4.7, 4.5, and 4.7 % lactose with no significant difference among treatments. The CLA content of milk in High CLA-1 and High CLA-2 treatments was 300 % more than Low CLA treatment. The high CLA milk was also high in unsaturated fatty acids compared with low CLA milk. Open panel evaluation of high and low CLA milk for mouth-fill, color, flavor, and overall quality suggested no differences among treatments including positive control (Table 2). On the hedonic scale, both high and low CLA milk were liked moderately by the consumers including the milk in positive control.
Table 2. Open panel consumer acceptability characteristics of CLA enriched milk.

| Characteristics | Store milk | Low CLA | High CLA- | High CLA- | SEM<sup>3</sup> | P = 
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouth-fill</td>
<td>6.5</td>
<td>6.6</td>
<td>6.7</td>
<td>6.7</td>
<td>0.2</td>
<td>0.76</td>
</tr>
<tr>
<td>Color</td>
<td>6.7</td>
<td>6.6</td>
<td>6.6</td>
<td>6.6</td>
<td>0.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Flavor</td>
<td>6.1</td>
<td>6.0</td>
<td>5.8</td>
<td>5.9</td>
<td>0.4</td>
<td>0.79</td>
</tr>
<tr>
<td>Overall quality</td>
<td>6.4</td>
<td>6.1</td>
<td>6.2</td>
<td>6.2</td>
<td>0.3</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<sup>1</sup>Higher score is better  
<sup>2</sup>Store bought milk was used as positive control  
<sup>3</sup>Standard error of mean

Evaluation by a trained panel of experts observed no significant differences in overall quality, evenness of color, and flavor characteristics of milk containing low and high CLA, except that barny flavor was perceived stronger in high CLA milks (Table 3). Trained judges also noticed stronger astringent, cowy, and oxidized flavors in positive control milk that was bought from the store compared with fresh experimental milk. All flavor attributes of low and high CLA milk were rated towards none or barely perceptible side of the scale.

Conclusion
Consumer acceptability characteristics (mouth-fill, color, flavor and overall quality) of milk naturally enriched with CLA were the same as milk with low levels of CLA, suggesting that milk with high CLA was acceptable to a majority of consumers.

References
Table 3. Overall quality, evenness of color, and flavor characteristics of CLA enriched milk

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Store milk</th>
<th>Low CLA 1</th>
<th>High CLA-1</th>
<th>High CLA-2</th>
<th>SEM 3</th>
<th>P =</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall quality</td>
<td>5.9</td>
<td>7.3</td>
<td>6.5</td>
<td>6.3</td>
<td>0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Quality</td>
<td>6.1</td>
<td>7.0</td>
<td>6.9</td>
<td>6.3</td>
<td>0.5</td>
<td>0.47</td>
</tr>
<tr>
<td>Color, evenness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid</td>
<td>1.3</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>0.1</td>
<td>0.94</td>
</tr>
<tr>
<td>Astringent</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Barny</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Bitter</td>
<td>1.2</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>0.1</td>
<td>0.98</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.3</td>
<td>1.8</td>
<td>2.1</td>
<td>1.9</td>
<td>0.2</td>
<td>0.19</td>
</tr>
<tr>
<td>Cowy</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Feed</td>
<td>2.3</td>
<td>1.6</td>
<td>2.1</td>
<td>2.2</td>
<td>0.2</td>
<td>0.35</td>
</tr>
<tr>
<td>Fermented</td>
<td>1.1</td>
<td>1.2</td>
<td>1.3</td>
<td>1.2</td>
<td>0.1</td>
<td>0.72</td>
</tr>
<tr>
<td>Flat/low flavor</td>
<td>1.6</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>0.1</td>
<td>0.32</td>
</tr>
<tr>
<td>Foreign</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
<td>1.7</td>
<td>0.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Garlic/onion</td>
<td>1.1</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.1</td>
<td>0.40</td>
</tr>
<tr>
<td>Lacks</td>
<td>2.3</td>
<td>1.8</td>
<td>2.0</td>
<td>2.4</td>
<td>0.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Freshness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malty</td>
<td>1.2</td>
<td>1.3</td>
<td>1.0</td>
<td>1.2</td>
<td>0.1</td>
<td>0.47</td>
</tr>
<tr>
<td>Oxidized</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Rancid</td>
<td>1.3</td>
<td>1.1</td>
<td>1.3</td>
<td>1.3</td>
<td>0.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Salty</td>
<td>1.3</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>0.1</td>
<td>0.75</td>
</tr>
<tr>
<td>Unclean</td>
<td>1.1</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
<td>0.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Others</td>
<td>1.3</td>
<td>1.4</td>
<td>1.7</td>
<td>1.5</td>
<td>0.2</td>
<td>0.79</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means with different superscripts in the same row are significantly different.

<sup>1</sup>Higher the score better the overall quality and color. Smaller the scores less perceived the flavor.

<sup>2</sup>Store bought milk was used as positive control.

<sup>3</sup>SEM = standard error of mean.
Western Dairy Center

Project Report

Reporting Period January 1, 1999 – December 31, 2002

Principal Investigators: Conly Hansen, Utah State University
Co-Investigators: Donald J. McMahon, Utah State University

Project Title: Controlling Chemical Composition and Functionality of Cheese

Institution Project #: 99204

Project Completion Date: 6/30/03

National Research Plan (1997): Priority: 4; Goal: 4; Tactic: 1

Modifications to Project/Budget:
None

Project Objectives: (Include any revisions to objectives)
To determine the influence of pH, calcium, salt and moisture content of cheese on shredability and meltability.

Objective 1: To develop a high pressure injection system for modifying the chemical composition of cheese.

Objective 2: To modify pH, calcium, and salt contents of cheese while keeping all other parameters constant, and determine their influence on functionality.

Objective 3: To determine the combinations of calcium, salt, and pH required for optimum shredding and melting of cheese.

Project Summary:
Chemical composition of cheese was modified by injecting concentrated solutions of calcium chloride, sodium chloride or glucono-δ-lactone into blocks of cheese. A high pressure injection system was used to repeatedly inject streams of the injectate fluid into the cheese such that approximately 1% (w/w) of the injectate was added to the cheese. Injections were repeated after 24 hours to produce cheese that had been injected up to 5 time. The cheese was then allowed to equilibrate and then tested for chemical composition, physical properties, melting properties. Changes in cheese properties were then explained using image analysis of electron micrographs of the cheese to monitor changes in protein structure. Calcium content was studied between 0.3 and 1.8% (w/w), salt content was studied between 0.1 to 2.7% (w/w) and pH was studied over the range 5.3 to 4.7. Overall, increasing calcium content caused a contraction in the cheese protein matrix resulting in syneresis, increased firmness...
and decreased melting properties. Salting of cheese had its most significant effect on initial salting when the salt level was increased to 0.6% (w/w) with very little further effect. Salting caused a slight increase in the meltability of the cheese presumably because of the increased hydration of the proteins that occurs upon addition of salt, but there was no evidence of any exchange between calcium and sodium ions.

Progress

2. Modifying cheese chemical composition by injecting ionic solutions into cheese

A. Effect of water and calcium injection on structure-function attributes of cheese

Objectives

Our objective was to determine how injection of calcium solutions into cheese affects cheese microstructure and to relate changes in structure to changes in hardness and melting of cheese.

Materials and Methods

Mozzarella cheese (48, 49, and 53% moisture, and 22% fat) was made by a direct-acid, stirred-curd procedure. Cheese was cut into 0.3 to 0.4-kg blocks, vacuum packaged and stored for 10 d at 4°C. Cheese blocks were then high-pressure injected (1 to 5 times) with either water or a 40% calcium chloride solution. Thus, ten treatments were defined, corresponding to five water and five calcium injection levels. A control, uninjected cheese block was also considered. Injections were performed in two opposite sides of the block, and according to a 1 x 1 cm pattern, with successive injections performed 24 h apart. Pressure of injection was set as 1400 psig, and burst injection time as 1 s. After 42 d of storage at 4°C, cheese blocks were analyzed for structural and functional attributes. Scanning electron micrographs, (1500 X magnification, from two different fields) were uploaded into Adobe Photoshop® 4.0 and their gray-scale values analyzed. Dark areas (corresponding to fat/whey pockets) were differentiated from light areas (corresponding to protein matrix) by applying a threshold function, and the proportion of pixels associated with dark and light areas determined. Texture profile analysis was performed using a two-bite compression test run on an Instron 5542 (Canton, MA). Samples, 20 mm by 16 mm diameter, were taken from the cheese immediately after removal from the refrigerator and tested at ~5°C.

Results and Discussion

Results of the statistical analysis are presented in Table 1. When water was injected, a slight increase in weight was observed. In contrast, when calcium was injected, the cheese lost weight and considerable serum was expelled from the cheese. Moisture content increased with water injection, and decreased with calcium injection. The control (uninjected) cheese had the typical structure of a stirred/pressed-curd cheese, with protein matrix interspersed with areas containing fat and/or serum. Injecting water increased the area occupied by the protein matrix (by 14% after 5 injections) as shown by an increase in the proportion of light pixels in the micrographs. Increasing the calcium content of
the cheese (from 0.3% to 1.8% after 5 injections) decreased the area occupied by the protein matrix (by 17%). This represents a contraction of the protein matrix and concomitant release of serum entrapped within the protein matrix. A decrease in cheese pH occurred upon injection of calcium, but it had been previously observed that pH did not affect cheese microstructure unless it was accompanied by a change in calcium content. Water injection decreased cheese hardness but did not affect any other functional attribute. Hardness increased when calcium was injected, but cohesiveness decreased. Adhesiveness and springiness were unaffected. Meltability of the cheese was inversely proportional to calcium content.

Table 1. Pr>F for the ANOVA sources of variation and specified contrasts by variable of interest.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calcium</th>
<th>Moisture</th>
<th>pH</th>
<th>Weight</th>
<th>Melting</th>
<th>Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Block</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.0634</td>
<td>0.0005</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Determination</td>
<td>0.6707</td>
<td>0.9705</td>
<td>0.8773</td>
<td></td>
<td>0.9841</td>
<td>0.7387</td>
</tr>
<tr>
<td>Contrast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-Calci um¹</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Control-Water²</td>
<td>0.6225</td>
<td>0.0253</td>
<td>0.0105</td>
<td>0.1418</td>
<td>0.9455</td>
<td>0.0254</td>
</tr>
</tbody>
</table>

¹. Contrast of control (uninjected cheese) against all calcium levels.
². Contrast of control (uninjected cheese) against all water levels.

Conclusions

Increasing calcium content of cheese alters how proteins in the cheese matrix interact. It appears that calcium promotes protein-to-protein interactions, probably through calcium bridging and charge neutralization. Such increased interactions between proteins cause contraction of the protein matrix and expulsion of serum from the matrix. More energy must also be applied to overcome these interactions and allow proteins to flow when heated. Thus,
cheese hardness is increased and meltability decreased when the calcium content of the cheese is increased.

**Presentations**


**Publications**


**B. Effect of salt on structure-function relationships of cheese**

**Objectives**

To determine the effect of salt on structural and functional properties of cheese.

**Materials and Methods**

Unsalted, commercial Muenster cheese (41% moisture, 29% fat, 0.7% calcium) was obtained on 1 d and cut into 0.5 to 0.6-kg blocks that were vacuum packaged and stored for 14 d at 4°C. Cheese blocks were then high-pressure injected 1, 3, or 5 times, with a 20% (wt/wt) sodium chloride solution. Successive injections were performed 24 h apart. After 40 d of storage at 4°C, cheese blocks were analyzed for chemical, structural, and functional attributes.

**Results**

Injecting sodium chloride increased the salt content of cheese. After 5 injections, the salt content was 2.7% compared to 0.1% in the control, uninjected cheese. At the highest levels, salt injection promoted syneresis, and residual moisture was observed inside cheese packages. After 3 injections the moisture content decreased from 41% to 39%. However, the increased salt content resulted in a net weight gain of 1.9% after 5 injections. Cheese pH, soluble nitrogen, and total and soluble calcium content was unaffected. Although only significant at \( P < 0.1 \), cheese injected 5 times had a 4% increased area of cheese matrix occupied by protein compared to uninjected cheese. Cheese hardness, adhesiveness, and initial rate of cheese flowing increased upon salt injection. However, the final extent of cheese flow was unaffected.
Conclusions

Adding salt to cheese alters protein interactions, such that the protein matrix becomes more hydrated and expands. However, increasing the salt content of cheese did not cause an exchange of calcium with sodium. Therefore, calcium-induced protein interactions would remain the limiting factor controlling cheese functionality.

Presentations


Publications


C. Effect of pH on structure-function relationships of cheese

Objectives

To determine the effect of pH on chemical and functional properties of cheese.

Materials and Methods

Commercial Cheddar cheese (34% moisture, 30% fat, 1.7% salt, 0.8% calcium) was obtained on 1 d and cut into 0.4 to 0.5-kg blocks that were vacuum packaged and stored for 14 d at 4°C. Cheese blocks were then high-pressure injected 1, 3, or 5 times, with a 20% (wt/wt) glucono-delta-lactone solution. Successive injections were performed 24 h apart. After 40 d of storage at 4°C, cheese blocks were analyzed for chemical and functional attributes.

Results

Injection of glucono-delta-lactone solution decreased cheese pH. After 5 injections, cheese pH was 4.7 compared to 5.3 in the control, uninjected cheese. Decreased pH increased the content of soluble calcium and decreased the total calcium content of cheese. At the highest level, injection of acid promoted syneresis, and residual moisture was observed inside cheese packages. Thus, after 5 injections the moisture content of cheese decreased from 34% to 31%. This resulted in decreased cheese weight, 2.5% after 5 injections. Injecting acid decreased cheese hardness, and at the highest levels of injection, decreased pH and moisture content caused the cheese to become brittle. Thus, the cheese lost structural cohesion, fracturing during testing. When heated, the initial rate of
cheese flow increased when pH was lowered from 5.3 to 5.0. However, lowering cheese pH to 4.7 caused decreased flowing rate. Also, the final extent of cheese flow was unaffected by lowering pH to 5.0, but it decreased when cheese pH was lowered to 4.7.

Conclusions

Lowering the pH of Cheddar cheese by injecting an acidulant solution alters protein interactions, which then affects cheese functionality. Decreased pH not only promotes calcium solubilization and decreased calcium content of cheese, which impair interactions between proteins, but it also leads at low pH to isoelectric precipitation of caseins, which favors interactions between proteins. At low levels of acidulant injection, calcium solubilization is the predominant factor, and interactions between proteins decrease. Thus, the content of bound calcium would direct cheese functionality when the pH of cheese is above 5.0. In contrast, at high levels, acidulant injection promotes protein-to-protein interactions as the caseins approach their isoelectric point. Thus, at pH values below 5.0, the acid precipitation of caseins overcomes the opposing effect caused by increased calcium solubilization and decreased calcium content of cheese, and there is a net increase of protein-to-protein interactions.

Presentations

Publications
Western Dairy Center
Project Report
Reporting Period February 1, 2001 — December 31, 2002

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

Project Title: Rehydration and structure of reconstituted casein micelles.

Institution’s Project #: 01129

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.
2. Investigate any differences in coagulation properties of milk containing reconstituted casein micelles.

Project Summary: (Suitable for inclusion in Center documents released to the public)
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 _-casein. The irregular supramolecular structure for the colloidal casein particles in milk, 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.
2. Methods:

A technique for preparing casein supramolecules for transmission electron microscopy, based upon freeze drying enables viewing of its structure with minimal variation from its native form. Milk was diluted with water (1:100), and casein supramolecules were immediately adsorbed onto poly-L-lysine-treated, parlodion-coated copper grids. The grid with adsorbed proteins was rinsed to remove secondary adsorbed material, then placed on top of a drop of 12 mM solution of uranyl oxalate (50:50 uranyl acetate and oxalic acid), rinsed with water, then flash frozen in liquid nitrogen-cooled, liquefied Freon 22, and freeze dried. Samples were then viewed with a Zeiss 902 (Thornwood, NY) microscope at 80 kV. Multiple images were captured at varying tilt angles and stereopairs generated.

3. Significant Conclusions

Based upon the potential functionalities of the caseins (and the calcium phosphate nanoclusters), we developed a model of the colloidal casein particle as an irregular supramolecule. The flexibility of the caseins permits various structures, such as clumps, loops and linear strands, to be formed based upon random associations during cellular synthesis of the casein supramolecule. This results in an irregular structure being developed rather than the repeated structures that form the basis for the formation of regular supramolecules.

Publications: None

Theses: None

Published Abstract:

Technology Transfer Activities
For information on licensing contact:
Western Dairy Center

Project Report

Reporting Period: January 1, 2002—December 31, 2002

Year-end report 2002

Note: New data in bolded Ariel font

Principal Investigators: Ann W. Sorenson

Co-Investigators: Gary Straquadine, Judith Hallfrisch

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: 12/31/03

<table>
<thead>
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<th>Goal:</th>
<th>Tactic:</th>
</tr>
</thead>
</table>

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

1. Significant Progress against Objectives:

Methodology: All aspects of the survey protocol have been developed and checked. 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 maintained by USDA. The diet analysis program is much more flexible than the popular commercial packages that are available to the public.

Data collection: The first wave of questionnaires was sent out in March 2002 to people 50 through 64 years of age. To date 184 usable questionnaires have been returned and coded (approximately 30% to date). Participants are still returning their completed questionnaire in response to follow-up with a reminder postcard. Packets for the second wave of questionnaires, which will sample will over sample people 65 years of age an older, are current being prepared for mailing in September (see item 3 below).

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 to date. Some questionnaires are still being returned after being sent a reminder card. Of the 462 questionnaires, 414 have been coded (179 men and 235 women). The sample was stratified for urban and rural status, gender and age.

Data Analysis: The questionnaires collected in 1999-2000 have been coded and the data analyzed for several for several studies that compared aspects the diets of the Utah cohort compared to the National survey NHANES III and to a population of the same age in Geneva Switzerland. Preliminary data analysis on the March cohort will begin as soon as the second wave of questionnaires is sent out.

In preparation for studies using inferential analysis, the same variables were compared between the 1999-2000 (550 people) and the 2002 (462 people) cohorts. To increase the sample size, variables, which are not significant from the 1999 to the 2002 survey, were combined for a total sample of 1012.

A study is in progress to determine the association of dairy products and individual dairy foods with selected health conditions including, osteoporosis, heart disease, diabetes, cancer and obesity. The data must be adjusted to eliminate the effects of other factors that may be risk modifiers for the outcomes (diseases). These confounders can bias the results of the diary products on the outcomes. The risk modifiers include 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) and Women who use alcohol (ages 50-64; p = 0.04).

The intake of fourteen dairy foods was compared between the 1999 and 2000 cohorts. In the 2002 group, 29 men and 27 women didn’t fill-out the food frequency. 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).

In the combined data set, 14 percent of the participants did not eat any dairy products. The following table shows the percent of participants who consumed dairy products (not age adjusted).

PERCENT OF PARTICIPANTS CONSUMING 14 DIFFERENT DAIRY FOODS

<table>
<thead>
<tr>
<th>Dairy Food</th>
<th>Participants Consuming</th>
<th>Not Consuming</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cottage cheese</td>
<td>65.7%</td>
<td>69.9%</td>
</tr>
<tr>
<td>Hard cheese</td>
<td>87.1%</td>
<td>87.8%</td>
</tr>
<tr>
<td>Yogurt</td>
<td>40.2%*</td>
<td>61.6%</td>
</tr>
<tr>
<td>Use all milk types</td>
<td>36.4%**</td>
<td>41.3%**</td>
</tr>
<tr>
<td>Skim milk</td>
<td>16.4%</td>
<td>22.1%</td>
</tr>
<tr>
<td>1% milk</td>
<td>18.2%</td>
<td>17.9%</td>
</tr>
<tr>
<td>2% milk</td>
<td>32.0%</td>
<td>29.7%</td>
</tr>
<tr>
<td>Whole milk</td>
<td>8.6%</td>
<td>4.15%</td>
</tr>
<tr>
<td>Hot cocoa</td>
<td>37.3%</td>
<td>43.6%</td>
</tr>
<tr>
<td>Butter</td>
<td>50.0%</td>
<td>61.1%</td>
</tr>
<tr>
<td>Ice cream</td>
<td>76.1%</td>
<td>77.3%</td>
</tr>
<tr>
<td>Ice milk/Frozen Yogurt</td>
<td>30.5%</td>
<td>35.6%</td>
</tr>
<tr>
<td>Pudding</td>
<td>45.7%</td>
<td>50.5%**</td>
</tr>
<tr>
<td>Cream based soups</td>
<td>26.1%</td>
<td>28.6%</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01

The Questionnaire will be revised before the next round of data is collected. Since we do not have access to a sampling pool for the oldest age groups, we are planning on adding a younger age group, 35 to 49 years of age to the 50 to 64 year and the 65 to 75 year age groups. The questionnaire will be adjusted for people 35 to 49 years according to the NHANES III questionnaires for the same group. The original questionnaire will be used for the older groups. The Food Frequency Questionnaire will be revised for all age groups to reflect the foods that have entered the diet over the past 5 years and remove those foods that are no longer consumed. The food groupings will also be revised.

Preliminary Results:
This preliminary report is based on analysis of the 550 questionnaires collected in 1999-2000 and coded during the past year. 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. 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 was divided in to types
available at most retail outlets. Figure 3 shows that more 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.
The older people in Utah are a population of dairy food users. Figure 2 is an age-
adjusted comparison of the percent of men and women over 50 years of age
using the dairy foods. Over 90 percent report using hard cheeses and more that
80 percent drink milk. Even cream cheese, the dairy product eaten least is
consumed by an average of 40 percent of the older population.

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
2 shows the rank of dairy products compared to the top 10 of all foods as sources
of Vitamin A, Calcium and energy (Kcals). 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 liquids 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 supplement on the diet of the total cohort, Table 3 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.

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. Dairy products are good sources Calcium, Vitamin A, Vitamin
B12, Vitamin D, Zinc and Magnesium
Tables and figures:

Table 1
Rank Order of Top Ten Dairy Products by Gender

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

Table 2
Dairy Food rankings from Top 10 Sources of All Foods
For 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>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>Skim milk (3)</td>
<td>Skim milk (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1% milk (4)</td>
<td>1% milk (5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yogurt (5)</td>
<td>Yogurt (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ice cream (6)</td>
<td>Ice cream (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot chocolate (10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Instant brkfast. (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole milk (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>65-79</td>
<td>Hard cheese (5)</td>
<td>Hard cheese (1)</td>
<td>Hard cheese (1)</td>
</tr>
<tr>
<td></td>
<td>Skim milk (10)</td>
<td>2% milk (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skim milk (4)</td>
<td></td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td>Yogurt (6)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hot chocolate (7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ice cream (10)</td>
<td></td>
</tr>
<tr>
<td>80+</td>
<td>Hard cheese (6)</td>
<td>Hard cheese (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2% milk (7)</td>
<td>2% milk (3)</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>1% milk (4)</td>
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<tr>
<td></td>
<td></td>
<td>Whole milk (5)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Skim milk (6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spec. milk (9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot chocolate (10)</td>
<td></td>
</tr>
</tbody>
</table>

WOMEN

<p>| 50-64          | 2% milk (8)       | Hard cheese (1)| Hard cheese (1)      |
|                | Skim milk (9)     | 2% milk (2)    |                      |
|                |                  | Skim milk (4)  |                      |</p>
<table>
<thead>
<tr>
<th></th>
<th>1% milk (5)</th>
<th>Yogurt (6)</th>
<th>Hot chocolate (7)</th>
<th>Ice cream (9)</th>
<th>65-79</th>
<th>Hard cheese (5)</th>
<th>Skim milk (7)</th>
<th>1% milk (8)</th>
<th>2% milk (10)</th>
<th>Hard cheese (1)</th>
<th>1% milk (2)</th>
<th>Skim milk (3)</th>
<th>2% milk (4)</th>
<th>Yogurt (6)</th>
<th>Hot chocolate (7)</th>
<th>Spec. milk (9)</th>
<th>Hard cheese (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>65-79</td>
<td>Hard cheese (5)</td>
<td>Skim milk (7)</td>
<td>1% milk (8)</td>
<td>2% milk (10)</td>
<td>Hard cheese (1)</td>
<td>1% milk (2)</td>
<td>Skim milk (3)</td>
<td>2% milk (4)</td>
<td>Yogurt (6)</td>
<td>Hot chocolate (7)</td>
<td>Spec. milk (9)</td>
<td>Hard cheese (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80+</td>
<td>2% milk (7)</td>
<td>Hard cheese (8)</td>
<td>Hard cheese (2)</td>
<td>2% milk (3)</td>
<td>Whole milk (4)</td>
<td>Skim milk (5)</td>
<td>1% milk (6)</td>
<td>Ice cream (7)</td>
<td>Yogurt (9)</td>
<td>*Inst. Brkfast. (5)</td>
<td>2% milk (10)</td>
<td>Hard cheese (3)</td>
<td>*Inst. Brkfast. (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Instant breakfast is usually prepared with milk although it is not counted as a dairy product.
Table 3
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>
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</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>

Figure 1

Percent of Milk Users in the Utah Population 50 Years of Age and Older

People having no preference of type of milk consumed is Designated As “Mixed Milk”
Figure 2

Percent of Men and Women 50 Years and Older in Utah Consuming Various Dairy Foods

- Hard cheese
- Milk, all types
- Ice cream
- Cottage cheese
- Butter
- Yogurt
- Hot chocolate
- Froz yogurt
- Cream cheese

Figure 3

Percent of People in Utah Using Liquid Supplements by age Group

- % of Users
- % Total Sample

Age groups
- 50-64
- 65-79
- 80+
Figure 4

Percent of Population Ages 59 to 79 Years*
Using Supplements by Type in Two Populations, Utah and NHANES III

* Age and gender adjusted

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

2. Significant Conclusions:
The survey of older people in Utah provides the only state and local data on the use of dairy products and provides the only state-wide data set designed to explore the effects of dairy products on health and as risk modifiers in diet related chronic diseases. This data is not included in the National Health and Nutrition Examination Survey, the only national survey that includes data on health and diet. As of 1999, the NHANES series was combined with the USDA Continuing Survey Food Intakes of Individuals (CSFII) to make one national surveillance system. The data collected in the Utah survey can be compared directly to the national survey because it was derived directly from the NHANES III.
Since dairy products are consumed in quantities great enough to meet the needs of most 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%).

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 is being adjusted to increase the upper age limit of people selected from driver's license files but this source will limit the response from the oldest group because the number of people from this old age cohort in the Driver's license database decrease with age. The adjusted sample is being finalized and the questionnaire packets will be sent out early in September.

Published Abstract:
Vitamin A Intakes in Two Populations

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 RE/day for women and 347 RE/day for men. The mean intake from food sources alone is 2113 RE/day (246% RDA) for Utah women compared to 953 RE/day (119%) for women in Geneva. For Utah men, the vitamin a intake is 2001 RE/day (200% RDA) verses 1018 RE/day (102%) for men in Geneva. (US means for women = 1170 RE/day, men = 1419 RE/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

Presentations:

* 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

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</tbody>
</table>

Visitors Hosted:
Judith Hallfrisch, Ph.D., Senior Scientist, Human Nutrition Research Center, Cecile Delhumeau, Ph.D., Post Doctoral Student, University of Grenoble, France, Biostatistics
Western Dairy Center

Project Report
Reporting Period January 1, 2001 — December 31, 2002

Principal Investigators: Barry Swanson, WSU
Co-Investigators: Joseph Powers, WSU
Stephanie Clark, WSU
Lloyd Luedecke, WSU

Project Title: Nonthermal attenuation of Lactobacilli to accelerate cheese ripening

Institution’s Project #: 01125

Project Completion Date: June 30, 2003


Modifications to Project/Budget: n/a

Project Objectives: (Include any revisions to objectives) (Revised 02.28.02)

1. Determine the relative increase in protease, aminopeptidase and flavor development in full and low fat Cheddar cheese resulting from high hydrostatic pressure (HHP) attenuation of Lactobacillus adjunct cultures; and

2. Determine the potential for HHP attenuation of adjunct Lactobacilli to accelerate proteolysis and intensify the flavor of full fat and low fat Cheddar cheese during reasonable aging times.

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

The ultra high pressure attenuation of Lactobacillus helveticus W260R successfully retarded acid production by 8 h and enhanced aminopeptidase specific activities. Experimental full fat and low fat Cheddar cheeses were manufactured with and without Lactobacillus helveticus strains WSU19, W260R or high pressure attenuated W260R adjunct cultures. High pressure attenuation of Lactobacillus helveticus strain W206R did not enhance autolysis of W260R in Cheddar cheese. At 18 wks all cheeses contained ~ 10^4 CFU Lactobacilli /g regardless of adjunct. Proteolysis in full fat aged cheeses containing high pressure treated W260R was less than proteolysis in full fat cheeses containing control W260R. Sensory evaluation of aged cheeses demonstrated that cheeses containing adjuncts were less bitter than cheeses without adjunct cultures. No significant differences in flavor were noted among Cheddar cheeses made with untreated or high pressure attenuated adjunct Lactobacilli helveticus cultures.
Significant Progress against Objectives:

Experiments conducted since April 2002:
1. Microbiology assay of experimental Cheddar cheeses.
2. Proteolysis and aminopeptidase assays in cheese.
3. Sensory analyses of Cheddar cheeses.

Objectives for the experiments:
1. Evaluate the rates of selected high hydrostatic pressure treatments of *Lactobacillus helveticus* W260R autolysis, proteolysis, and aminopeptidase activity during Cheddar cheese ripening; and
2. Assess sensory characteristics in Cheddar cheese made with the selected high pressure treated *Lactobacillus helveticus* W260R adjunct culture.

Results:

Table 1. Proximate analysis of experimental Cheddar cheeses.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Moisture (wwb)</th>
<th>TS</th>
<th>Fat (wwb)</th>
<th>Fat (dwb)</th>
<th>Protein (wwb)</th>
<th>Salt (wwb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Fat 98</td>
<td>35.5</td>
<td>64.5</td>
<td>34.5</td>
<td>53.3</td>
<td>25.1</td>
<td>1.22</td>
</tr>
<tr>
<td>98 - WSU19</td>
<td>34.9</td>
<td>65.1</td>
<td>35.3</td>
<td>54.2</td>
<td>24.0</td>
<td>1.40</td>
</tr>
<tr>
<td>98 - W260R</td>
<td>37.0</td>
<td>63.0</td>
<td>34.6</td>
<td>54.9</td>
<td>23.0</td>
<td>1.29</td>
</tr>
<tr>
<td>98 - W260R HHP</td>
<td>36.1</td>
<td>63.9</td>
<td>34.8</td>
<td>54.5</td>
<td>23.5</td>
<td>1.25</td>
</tr>
<tr>
<td>Low Fat 98</td>
<td>39.1</td>
<td>60.9</td>
<td>24.3</td>
<td>39.9</td>
<td>30.3</td>
<td>1.39</td>
</tr>
<tr>
<td>98 - WSU19</td>
<td>38.0</td>
<td>62.0</td>
<td>24.3</td>
<td>39.2</td>
<td>30.6</td>
<td>1.71</td>
</tr>
<tr>
<td>98 - W260R</td>
<td>38.1</td>
<td>61.9</td>
<td>24.4</td>
<td>39.4</td>
<td>31.0</td>
<td>1.60</td>
</tr>
<tr>
<td>98 - W260R HHP</td>
<td>38.2</td>
<td>61.8</td>
<td>24.3</td>
<td>39.3</td>
<td>30.4</td>
<td>1.78</td>
</tr>
</tbody>
</table>

Values are average of duplicate analyses from duplicate cheese (except pH).

Values in a column followed by the same letter are not significantly different (p<0.05)

1. TS: total solids
2. S/M: salt in moisture
3. wwb: wet weight basis
4. dwb: dry weight basis

The Cheddar cheeses were analyzed one day after manufacture. Addition of starter culture with or without adjunct did not affect cheese physical composition (Table 1). However, as expected, reducing fat in milk significantly affected cheese composition. LF cheese exhibited higher moisture, protein, and salt content than FF cheeses. Fat was replaced by moisture resulting in higher protein concentrations in LF cheese than in FF cheese. LF cheeses exhibited slightly higher pH than FF cheese.

Microbiological assays of cheese

Table 2. Lactococci in FF Cheddar cheese (log CFU/g cheeses) on M17 media
The number of lactococci in experimental cheeses was measured on M17 Lactose medium. Initially, experimental cheeses contained similar amounts of lactococci (Table 2, Figure 1). The numbers of lactococci in experimental cheeses decreased throughout the ripening period. In general, cheeses containing no adjunct exhibit larger lactococci numbers than cheeses with adjunct. At 28 weeks aging time, lactococci populations in FF and LF cheeses without adjunct were $10^4$ and $\sim10^5$ CFU/g, respectively. FF cheeses with adjunct cultures contained lactococci numbers $<10^4$ CFU/g. Fat contents did not greatly affect number of lactococci in cheese containing W260R adjunct (Table 2, Figure 1). At 28 weeks aging, FF and LF cheese with W260R exhibited $4 \times 10^5$ and $5 \times 10^5$ CFU/g, respectively. A similar trend was also observed in cheese containing treated W260R.

Figure 1. Enumeration of lactococci from (A) Full fat Cheddar cheese and (B) Low fat Cheddar cheese on M17 Lactose media during ripening with or without addition of Lb. helveticus WSU19, W260R, or HHP treated W260R. Plates were incubated at $25^\circ$C for 4 d. Each point represents the log CFU/g of average of duplicate analyses of duplicate cheeses.
A. Lactococci in FF cheese

B. Lactococci in LF cheese
Table 3. Lactobacilli in Cheddar cheese (log CFU/g cheese)

<table>
<thead>
<tr>
<th>Fat</th>
<th>Culture</th>
<th>Aging time (week)</th>
<th>log CFU/g cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FF 98 - WSU19</td>
<td>7.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>98 - W260R</td>
<td>8.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>98 - W260R HHP</td>
<td>8.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LF 98 - WSU19</td>
<td>7.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.24&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>98 - W260R</td>
<td>7.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>98 - W260R HHP</td>
<td>7.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are average of CFU on duplicate plates from duplicate cheese batches. *a,b,c, Means in a column for each fat level followed by same letter are not significantly different (p<0.05).

Statistical analyses on FF and LF cheeses were performed separately.

Initial populations of lactobacilli in cheeses containing WSU19 and W260R were similar (Table 3). The numbers generally decreased throughout the ripening (Figure 1). An exception was in cheeses containing WSU19 in which lactobacilli increased slightly at 28 weeks. It was expected that lactobacilli populations in cheeses containing WSU19 or HHP-treated W260R would decrease more rapidly than populations in cheeses containing untreated W260R. However, the results indicate that untreated W260R populations decreased as fast as WSU19 and HHP treated W260R. In LF cheeses, W260R decreased in number faster than WSU19 and HHP treated W260R throughout 18 weeks ripening. After 18 weeks of aging, in FF cheese, lactobacilli populations were not significantly different among cheeses containing different adjuncts. At the end of aging time (28 weeks), cheeses containing HHP treated W260R exhibited significantly lower lactobacilli populations than cheese containing WSU19 or untreated W260R adjunct culture.

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Aminopeptidase activities in Cheddar cheese

PepN exhibits broad specificities on oligopeptides and generate di-tripeptides, and amino acids. At time zero, cheeses containing WSU19 and untreated W260R exhibited a greater PepN activity than cheeses containing no adjunct or HHP treated W260R (Table 4). An exception was in FF cheeses containing WSU19. Cheeses with no adjunct showed a lesser increase in PepN activity throughout ripening, suggesting that adjunct bacteria contributed to the PepN activities in cheese. Most cheeses with adjunct exhibited a big increase of PepN activity after weeks ripening time and exhibited PepN activity throughout 22 weeks.
ripening. An exception was FF cheese made with W260R in which PepN activity decreased after 18 weeks aging. At 0 week, cheeses contained ~10⁸ lactobacilli/g. At 4 weeks aging time, reductions of 1-2 log of lactobacilli populations was observed during the first month of cheese ripening that represents lyses of 9 x 10⁷/g lactobacilli cells. At 18 weeks ripening, FF cheeses containing WSU19 adjunct exhibited ~10⁴ lactobacilli/g and decreased to 5 x 10³ CFU/g that represented lyses of 5 x 10³/g lactobacilli cells. A decrease of PepN activity after 22 weeks ripening time may indicate that some PepN were inactivated during extended ripening time of cheese, or the number of cells that lysed decreased during ripening.

Table 4. PepN activity in Cheddar cheese

<table>
<thead>
<tr>
<th>Fat</th>
<th>Culture</th>
<th>Aging time (week)</th>
<th>Nanomoles/h/g cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FF</td>
<td>98</td>
<td>188.9b</td>
<td>126.8b</td>
</tr>
<tr>
<td></td>
<td>98-WSU19</td>
<td>191.3b</td>
<td>934.7a</td>
</tr>
<tr>
<td></td>
<td>98-W260R</td>
<td>370.3a</td>
<td>980.7a</td>
</tr>
<tr>
<td></td>
<td>98-W260R HHP</td>
<td>119.3b</td>
<td>436.9ab</td>
</tr>
<tr>
<td>LF</td>
<td>98</td>
<td>106.4b</td>
<td>190.4c</td>
</tr>
<tr>
<td></td>
<td>98-WSU19</td>
<td>425.5a</td>
<td>505.2b</td>
</tr>
<tr>
<td></td>
<td>98-W260R</td>
<td>346.8a</td>
<td>1226a</td>
</tr>
<tr>
<td></td>
<td>98-W260R HHP</td>
<td>82.3b</td>
<td>436.8b</td>
</tr>
</tbody>
</table>

Values are average of duplicate assays of duplicate cheese batches. *a,b,c Means in a column for each fat level followed by same letter are not significantly different (p<0.05). Statistical analyses on FF and LF cheeses were performed separately.
Cheese pH (± 5.0) and NaCl (1.2 – 1.8 %) content may have decreased PepN activities from WSU19 more than PepN from W260R. The increase of PepN activities in FF cheeses paralleled the decrease in cell numbers (Figure 13A). Lactobacilli cell lysis decreased after 18 weeks ripening (except for HHP treated W260R). In LF cheeses, lactobacilli continually lysed until the end of ripening time (28 weeks) (Figure 13B). However, PepN in LF cheeses beyond 22 weeks decreased, suggesting that the cell numbers on MRS was not related to the release of PepN into LF cheeses. A possibility include the cells lysed but maintained their rod shape, avoiding the complete release of PepN into cheeses. PepN released by cells beyond 18 weeks may be unstable in cheese.

PepX exhibits specificity towards dipeptides that contain proline that are abundant in β-casein (Hutkins 2001). PepX activities in cheese exhibited similar trends to PepN (Table 5). PepX activity in cheeses containing W260R adjunct were greater than PepX activities in other cheeses. HHP treatments of W260R resulted in a decrease of PepX activities in cheeses. Throughout aging time, FF and LF cheeses containing adjuncts exhibited greater PepX activities than cheeses without adjunct. The results suggested that Lb. helveticus adjuncts are better PepX producers than the starter used.

Table 5. PepX activities in Cheddar cheese

<table>
<thead>
<tr>
<th>Fat</th>
<th>Culture</th>
<th>Aging time (week)</th>
<th>nanomoles/h/g cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FF</td>
<td>98</td>
<td>585.3a</td>
<td>274.3b</td>
</tr>
<tr>
<td></td>
<td>98 - WSU19</td>
<td>452.4c</td>
<td>803.4a</td>
</tr>
<tr>
<td></td>
<td>98 - W260R</td>
<td>740.5a</td>
<td>944.0a</td>
</tr>
<tr>
<td></td>
<td>98 - W260R HHP</td>
<td>531.3bc</td>
<td>414.4b</td>
</tr>
<tr>
<td>LF</td>
<td>98</td>
<td>561.2bc</td>
<td>230.9c</td>
</tr>
<tr>
<td></td>
<td>98 - WSU19</td>
<td>932.2a</td>
<td>741.4a</td>
</tr>
<tr>
<td></td>
<td>98 - W260R</td>
<td>692.2ab</td>
<td>809.6a</td>
</tr>
<tr>
<td></td>
<td>98 - W260R HHP</td>
<td>365.4c</td>
<td>526.2b</td>
</tr>
</tbody>
</table>

Values are average of duplicate assays of duplicate cheese batches
*a,b,c, Means in a column for each fat level followed by same letter are not significantly different.
However, PepX activities did not always increase throughout the ripening period (Figure 15). In FF cheeses, rapid increases of PepX activities were observed after 4 weeks ripening in cheeses containing WSU19 and control W260R (from 452 to 803 and from 740 to 944 nanomoles/h/g, respectively). Beyond 4 weeks, activities either increased or decreased slightly. In FF cheeses without adjunct or with HHP treated W260R, greatest PepX activities were observed at 0 week ripening time (585 and 531 nanomoles/h/g cheese, respectively) and decreased throughout ripening. The results suggest that PepX from starter culture is unstable in experimental cheese. PepX activities in FF cheeses containing WSU19 and W260R did not decrease markedly after 22 weeks, possibly due to continuous cell lysis contributing to a higher availability of PepX in cheese. However, even when HHP treated W260R exhibited continuous lysis, PepX activities in cheeses never reached the 0 week value (531 nanomoles/h/g cheese). The results indicated that HHP did not markedly reduce PepX activity at initial ripening, but may alter PepX stability during cheese ripening.

In LF cheeses, PepX activities in cheeses containing WSU19 decreased throughout the first 18 weeks ripening before increasing at 22 and 28 weeks. The decrease in PepX activities suggested that PepX from WSU19 was less stable in LF cheese than in FF cheeses. There is no clear explanation why PepX activities increased only in LF cheeses with WSU19 adjunct beyond 22 weeks. However, an increase of lactobacilli cells was observed in LF cheese containing WSU19 adjunct at 28 weeks ripening time that may indicate the growth of thermophilic NSLAB. Crow and others (1995) summarized that adventitious NSLAB may utilize lysate of starters for nutrients and start to grow after 3 mo of ripening time. In this study, no specific enumeration of NSLAB was conducted to confirm the growth of NSLAB in experimental cheeses. Johnson (2001) mentioned that addition of Lactobacillus adjunct helps to control the growth of NSLAB, at least initially. However, depending on the strain, the adjunct lactobacilli may die or may not compete well against NSLAB that change microorganism populations as the cheese matures (Johnson 2001). In our experimental LF cheeses, WSU19 may not compete well with NSLAB (after 18 wk). When NSLAB cells lyse, some of the intracellular PepX activity may have contributed to the increased of PepX activities at 28 wk ripening time.

**Proteolysis in Cheddar Cheese**

The products of proteolysis in experimental cheeses was analyzed as total free amino groups (FAG) obtained from 12% TCA/TNBS (Kuchroo and others 1982). All cheeses exhibited increasing numbers of FAG throughout ripening (Table 6). Cheeses without adjunct had the smallest FAG. However, it was obvious that starter culture was capable of hydrolyzing caseins into peptides and amino acids. O'Reilly and others (2002) observed that Lactococcus lactis strains 303, 227, 223 and AM2 starters were capable of producing free amino acids of 801, 1116, 689, and 787 µg/g, respectively after 1 mo ripening.

Addition of adjuncts significantly increased proteolysis in Cheddar cheese. In FF cheeses 14 wk and older, WSU19 and control W260R adjuncts
contributed to the greatest FAGs, exhibiting 2091 and 2241 mM Gly/g, respectively at 18 wk. However, control W260R in LF cheeses exhibited greater FAG than WSU19 adjunct (except at 28 weeks). At 18 wk, LF cheeses containing WSU19 or control W260R had 2443 and 2909 mM Gly/g, respectively. In general, FAGs in LF cheeses were greater than FAGs in FF cheeses. Greater protein content (Table 6) in 1 d old LF cheeses might be responsible for the greater accumulation of free amino groups in LF cheeses. Larger amount of casein provides larger amount of substrate for proteolysis by starter and adjunct bacteria in LF cheeses than proteolysis in FF Cheddar cheese.

In this study, proteolytic activity during ripening as measured by FAG production, decreased in cheeses containing HHP treated W260R. In LF specifically, proteolytic activity decreased from the beginning of ripening period. At 0 wk, LF cheeses containing control W260R and HHP treated W260R showed FAGs of 143 and 88.2 mM Gly/g, respectively. HHP attenuation may alter activities of cell wall related proteinase. However, greater fat content in FF cheeses may help cells to recover after HHP application. There is no clear relationship between FAG numbers and lactococci cell numbers. For example, in 28 wk old FF cheeses without adjunct, the number of lactococci cells slightly increased after 22 wk, but FAG increased from 1621 to 2268 mM Gly/g. However, in FF cheeses containing HHP treated W260R, lactobacilli cells decreased from ~10^6CFU/g (18 weeks) to ~10^2CFU/g (22 wk) followed by a FAG increase from 1527 to 2074 mM Gly/g cheese.

PepN and PepX activities decreased in cheeses after 20 wk ripening. However, FAG numbers continued to increase throughout ripening. The observations indicate that there were some peptidases not analyzed in this study, which continued to degrade oligopeptides into peptides and amino acids. Smaller rate of FAG production after 18 wk ripening than in earlier aging time was observed. Slowing down of FAG production is possibly due to a smaller number of lysed cells during late ripening (> 18 wk) and resulted in small degradations of peptides in cheese.

From PepN, PepX and proteolysis analyses, cheeses containing control W260R exhibited generally greater activities than other experimental cheeses. The results indicate that the cheeses with control W260R may have increased flavor intensity. However, cheeses with increased proteolysis may have unpleasant off-flavors and body defects (Fox 2000). Consequently, chemical analyses need to be accompanied by sensory study to evaluate cheese quality.
Table 6. Free Amino Groups in Cheddar cheese

<table>
<thead>
<tr>
<th>Fat</th>
<th>Culture</th>
<th>Aging Time (wk)</th>
<th>mM in Glycine equivalent/ g cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>FF</td>
<td>98</td>
<td>81.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>156.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>98 – WSU19</td>
<td>76.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>271.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>98 – W260R</td>
<td>86.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>303.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>98 – W260R HHP</td>
<td>87.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>238.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LF</td>
<td>98</td>
<td>78.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>272.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>98 – WSU19</td>
<td>131.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>316.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>98 – W260R</td>
<td>143.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>414.0&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>98 – W260R HHP</td>
<td>88.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>240.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: numbers are average from three readings of duplicate cheeses.
*a,b,c,d Means in a column that followed by same letter are not significantly different (p<0.05)

**Sensory analysis of Cheddar cheese**

In general, all cheeses containing an adjunct culture were less bitter than cheeses that did not contain an adjunct culture. Adjunct cultures also helped to develop Cheddar flavor faster in FF cheeses. Control W260R significantly reduced the weak body defect in LF Cheddar cheese and facilitated flavor development at the similar level to WSU19 adjunct culture. HHP did not significantly decrease the ability of W260R adjunct culture to reduce bitterness. However, HHP decreased the ability of W260R to reduce weak body defect and to develop sweet flavor in cheese.

**Future Experiments:**
1. Screen selected primary and adjunct Cheddar cheese cultures for a) rate of acid production; b) rate of autolysis; and c) aminopeptidase and proteolysis activities;

2. Identify and validate improved cell lysis detection methods; and

3. Develop a model system that will simulate Cheddar cheese to assay culture autolysis and proteolysis.

2. Significant Conclusions:

The current study demonstrated that HHP at 410 MPa for 20 min at ambient temperature did not reduce cell viability in Cheddar cheese during ripening. The treatment decreased AP and proteolytic activity in Cheddar cheese during ripening. However, with several exceptions, sensory study did not exhibit significant differences among cheeses made with control W260R or HHP treated W260R. Control W260R contributed to flavor development in Cheddar cheese during ripening without attenuation.

3. Anticipated Problems/Delays: Washington State University received an extension of the research project end date until June 30, 2003. Receipt of the extension has greatly facilitated continuation of research experiments.

Theses:


Published Abstract:

Western Dairy Center
Final Project Report
Reporting Period January 1, 2001 to December 31, 2002

Principal Investigators: J.A. Torres, OSU

Co-Investigators: D.F. Farkas, OSU
G. Vel-zquez, OSU, Mexico
J. Salas, CICATA, Mexico
M. McDaniel, OSU
E. Morales, U. Austral, Chile
J.A. Ramírez de LeUn, U.A. Tamaulipas, México
J. Serrano, OSU & U.A. QuerEtaro, México
R. Alfaro, OSU & I.T.E.S. de Monterrey, México

Project Title: Shredded Cheddar cheese: accelerating shreddability by moderate hydrostatic pressure (MHP)

Institution’s Project #: 2002-2-13-12-02

Project Completion Date: 12/31/02


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

Objective 1: Determine influence of moderate hydrostatic pressure (MHP) treatments on the mechanical properties of Cheddar cheese including shreddability.

HYPOTHESIS/TASK: Commercial units for high pressure processing operate in the 40,000 to nearly 100,000 psi pressure range. Complex construction technologies (e.g., wire-wound pressure vessels) and material strength constraints increase the cost and limit the size of pressure vessels capable of exceeding 60,000 psi. Our previous work had shown that MHP treatments of fresh curd under this pressure limit yield immediately cheese with a microstructure similar to aged Cheddar cheese. Our work hypothesis for this project was that MHP treatments could shorten the storage needed before shredding Cheddar cheese, an opportunity that we were uniquely qualified to evaluate using our large 20-liter pressure vessel and close collaboration with Avure Technologies and Tillamook County Creamery Association. Mechanical properties of Cheddar cheese for shredding: We determined the pressure effect on the mechanical properties of Cheddar cheese by compression and TPA tests. Characterization of shredded Cheddar cheese: MHP-treated,
control and commercial shredded cheese were analyzed for moisture and chemical composition (fat, protein, proteolysis, pH and salt). Characterization by particle size/shape and meltability indicators could not be reliably assessed as planned because the image analysis software/hardware setup will need further development work. Stability of the setup made it impossible to obtain reliable values.

Objective 2: Sensory analysis of shredded Cheddar cheese

HYPOTHESIS/TASK: Objective measurements of shreddability were combined with sensory parameters to generate a full characterization of shredded Cheddar cheese as affected by MHP treatments. MHP-treated, control and commercial shredded cheese were analyzed by descriptive analysis sensory methods focusing on appearance and consumer use factors during the first 30 days after cheese production and pressure treatment. Pressure effects on flavor are expected to be none or positive but this was not confirmed experimentally.

Objective 3: Recommend MHP treatments to reduce the production costs of shredded Cheddar cheese

HYPOTHESIS/TASK: A survey of shredded Cheddar cheese in U.S. markets showed large differences in the characteristics of the products on the market. Quality assurance managers for commercial shredded cheese producers were phone interviewed on the desirable properties of cheese for shredding and the characteristics of the shredded cheese obtained. This industry information and the experimental data obtained guided our selection of MHP treatments. Consumer tests of melted and shredded MHP-treated Cheddar cheese for ease of use, appearance and flavor were not conducted. Instead, experimental work was expanded to include stirred curd Cheddar cheese produced by Glanbia Foods in Idaho in addition to milled curd Cheddar cheese produced at Tillamook County Creamery Assoc. in Oregon. This allowed us to demonstrate that the pressure effect on cheese microstructure applies to cheeses produced by these very different technologies.

Project Summary:
Cheese production in the United States has increased to ~730 million tons/month due in significant part to the demand for natural shredded cheese. There is great consumer interest in shredded cheese not only in the US but also elsewhere in the world. Shredded cheese has become the most common ingredient cheese sold through U.S. retail, food-processing and foodservice channels. Many natural cheeses can be shredded but storage costs before a given variety reaches shreddability are significant. These costs include refrigerated storage space and inventory keeping which require a large capital investment. In previous work, we had discovered that moderate pressure treatments applied to fresh curd yield immediately a microstructure similar to aged Cheddar cheese. In this project we determined that these pressure treatments and microstructure effect can eliminate the storage needed before shredding Cheddar cheese. We were uniquely qualified to evaluate this opportunity as our pilot plant is equipped with a 20-liter pressure vessel which was ideal to prepare the large
number of samples required for sensory and objective measurements. In addition, we worked closely with Avure Technologies (formerly Flow International Inc., Kent, WA) and used their commercial high-pressure prototype units whenever appropriate. Relatively little information has been published on the mechanical properties making cheese blocks suitable for shredding. Texture measurement procedures found in the literature differed in test parameters and those had to be evaluated to find the most adequate conditions for our experimental samples. Also missing was a definition of consumer-acceptable shredded cheese. This information was generated by a trained descriptive sensory panel that examined the appearance of pressure treated, control and commercial shredded Cheddar cheese. Objective measurements of shreddability include a computerized vision system (Figure 1) built by co-PI Salas and microstructure determinations by electron microscopy. Unfortunately our experimental vision unit had instability problems and we could not characterize the size/shape of the shredded cheese nor use its area measurement tool to assess cheese meltability.

Examination of the first runs in our experimental design led to an important project revision: (1) project scope expansion by including Cheddar cheese manufactured by milled and stirred curd technologies; (2) pressure treatment of cheese blocks collected right after cooling which were shredded immediately and after storage for up to 27 days; (3) selection of pressure treatments of 3 and 7 minutes at 50,000 and 70,000 psi to include moderate pressure levels below and slightly above the 60,000 psi pressure technology barrier; (4) improvement of the evaluation of mechanical properties; (5) improvement attempts to solve the image analysis software stability problems. Trained sensory panel evaluations following the modified project procedures demonstrated that MHP can streamline the production of shredded cheese. Cheese blocks coming from the cooling unit can be pressure treated, shredded and packaged immediately for distribution to consumers. Most importantly, this technology will work with relatively low pressure (i.e., 50,000 psi) and short time (i.e., 3 minutes or less). Furthermore, the properties of pressure treated and control samples are practically identical after 15 days of refrigerated storage meaning that we can accelerate and streamline the production of shredded cheese with the properties known to be acceptable to consumers. Conclusions based on the trained sensory panel observations were confirmed by objective measurements.

1. Completed Objectives:
Commercial Cheddar cheese samples were used for the development of experimental procedures required for this project. This development included (1) construction of an optical and sample handling set up for the image analysis of shredded cheese particles and the evaluation of cheese melting which will need further refinements to solve computer stability problems affecting data acquisition reliability; (2) training of a descriptive sensory panel and the development of a sample evaluation ballot; and (3) texture measurement and data analysis procedures. These methods were applied for a first experimental run using 3-day old blocks of Cheddar cheese obtained from the Oregon Tillamook County Creamery Association.
All experimental procedures worked well except the image analysis system. The sensory analysis was conducted on two shred sizes but this was found to be unnecessary to see the effect of pressure on shreddability. It was also found to be overwhelming to the panelists. A second experimental run used 0-day old blocks obtained from the same plant (milled curd Cheddar) and from the Glanbia Foods plant in Twin Falls, Idaho (stirred curd Cheddar). The treatments were run using two blocks from the same production batch. The rationale to run samples produced by these two technologies was that quality managers at shredding cheese plants had observed differences in the quality of the shreds obtained. Day 0 was chosen as an earlier pressure treatment would provide a commercial advantage. A wire cheese cutter was used to cut Cheese blocks into 18x9x3 cm chunks packaged and vacuum-sealed at the plant (first run) or the pressure processing facility (second run). These chunks were pressure treated at Avure Technologies (formerly Flow International Inc., Kent WA) and transported in ice coolers with ice packs to our OSU laboratory where they were stored at 34 o°F. Two sets of samples obtained randomly from the same cheese blocks were subjected to pressure treatments or left as untreated controls. The first set was used for chemical analysis, microstructure determinations by scanning and transmission electron microscopy (day 1 and 4), and determination of mechanical properties and evaluation of cheese meltability. The second set was used to prepare shredded samples that were analyzed by a digital image analysis set up and a trained descriptive sensory analysis panel. Shredding and all measurements (except when noted) were done after 1, 6, 17, and 27 days of storage.

1) Measurements on shredded Cheddar cheese
Two shredder plates (3/32" and 3/16") were used on the Hobart AS200T (The Hobart Mfg. Co., Troy, Ohio) to produce thick and thin (first run of this study) or only thick (second run of this study) shredded cheese particles similar to the ones most frequently produced commercially for instrumental and sensory analysis methods. A trained panel evaluated commercial and experimental samples of shredded Cheddar cheese to find attributes useful to evaluate the effect of pressure treatments. A copy of the ballot developed was presented in a previous report.

2) Chemical Analysis
The chemical composition of MHP-treated milled and stirred Cheddar cheese was determined at 1, 13, and 27 d. As expected, the moisture, fat, protein and sodium contents in milled and stirred curd (Table 1) were not significant different among the pressure treatments and did not change with storage time.
Table 1.
Milled and stirred curd Cheddar cheese composition at different storage time

<table>
<thead>
<tr>
<th>Composition</th>
<th>milled curd</th>
<th>stirred curd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 13</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>28.4 ± 0.4</td>
<td>28.1 ± 2.2</td>
</tr>
<tr>
<td>Fatt (%)</td>
<td>35.2 ± 0.7</td>
<td>35.5 ± 0.4</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>30.8 ± 1.2</td>
<td>32.8 ± 1.2</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>193.5 ± 2.0</td>
<td>197.5 ± 5.6</td>
</tr>
</tbody>
</table>

3) Textural Properties
Cubical 2x2x2 cm samples for textural properties were obtained using the stainless steel wire cheese cutter built specifically for this project (Figure 5). Compression tests were performed using a TA.XT2 Texture Analyzer (Stable Micro Systems, U.K.) Texture profile analysis (TPA) at 50% was performed at 1.0 mm/s of crosshead speed with ten samples analyzed for each treatment. The hardness, springiness and cohesiveness values were calculated. Data is presented in a summarized form in Table 2 (milled curd, Tillamook County Creamery Assoc.) and 3 (stirred curd, Glanbia Foods Inc., Twin Falls, ID).

Table 2.
TPA analysis of milled curd Cheddar cheese throughout ripening time.

<table>
<thead>
<tr>
<th>Texture Parameter</th>
<th>Storage (days)</th>
<th>Control</th>
<th>345 MPa</th>
<th>483 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>1</td>
<td>16.5a,1</td>
<td>15.0 a,2</td>
<td>14.1 a,2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>15.9 a,1</td>
<td>17.0 b,2</td>
<td>14.6 a,3</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>13.6 b,1,2,3</td>
<td>14.7 a,1</td>
<td>14.1 a,1,2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>13.5 b,1</td>
<td>12.0 c,2</td>
<td>11.4 b,2,3</td>
</tr>
<tr>
<td>Springiness</td>
<td>1</td>
<td>0.76 a,1</td>
<td>0.72 a,2</td>
<td>0.74 a,1,2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.77 a,b,1</td>
<td>0.74 a,1,2</td>
<td>0.77 a,b,1</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.74 a,1</td>
<td>0.74 a,1</td>
<td>0.72 a,2</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.66 c,1</td>
<td>0.73 a,2</td>
<td>0.73 a,1</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>1</td>
<td>0.46 a,1</td>
<td>0.46 a,1</td>
<td>0.48 a,1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.47 a,1</td>
<td>0.46 a,1</td>
<td>0.47 a,1</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.44 a,1</td>
<td>0.45 a,b,1,2</td>
<td>0.46 b,2,3</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.36 b,1</td>
<td>0.44 b,2</td>
<td>0.45 b,2</td>
</tr>
</tbody>
</table>

a, b, c, Mean values (n = 8) in column with different superscript letters differ (P < 0.05).
1,2 Mean values (n = 8) in rows with different superscript numbers differ (P < 0.05).
Table 3 - TPA analysis of stirred Cheddar cheese throughout ripening time.

<table>
<thead>
<tr>
<th>Texture Parameter</th>
<th>Storage (days)</th>
<th>Control 345 MPa</th>
<th>7 min</th>
<th>483 MPa</th>
<th>3 min</th>
<th>7 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 min</td>
<td>7 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.2 a,1</td>
<td>15.4 a,2</td>
<td>16.9 a,3</td>
<td>15.0 a,2</td>
<td>17.0 a,3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>14.3 a,1</td>
<td>14.2 b,1</td>
<td>13.0 b,2</td>
<td>14.2 a,1</td>
<td>14.9 b,1</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>12.3 a,b,1</td>
<td>10.5 c,2</td>
<td>11.0 c,2</td>
<td>17.7 b,1</td>
<td>12.8 c,3</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>11.3 b,1</td>
<td>10.7 c,1</td>
<td>9.8 d,2</td>
<td>11.0 c,1</td>
<td>13.2 c,3</td>
<td></td>
</tr>
<tr>
<td>Springiness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.75 a,1</td>
<td>0.77 a,1,2</td>
<td>0.81 a,3</td>
<td>0.77 a,1,2,3</td>
<td>0.79 a,2,3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.70 b,c,1</td>
<td>0.76 a,b,2</td>
<td>0.76 b,2</td>
<td>0.79 a,b,2</td>
<td>0.78 a,2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.72 a,b,1</td>
<td>0.75 a,b,1,2</td>
<td>0.75 b,1,2</td>
<td>0.82 b,3</td>
<td>0.77 a,2</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.67 c,1</td>
<td>0.73 b,2,3</td>
<td>0.71 c,2,3</td>
<td>0.76 a,3,4</td>
<td>0.78 a,4</td>
<td></td>
</tr>
<tr>
<td>Cohesiveness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.42 a,1</td>
<td>0.48 a,1</td>
<td>0.51 a,2</td>
<td>0.50 a,2,0</td>
<td>0.51 a,2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.44 a,1</td>
<td>0.48 a,1</td>
<td>0.48 a,2</td>
<td>0.50 a,2</td>
<td>0.50 a,2</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.44 a,1</td>
<td>0.48 a,b</td>
<td>0.48 a,2</td>
<td>0.50 a,2</td>
<td>0.49 a,2</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0.38 b,1</td>
<td>0.43 b,1</td>
<td>0.43 b,2</td>
<td>0.46 b,3</td>
<td>0.45 b,2,3</td>
<td></td>
</tr>
</tbody>
</table>

a, b, c. Mean values (n = 8) in column with different superscript letters differ (P < 0.05).
1,2 Mean values (n = 8) in rows with different superscript numbers differ (P < 0.05).

4) Microstructure Determinations

Scanning electron microscopy determinations were completed for samples collected at day 1 and confirmed the immediate transformation of cheese microstructure for the MHP treatments selected in this study (Figure 4). Day one was the earliest observation possible because of project logistics, i.e., cheese transportation from production facilities (Tillamook, OR and Twin Falls, ID) to MHP vessel (Kent, WA) and sample analysis location (Corvallis, OR). Untreated control cheese shows the characteristic discontinuous and porous cheese matrix while the MHP cheese (50,000 psi, 3 min) shows a more continuous matrix usually observed in aged Cheddar cheese.

5) Sensory Analysis

Stirred and milled Cheddar cheese samples showed remarkable consistency in the seven parameters chosen to characterize shredded Cheddar cheese. At day 1, control samples were found to exhibit Figure 4. SEM micrographs of MHP (50,000 psi, 3 min) cheese at day 1 a much higher presence of crumbles, had lower length uniformity and the presence of small pieces lowered its mean length. Surface examinations showed that the pressure treated samples were smoother and oilier. Finally, when handled by hand, panelists found control samples to differ from pressure treated samples in the cohesiveness of individual shred pieces and in the oily hand residue.

As storage time progressed, we observed for all treatments and all factors used by the trained panel that the difference between pressure-treated cheese and
control samples disappeared. This is an important observation as it means that Day II pressure treated samples (earliest evaluation possible) have sensory properties similar to the ones produced today by conventional methods. The sensory characteristics that consumers are finding acceptable in cheese aged for shredding are the same as the ones generated using pressure treatments and without need for aging. Also important is to note that ALL pressure treatments were equally effective; even the lower pressure, and thus lower cost alternatives, can eliminate the need for cheese storage before shredding. The rapid seafood industry acceptance for HPP technology is worth mentioning here; the rapid introduction of HPP treatments for oyster shucking reflects the effectiveness of pressures below 60,000 psi. The same low pressure requirement for accelerating shreddability could help introduce HPP into dairy plants.

Finally, pressure treatment of stirred curd Cheddar cheese samples (Fig. 6) resulted in the same effect as the one observed for milled curd samples (Fig. 5). This is another significant finding as it suggests that the microstructure change by HPP appears to be a general effect that could be used for other cheese varieties used for shredding today or identified as new products of interest to consumers. A barrier to the introduction of other shredded cheese varieties has been aging cost considerations.

2. Significant Conclusions:
We developed and implemented all relevant analytical techniques to study cheese shreddability, particularly: (1) training and development of descriptors for a trained panel for shredded cheese; (2) development of an image analysis set up to characterize objectively shredded cheese particles and cheese melting; and (3) evaluation of testing conditions for determination of mechanical properties and cheese microstructure. Most of this information was not available in the literature and publications are being prepared for dissemination to industrial users and other dairy researchers. The proposed moderate HPP treatments modified the microstructure and texture properties of Cheddar cheeses manufactured by two different technologies. These modifications were associated to a modification of protein structure without inhibiting the proteolytic activity needed to ripe the cheeses during cold storage as shown by proteolysis measurements during subsequent storage time. MHP treated cheeses showed immediately the sensory attributes observed in 27-day untreated cheese which means that we can eliminate storage as a requirement for cheese shredding. A treatment of 50,000 psi (345 MPa) during 3 min was sufficient to induce at day one the same characteristics of day-27 ripened cheese. Two full experimental runs were used to evaluate pressure treatment effects on shredded Cheddar cheese. Trained sensory panel evaluations following the modified project procedures demonstrated that MHP could be used to generate a streamlined production technology for shredded cheese. Conclusions based on the trained sensory panel observations were confirmed by objective measurements. Cheese blocks coming from the cooling unit can be pressure treated, shredded and packaged immediately for distribution to consumers. Most importantly, all pressure treatments were successful and that is important because the relatively low 50,000 psi pressure is more likely to succeed as a commercial application. Furthermore, the properties of pressure treated and
control samples are practically identical after 15 days of refrigerated storage meaning that the acceleration and streamlining of the production of shredded cheese will not change the properties known to be acceptable to consumers. Estimates for capital and operational costs for curd processing by MHP and refrigerated warehouse costs for cheese storage for pressure treatments at 50,000 psi confirmed the financial advantages of MHP-treated cheese for shredding. Expected savings are $15/1000 lb shredded cheese based on a one-month reduced storage time at refrigeration temperature. Based on our findings, a plant would need to consider other savings in the form of estimates for the savings possible from streamlining shredded cheese production. MHP treatments could eliminate the handling of cheese blocks sent to storage, retrieval from storage, and subsequent opening up of cheese boxes for shredding. Finally, the observation that MHP worked for both milled and stirred curd cheese suggests that the technology may allow the early shredding of other cheese types providing further opportunities for marketing milk.

3. Problems:
As reported above, the image vision system to measure objectively particle size was unstable and could not be used in a reliable manner. Further refinements will be necessary to solve the software stability problems.

Publications:

Theses:

Published Abstract:

Presentations:
Technology Transfer Activities
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Professor Elton Morales, Assistant Professor of Food Engineering, Universidad Austral de Chile, Valdivia, Chile

Dr. Jorge O. Bouzas, Senior Manager, Ingredients Research, Technical Center, Hershey Foods, Hershey, PA 17033
Western Dairy Center

Project Report

Reporting Period November 1, 2000 — December 31 2002

Principal Investigators: Marie K. Walsh
Charles Carpenter

Co-Investigators:

Project Title: Characterization of textured whey protein used as a meat extender

Institution’s Project #: 01127

Project Completion Date: December 31, 2001


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)
1. Characterize TWP produced from different sources of WPC and starch. In vitro measures of stability, water holding capacity, thermal stability, pH stability, fat binding, and shelf life will be done.
2. TWP produced and analyzed in Objective 1 which show similar characteristics to our current product will be further characterized in hamburger patties containing 30% TWP (g/g). In situ measures of shelf life, freeze thaw, cook yield and sensory analysis will be done.
3. Determine the nutritional profile, shelf life and market areas for TWP as a meat extender.

Project Summary: (Suitable for inclusion in Center documents released to the public)
We have previously employed thermoplastic extrusion to produce a textured whey protein (TWP) from whey protein concentrate (WPC). The TWP showed significant promise as an extender in ground beef patties. Commercial sources of WPC and starch vary, therefore this research will investigate the influence of various brands of WPC and starch on TWP performance as a meat extender. TWP samples will also be characterized with respect to thermal and pH stability, water and fat binding, shelf life and freeze thaw stability. The nutritional profile will be developed and sensory evaluation in hamburger patties will be conducted.
Significant Progress against Objectives:

Seven commercial whey sources (WPC 80) have been extruded at three protein levels (48%, 53% and 64%) as shown in Table 1. The last three sources (Land'O Lakes, Calpro and Plain View Milk) did not produce successful products at any of the three protein levels tested.

Table 1. TWP Blends and WPC Commercial Sources

<table>
<thead>
<tr>
<th>Source</th>
<th>2/1 (53% protein)</th>
<th>3/2 (48% protein)</th>
<th>4/1 (64% protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proliant</td>
<td>3/7/01 ANALYZED fibrous, stable</td>
<td>3/19/01 ANALYZED, lower water fibrous, stable</td>
<td>CURRENT ANALYZEDS fibrous, stable</td>
</tr>
<tr>
<td>Century Instant</td>
<td>4/4/01 ANALYZED some fibrous, stable</td>
<td>4/9/01 ANALYZED, 300 rpm, water equals 2.65 fibrous, stable</td>
<td>5/01 Analyzed fibrous, stable</td>
</tr>
<tr>
<td>Davisco Foods</td>
<td>4/16/01 ANALYZED, water 2.3, negative 10-30 psi fibrous, stable</td>
<td>4/19/01 ANALYZED not as fibrous as 2:1, water 2.2, -10 psi,</td>
<td>6/01 sticky, no fiber formation</td>
</tr>
<tr>
<td>Warnambool</td>
<td>3/12/01: 4/23/01 ANALYZED</td>
<td>4/25/01 ANALYZED, little fibrous texture and sticky</td>
<td>6/12/01 ANALYZED, can produce, sticky, difficult to extrude, different smell</td>
</tr>
<tr>
<td>PlainView Milk</td>
<td>3/21/01 NO ANALYSIS Expanded product</td>
<td>3/26/01 NO ANALYSIS Expanded product</td>
<td>6/15/01 ANALYSIS VERY dark, different smell</td>
</tr>
<tr>
<td>Calpro</td>
<td>4/2/01 NO ANALYSIS, no fibrous texture, sticky</td>
<td>4/3/01 NO ANALYSIS, sticky, not consistent, did not extrude well</td>
<td>6/18/01 NO ANALYSIS, product orange, foamy and non-textured</td>
</tr>
<tr>
<td>Land'O Lakes</td>
<td>49/00 NO ANALYSIS, no fibrous texture, sticky</td>
<td>9/00 NO ANALYSIS, sticky, not consistent, did not extrude well</td>
<td>not enough whey sample to evaluate in 4:1 ratio</td>
</tr>
</tbody>
</table>

The first three samples (Proliant, Century Instant and Davisco Foods) did produce suitable TWP as determined visually and Warnambool was successful at the 48% and 53% protein level.

The products produced from the first three commercial sources were analyzed for water holding capacity and % solids lost as compared to a textured soy product (TVP). This data is shown in the graphs in the appendix. On average there was a higher water holding capacity with an increase in pH and temperature with TVP having a higher water holding capacity.

The % solids lost for each of these samples ranged from 10 to 15% with TVP having values greater then 20%.

This data is being analyzed statistically for the next report.
TWP can have different markets depending on the cost which can be determined by the protein level. With a higher protein level, the TWP is more expensive but may have a different market. In order to determine the maximum and minimum amount of whey protein needed to form a TWP, TWP was extruded at the protein values listed in table 2.

Table 2. Protein Concentrations in TWP* production

<table>
<thead>
<tr>
<th>WPC/Starch*</th>
<th>Protein (%)</th>
<th>TWP product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>40</td>
<td>No fibrous texture formed</td>
</tr>
<tr>
<td>3:2</td>
<td>48</td>
<td>Fibrous texture formed</td>
</tr>
<tr>
<td>2:1</td>
<td>52</td>
<td>Original Product, Fibrous texture formed</td>
</tr>
<tr>
<td>3:1</td>
<td>60</td>
<td>Fibrous texture formed</td>
</tr>
<tr>
<td>4:1</td>
<td>64</td>
<td>Fibrous texture formed</td>
</tr>
<tr>
<td>5:1</td>
<td>66</td>
<td>Very difficult to extrude, Can get fibrous texture</td>
</tr>
<tr>
<td>6:1</td>
<td>69</td>
<td>Too difficult to extrude</td>
</tr>
<tr>
<td>9:1</td>
<td>72</td>
<td>Too difficult to extrude</td>
</tr>
</tbody>
</table>

*Proliant WPC 80 and National Melogel used for each sample

2. Significant Conclusions:
Whey protein levels from 48 to 64% can lead to the production of a stable TWP. The water holding capacity and % solids lost of TWP produced at levels from 48-64% was determined and compared to a commercial source of TVP. Of the 7 different commercial sources of WPC 80, three sources were consistent in the production of TWP at three protein levels.

3. Anticipated Problems/Delays:
None

Presentations:
Invited presentation at the 5th Symposium on Advances in Dairy Product Technology, Cal Poly
Invited presentation at the Oregon Dairy Industries Conference, Portland Oregon

Patent/Invention Disclosures:
This technology is patent pending
Western Dairy Center
Project Report
January 1, 2001 to December 31, 2002

Principal Investigators: Bart Weimer, Utah State University
Co-Investigators:

Project Title: Importance of glutamic acid and α-keto acids in cheese flavor development

Institution's Project #: 01123

Project Completion Date: December 31, 2002


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

Hypothesis: The first catabolic step for amino acids is catalyzed by aminotransferases using α-keto acids as co-factors (amino donors and acceptors) to form flavor compounds precursors in cheese (also keto acids). The most common co-factor is important in the rate of flavor development in cheese, but additional keto acids need to be studied in relation to flavor formation potential.

Objective 1: Determine the diversity of transferase reactions that use acids in LAB.
Objective 2: Determine the role of this transformation in production of cellular energy and flavor compound production from each class of amino acids (branched chain, aromatic, acidic, neutral, basic).
Objective 3: Determine the rate of product formation with the addition of keto acids in relation to the amino acid precursor concentration. (Aminotransferases are bidirectional enzymes based on the products and reactant concentrations. If this reaction increases in the forward direction it may lead to a method to accelerate cheese flavor.)
Objective 4: Determine the environmental triggers that induce each aminotransferase in lactococci and lactobacilli with transcription analysis.

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

During this study, keto acids spontaneously degraded in solution to fatty acids. All bacteria tested produced fatty acids from keto acids at levels above spontaneous degradation. Brevibacteria catabolised amino acids only in carbohydrate starvation conditions. Lactic acid bacteria utilized amino acids and keto acids to produce fatty acids above flavor threshold levels. Higher amounts of fatty acids were produced from individual amino acids than a mixture. Addition of amino acids or keto acids as substrates yielded similar fatty acids, but different quantities during the incubation time. Lactococci, lactobacilli, and an ilvE deletion mutant of Lactococcus lactis ssp. lactis LM0230 utilized precursor amino acids and their corresponding keto acids differently; yet the ilvE deletion mutant retained the ability to produce branched chain fatty acids. These results indicate that bacteria associated with cheese production metabolize amino acids to fatty acid associated with desirable flavor. Production of BCFAs by the ilvE mutant indicate that multiple aminotransferases are involved in production of branched chain fatty acids in lactococci.

Lactococci withstand long-term (months) of carbohydrate starvation and remain metabolically active in a chemically defined medium. This metabolic capability is relevant in fermented foods when lactococci are entrapped in a protein–rich matrix with low pH, high NaCl, and a low oxidation/reduction potential. The aim of this work was to determine if these characteristics are possible in different subspecies of lactococci and to examine the length of time needed to induce these metabolic shifts. The present work examined starvation profiles of Lactococcus lactis ssp. cremoris SK11 and Lactococcus lactis ssp. lactis IL1403, a lactose and protease deficient strain. IL1403 provides insights into how lactose metabolism is related to starvation and fatty acid production since it lacks the plasmids associated with these functions. Each culture survived during carbohydrate starvation, but varied in their ability to maintained their ability to divide and form colonies. However, they were determined to be viable using staining dyes and they maintained similar levels of ATP as actively growing cultures. They catabolized branched-chain amino acids to branched-chain fatty acids, an activity they do not conduct in laboratory conditions or with carbohydrate present. The intracellular aminotransferase activity was similar during incubation, but decreased. These data indicate that multiple lactococcal strains are capable of surviving and metabolizing protein substrates during carbohydrate starvation.

2. Significant Conclusions:

A diverse set of keto acid intermediates were found in all bacteria studied. Deletion of a single aminotransferase (ilvE) did not significantly reduce the total VFA’s produced, but it did change the ratios.

Metabolism of branched chain amino acids can provide energy (ATP) for survival in cheese-like conditions without a carbohydrate source.
3. Anticipated Problems/Delays: none

Publications:

Theses:
Balasubramanian Ganesan – Ph.D. in progress

Published Abstract:

Presentations:
Balasubramanian Ganesan and Bart Weimer. 2002. Fatty acid production by lacticocci during carbohydrate starvation in a chemically defined medium. American Society for Microbiology, Salt Lake City, UT.
Western Dairy Center

Project Report
January 1, 2001 to December 31, 2002

Principal Investigators: Dr. Bart C. Weimer, Utah State University
Co-Investigators: Dr. Marie Walsh, Utah State University

Project Title: Rapid detection of *Listeria* in dairy products

Institution’s Project #: 01124

Project Completion Date: June 30, 2002

Combine the patented ImmunoFlow system with PCR to obtain a final result about the presence of *Listeria monocytogenes* in the food product

**Modifications to Project/Budget:**
None

**Project Objectives: (Include any revisions to objectives)**

**Hypothesis:**
Contamination of *Listeria* in dairy products is difficult to detect and is responsible for a large proportion of deaths from foodborne pathogens. False positives are a problem in detection of this organism. Rapid detection of *Listeria* is needed to aid in limiting distribution of contaminated milk and dairy products combined with genetic verification will reduce or eliminate false positive tests.

A. Optimize addition of secondary reagents for detection of *Listeria* in raw milk and soft cheese to produce a presumptive test that takes 15 to 30 minutes.
B. Couple the presumptive result to a PCR test to determine the species (and potentially) the strain.
C. Determine the detection limit and total test time to detect *Listeria*.

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

*Listeria monocytogenes* is a pathogenic microbe that causes serious illnesses and even death. Each year, the bacteria cause at least 2,493 cases of listeriosis. Of these, 2,298 persons are hospitalized and 499 persons die. The case fatality rate is high, i.e. 20 deaths per 100 cases of illness. Although HACCP and GMP are increasingly common in food industry companies, product recalls because of the chance of contamination are still the case. *Listeria monocytogenes* remains the primary agent in those recalls (25 out of 55 in 1999). This information and the fact that this organism grows well at refrigeration temperatures, suggest that a
rapid detection technique for *Listeria monocytogenes* in food is needed.

A 2-stage test will be created. A preliminary (presumptive) result will be generated quickly (15 minutes). If the presumptive test is positive, the sample will be further processed to extract the DNA from the cells on the bead surface. This will be used in a PCR reaction to confirm the presumptive result and differentiate *L monocytogenes* from other species. The combined use of the ImmunoFlow system and PCR allows a presumptive and confirmed diagnostic test in a rapid (30 minutes) and sensitive format (~100 cells). The easy use of the system will also be a big advantage.

The basis of this system involves the use of a patented flow-through capture cartridge that binds the pathogen onto the surface for subsequent detection. In the cartridge, a flow through fluidized bed module containing large (3 mm) glass beads was created. The glass beads are coated with covalently-bound antibodies against *Listeria*. By pumping contaminated samples through the bed, bacterial cells are captured and concentrated on the beads. This lead to three advantages:

1) Larger sample volumes of milk can be tested.
2) No pre-enrichment step is needed.
3) Lower amounts of *Listeria monocytogenes* in food samples is concentrated on the beads for detection.
4) The presumptive step is automated.
5) Subsequent verification with PCR is done in an inhibitor-free environment.

1. **Significant Progress against Objectives:**

Antibodies were screened for capture and background production. A limited number of Ab were selected to optimize the concentration for the presumptive step, that provides a system with low background and good binding. An indirect sandwich ELISA was found to be a good test format to maximize signal and minimize the background.

Optimization of the covalent binding step is being done by comparing two spacers (poly-Dextran and poly-PEG). Experiments are in progress to test these in static and ImmunoFlow formats.

Cell capture efficiencies have defined a reselection of the antibody system to allow greater cell capture. This is done and the reagent concentrations are being optimized.

Methods to extract the DNA from cells attached to beads are defined to standard methods without organic extraction. Verification of this result in a full ImmunoFlow test is underway. PCR verification for the capture and lysis is underway.
2. Significant Conclusions:
A combination of antibodies in the indirect sandwich ELISA were identified. Preliminary conditions to lyse the cells and DNA extraction will influence the choice of beads.

3. Anticipated Problems/Delays:
None

Theses:
Wim Lippens – in progress

Patent/Invention Disclosures:
Some is covered in previous patents, but new IP may be generated in the last portion of the work.

Technology Transfer Activities
For information on licensing contact:
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