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2006 Annual Report

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Western Dairy Center
Annual Report
2006

Western Dairy Center
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# Western Dairy Center
## Annual Report 2006

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Western Dairy Center

Introduction

The Western Dairy Center is a consortium of researchers devoted to improving the dairy industry in the United States, and specifically in the Intermountain West, by conducting research in all areas of dairy foods. The Center includes 15 researchers from Utah State University, University of Idaho, and Oregon State University, who are nationally know experts in the area of dairy processing, chemistry, microbiology, safety, proteins, and sensory evaluation. The Western Dairy Center has a fully operational, USDA inspected dairy processing plant, equipped with resources to conduct cutting-edge research and product development.

The Intermountain West accounted for approximately 16% of the US milk production in 2005. Several of the states in this area (Idaho, Arizona, Colorado, New Mexico, Oregon) have seen grater than a 40% increase in milk production since 1998. In addition to the increased milk production in the Western US, there has been a concomitant increase in cheese production. Specifically, Idaho is ranked fifth in the nation for total cheese production, which is a 50% increase since 1998, while Utah ranks eleventh for total cheese production. The combined states of Idaho and Utah are home to over 40 dairy manufacturing plants.

There is a need to support the increased milk and cheese production in the Intermountain West by utilizing the Western Dairy Center to resolve research and technical issues. To do so, the Center will enhance its services and focus in areas that will be most beneficial to the industry. The initial areas, to receive attention are consumer and descriptive sensory analysis, and new product development. Other functions dealing with processing, quality and co-product utilization will be addressed as needed.

The Western Dairy Center has defined three research initiatives to meet the growing milk and cheese production in Western US. The projects listed in these initiatives will need consistent DMI support to allow continued growth of the dairy industry in the region, and to allow industrial adoption of the research.

Western Dairy Center Research Initiatives

1. Discovery Initiative. This initiative will focus on novel research related to dairy products including cheese and its co-products (e.g. lactose, whey proteins, and whey permeate).

Projects currently active in this category include:
- Development and characterization of lactose hydrogels
- Synthesis, characterization and bioactivity of lactose lauryl esters
- Characterization of milk mineral as an antioxidant
- Characterization of levulinic acid as an antimicrobial agent in dairy foods
- Development of low fat cheese
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:

Eric Bastian
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Gulhan U. Yuksel
University of Idaho
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2. Technology Transfer Initiative. The Technology Transfer Initiative will focus on expanding the use of the technologies developed in the Discovery Initiative into other dairy products and/or other food systems. The Technology Transfer Initiative will rely on product development in the USU Dairy Products Laboratory, descriptive sensory analysis, and consumer acceptance panels. An objective of this initiative is to develop prototypes of dairy products from the research conducted in the discovery program.

Possible projects to include in this initiative are:
- Expand the use of textured whey protein containing added fiber as an extender in products including fermented sausages and comminuated meat products.
- Expand the use of milk mineral into meat systems other than beef and into high fat dairy products.
- Support the evaluation and production of low fat Cheddar cheese.
- Add probiotics to cheese.
- Development of omega-3 fortified reduced fat Cheddar cheese.

3. Technology Support Initiative. The Western Dairy Center can provide technical support to the industry to resolve current and manufacturing and product development problems. By working closely with our industry colleagues we can streamline their product development efforts and provide analytical and technical services, which they do not have. In addition, the Center can provide these same services to other DMI units and their clients.

These new initiatives will provide the research and technology needed to support the needs of the growing dairy industry in the Intermountain West.
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Western Dairy Center
Activities for 2006

In 2006, the Western Dairy Center continued our research program in the areas of cheese cultures, cheese technology, whey protein, and co-product utilization. Three projects were completed, one the production of exopolysaccharides by adjunct cultures, one on the development of low fat cheese, and one on the extrusion of whey proteins. Four new projects were started: two with the goal of improving the texture and flavor of low and reduced fat cheese; and two in the area of co-product utilization. Two projects were continued: one on lactose utilization, and one on extrusion of whey proteins.

The Center conducted the 17th Biennial Cheese Industry Conference on August 9, 2006, in Sun Valley Idaho. The conference focused on cheese flavor development and technology. Current research conducted at the Center was the focus of the conference.

Two sessions of the 23rd Cheese Making Short Course were conducted. One session focused on the cheese technology utilized in modern industrial cheese manufacturing plants, while the other addressed on the needs of artisan cheesemakers. Cheesemakers from throughout the western United States attended the courses.

Our work in low and reduced fat cheese was expanded to include probiotics and other value added ingredients. This will be the focus of our efforts in 2007.
PROJECT PROGRESS REPORT
of the
Western Dairy Center

1. Title of Project: Development of Lactose Hydrogels

2. Principal investigator: David Britt

3. Start Date: 1-1-05

4. End Date: 12-31-07

5. Executive Summary:

Since the project start date we have synthesized and characterized a library fatty-acid and fatty-amine based lactose-surfactants. The synthesis procedure was refined by determining the minimum reaction time (heating / cooling cycles) needed to achieve a stable gel with a defined gel transition temperature. The water holding capacity of the synthesized gels was also characterized as a function of reaction time. The influence of excess lactose (non-conjugated to fatty-acid or amine) on the gelation temperature was characterized using differential scanning calorimetry (DSC) to support visual observations of gelling. The DSC values were in excellent agreement with the visual method. With respect to biological activity, a quantitative assessment of lactose-surfactant biocidal activity as well as flocculating activity against E. coli, has been achieved in collaboration with Dr. Anne Anderson of the Biology Department. Emulsification properties of the lactose-surfactants have been investigated in collaboration with Dr. Marie Walsh of Nutrition and Food Science.


   a. Introduction. In this reporting period we have begun to investigate the emulsifying properties of the fatty-amine lactose surfactants in collaboration with Dr. Marie Walsh of the Nutrition and Food Science Department (Objective 2).

   b. Objective (s)

   **Objective 1:** Develop a library of lactose-surfactants having user-defined properties through conjugation with fatty amines, acids, and alcohols.

   **Objective 2:** Characterize lactose-surfactants physical properties: Critical micelle concentration, gel transition temperature, water activity, stability, gel structure and porosity.

   **Objective 3:** Tune lactose-surfactant water activity and gel-transition temperature to match the requirements of objectives 4-5:

   **Objective 4:** Improve water infiltration to controlled soils depths to avoid water
loss beyond the root zone for plant growth in laboratory scale soil columns.

**Objective 5:** Reduce erosion and improve runoff water quality in laboratory scale soil beds.

**Objective 6:** Quantify lactose-surfactant bioactivity.

**Objective 7:** Extend objectives 4-6 to field studies.

c. **Method and Materials. Emulsification protocol:**

Emulsions were made using 80 ml water and 20 ml pure canola oil. Water and oil mixtures were pre-emulsified for 3 min with a polytron (Ultra-Turrax T125, Janke and Kunkel) at a setting of 24,000. The samples were then emulsified with a microfluidizer (Microfluidizer 110S, Microfluidics Corp) at a pressure of 6900 psi for 5 passes. Samples were then diluted for an OD 500 less than 0.4 and the change in OD 500 was measured every 15 min for an hour. The rate of change was calculated and expressed as change/min. For monitoring emulsification, a known weight of lactose-surfactant was added to the oil/water mixture prior to pre-emulsification.

d. **Data or Results**

**Objective 1:** As with the previous periods, we continue to restrict our focus to lactose-fatty amine conjugates due to the covalent nature of conjugation and favorable gel self-assembly behavior. Surfactant amphiphilicity (hydrophobic / hydrophilic ratio) was systematically varied using fatty amines having alkyl-tails: C18, C16, C12. Conjugation to urea was also investigated as a negative control. Stable product was formed for all investigated alkyl-chain lengths.

**Objective 2:** A focus on the flocculating ability of the surfactants synthesized (Obj. 1) has indicated an optimal hydrophobic content is achieved for the C16 alkyl-tail surfactant. Flocculation of soil suspensions and bacterial suspensions have yielded positive results compared to the control suspensions devoid of lactose-surfactant as well as suspensions treated with commercial agents.

Emulsification properties of the hexadecylamine-lactose surfactant were investigated for the water / canola oil mixture described above. The data are presented in Figure 1.
Figure 1. Graph is showing the rate of oil/water emulsion destabilization (decrease in optical density at 600nm over 24 hrs) of the lactose-hexadecyl-amine surfactant (left, at 5 micrograms), C16-fatty amine (middle, at 5 micrograms) and control (right). The lactose-hexadecyl-amine surfactant samples were more stable than the emulsions prepared with just precursor fatty amine and the water and oil control, as indicated by a lower rate of emulsion destabilization. Graphs represent the mean of at least five trials.

From these preliminary data an emulsifying effect of the lactose surfactant is observed as compared to the controls. It is seen that 5 micrograms of lactose-hexadecyl-amine in 1 mL of the oil/water mixture stabilizes the emulsion 2.7 times (8.15/2.97) as compared to the control without surfactant, and 2.0 times (5.97/2.97) as compared to the control containing 5 micrograms of the precursor fatty amine. The next step will be to compare the product against standard surfactants and over longer time periods. In addition we are preparing lactose C16-amine product having a higher degree of conjugation, and the emulsification properties of these preparations will also be investigated.

Objective 3: Found that ions have an effect on hydrogel strength and water holding capacity. It appears that ions in water including hardness such as Ca\(^{2+}\) and Mg\(^{2+}\) hinder the formation of gels. It is hypothesized that negatively charged particles may promote stronger gel formation. This has been observed on a basic level by noting that soil containing anionic soil particles has been shown to promote gel formation and strength. These phenomena will be further investigated.
Figure 2. Water holding capacity curves for tap water and deionized water. The ions in the tap water are suspected of causing the reduction in water holding capacity of gels.

Objective 5: Used a filter to simulate the surface sealing of soil in the field and the effect of lactose on this infiltration reducing property. Confirmed that lactose reduced the clogging of the soil surface by small suspended particles.

Table 1. Average cumulative infiltration rates through a filter after 1.5 hours, for various lactose treatments of suspended sediment solutions. Lactose treatments caused large infiltration improvements. n=3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative Infiltration (ml/min)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: soil</td>
<td>0.42</td>
<td>0.03</td>
</tr>
<tr>
<td>Control: tap water</td>
<td>1.51</td>
<td>0.03</td>
</tr>
<tr>
<td>Standard lactose solution:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil in 300 mg/L</td>
<td>0.50</td>
<td>0.05</td>
</tr>
<tr>
<td>Soil in 3,000 mg/L</td>
<td>0.59</td>
<td>0.01</td>
</tr>
<tr>
<td>Soil in 30,000 mg/L</td>
<td>0.68</td>
<td>0.24</td>
</tr>
<tr>
<td>Pure lactose solution:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil in 3,000 mg/L</td>
<td>0.52</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Objective 6: The lactose-fatty amine conjugates (C16 alkyl tails) showed no intrinsic biocidal activity against E. coli or P. chlororaphis liquid cultures, as indicated in the Figure 2 below. Degradation of the product by bacteria was previously indirectly inferred by a sour smell and liberation of bubbles from soil columns treated with the product—the product degradation has been quantified as seen in Figure 3.
Optical Density at 600nm of E. coli in LB

Figure 3. Optical density at 600nm of E. coli (JM109) in LB broth with addition of hexadecylamine or LBMP. 10% hexadecylamine was the only treatment that affected growth. This data is consistent with data from growth on solid media (not shown).
CFUs of Bacteria Grown on Different Carbon Sources

Figure 4. Growth of soil microbes on minimal media with restricted carbon sources: glucose, lactose, and the lactose-surfactant (LBMP). Zero growth resulted from the use of hexadecylamine as a carbon source.

During our bacterial studies we also assessed the *E. coli* flocculation by the lactose-surfactant in comparison with PAM and C16 fatty amine, shown in Table 2.
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<table>
<thead>
<tr>
<th>ppm of flocculan t</th>
<th>PAM</th>
<th>Fatty Amine</th>
<th>Lactose-surfactant (1:1 amine:lactose)</th>
<th>Lactose-surfactant (1:2 amine:lactose)</th>
<th>Lactose-surfactant (1:10 amine:lactose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.7</td>
<td>14.7</td>
<td>14.7</td>
<td>14.7</td>
<td>14.7</td>
</tr>
<tr>
<td>20</td>
<td>17.6</td>
<td>23.5</td>
<td>23.1</td>
<td>27.5</td>
<td>14.4</td>
</tr>
<tr>
<td>50</td>
<td>14.9</td>
<td>16.2</td>
<td>26.5</td>
<td>29.5</td>
<td>18.4</td>
</tr>
<tr>
<td>100</td>
<td>17.4</td>
<td>18.5</td>
<td>26.8</td>
<td>29.0</td>
<td>8.5</td>
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<tr>
<td>500</td>
<td>16.1</td>
<td>30.8</td>
<td>72.9</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>38.4</td>
<td>73.1</td>
<td>83.1</td>
<td>61.4</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Percent E. coli (9x10^10 CFU) flocculated after 20 hours.
The influence of the lactose-hexadecyl-amine surfactant on cucumber seed ("straight eight") root growth was compared by spraying seeds with 5% solutions of the surfactant vs. a control (Pluronic P104 wetting agent) or water. Root growth was monitored at 4 days. Repeat experiments are underway to determine if the modest increase in root length for the lactose-surfactant acts to enhance root growth under these nutrient free growth conditions.

<table>
<thead>
<tr>
<th>Seed</th>
<th>P104</th>
<th>Lactose Surfactant</th>
<th>H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>4.6</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>4.2</td>
<td>4.6</td>
<td>4.9</td>
</tr>
<tr>
<td>4</td>
<td>5.5</td>
<td>4.4</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>3.9</td>
<td>5.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Average</td>
<td>4.36</td>
<td>4.66</td>
<td>4.26</td>
</tr>
<tr>
<td>Std.</td>
<td>0.58</td>
<td>0.33</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Table 3. Cucumber seed root lengths (cm) at 4 days growth nutrient free, unsaturated conditions.

Objective 7: Field studies have been carried out. Lactose-polymers were added directly water entering the head of irrigation furrows and the influence on water infiltration and furrow erosion were investigated. Control furrows were exposed to pure lactose, fatty-amine, or polyacrylamide (PAM). An interesting finding that we are investigating further is that raw lactose showed a significant effect as compared to the untreated furrow. Our lab scale studies did not predict this based on flocculation assays, so we are investigating this further in both lab and field-scale studies. Mr. Wes Hopwood successfully defended his MS thesis March 23, 2006. The committee (Drs. Lynn Dudley, Gary Merkely, and David Britt) recommended that the results be submitted for publication. Hopwood's thesis was approved and published. Mr. Hopwood is continuing with the research to conduct repeat
experiments and train an undergraduate student (Mr. Jon Lundell) to continue with the research. Ms. Tammy Anderson and Ms. Lindsay Robinson (BIE undergraduates) are continuing with studies on optimizing surfactant synthesis and characterizing gelling properties after being trained by Mr. Jerremy Rasband who has since graduated.

e. Discussion: From our fundamental characterization of the fatty-acid and fatty-amine based lactose hydrogels we have refined our synthesis procedures, verified our gel transition temperatures, and scaled up the process for batch synthesis. These efforts have lead to a journal publication (Dhruv et al., Chem. Mater 2005).

f. Efforts are being made to strengthen gel: including gelling in different organic solvents (organogels) as well as enhancing binding properties such hydrophobic interactions as well as "\( \pi-\pi \)" interactions. If an aromatic compound can be bound to the existing gel a stronger gel can be formed as a result of "\( \pi-\pi \)" stacking. Hydrophobic amino acids that contain an aromatic element are being considered because of their propensity to bind to lactose by the same manner as the fatty amine. Stronger gels could be used for slow release of water, nutrients and other soluble material as the gel is degraded by environmental conditions.

g. Conclusion and Next Step: We are encouraged by the reproducibility of our lactose-gels in terms of gel transition temperature, and we are working towards our original goals as well as developing systems with gel transition temperatures just above body temperature (37°C) for medically relevant applications such as controlled drug release matrices and wound healing scaffolds. We have been refining the gelation properties of our surfactants in order to determine the minimum amount of surfactant needed to completely gel and hold a given quantity of water. This is relevant for a wide variety of applications from soil treatment to cosmetics and medicine. We have discovered that excess lactose can significantly enhance the water holding capacity of a surfactant gel. Preliminary results are summarized in the graph below and ongoing experiments will investigate this over a greater range of concentrations and ratios:
Figure 5. Water holding capacity of lactose-fatty amine surfactant gels. A 150 mM solution of the surfactant (diamonds) was added in a volume indicated on the x-axis to 10 ml of tap water. The amount of water held by the resulting gel is indicated on the y-axis. The experiment was repeated with a 150 mM solution of the surfactant containing 100 mM excess lactose (squares). A dramatic increase in the water holding capacity is observed over the concentration range investigated. (J. Rasband, et al. unpublished)

7. Intellectual Property (if applicable). Please check the applicable box and provide additional explanation, if any.

Yes [ ] 
No [ X ] 
Possible [ ]

8. Anticipated Delays or Problems: None

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts

Materials Research Society Fall, 2004 meeting, Boston, MA.
Session GG: Mesoscale Architectures from Nano-Units-Assembly, Fabrication, and Properties.

Abstract #: GG10.5: http://www.mrs.org/meetings/fall2004/program/index.html (abstract attached)


Abby Tyler: Senior design report, Department of Biological Engineering, 2006.

PROJECT PROGRESS REPORT
of the
Western Dairy Center

1. Title of Project: Iron-binding by Milk Mineral – A Possible Antioxidant and Anti-microbial Mechanism

2. Principal investigator: Daren Cornforth

3. Start Date: 8/30/06

4. End Date: 12-31-08

5. Executive Summary:

Milk mineral (MM), the dried mineral fraction from whey, is a good calcium source, and has powerful antioxidant properties in cooked ground meats. Recent studies show that MM also prevents browning in raw ground beef. To receive acceptance for MM as a food ingredient, further studies are needed on antioxidant mechanism, possible antimicrobial effects, and sensory evaluation of products with added MM. Our hypothesis is that MM tightly binds trace levels of iron, preventing iron-stimulated rancidity, browning, and microbial growth in meat products. Demand for MM and related dairy products (nonfat dry milk) will increase as a result of a better understanding of MM properties.


   h. Introduction

   i. Objective (s)

   Objective 1: Measure iron-binding capacity of milk mineral (MM) by column chromatography. (Hypothesis – MM has high affinity for iron cations).

   Objective 2: Directly demonstrate iron binding to MM particles by scanning electron microscopy (SEM), using energy dispersive X-ray spectrometry to localize iron in relation to MM calcium and phosphate. (Hypothesis – negatively charged phosphates of MM have high affinity for iron cations).

   Objective 3: Measure possible antimicrobial effects of MM on total plate count and Enterobacter plate counts in ground beef. (Hypothesis – MM will inhibit bacterial growth by binding iron and slowing growth of iron-dependent bacteria, including spoilage and pathogenic organisms).

   Objective 4: Demonstrate a concentration-dependent effect of added iron on myoglobin oxidation (browning) in a model system at high and low oxygen concentrations (Hypothesis – Iron stimulates myoglobin
Objective 5: Determine the minimum level of MM needed to prevent myoglobin (Mb) oxidation in a model system of Mb with various iron levels. (Hypothesis – MM prevents myoglobin oxidation by binding iron).

Objective 6: Demonstrate that MM is more effective than Trolox (water soluble vitamin E), eugenol (clove antioxidant), or rosemarinic acid (rosemary antioxidant) for prevention of lipid and Mb oxidation in ground beef in high oxygen packaging. (Hypothesis – In raw and cooked ground beef, MM is more effective than other recognized antioxidants).

j. Method and Materials

Objective 1 - Iron Binding Column Preparation. Milk mineral, sodium tripolyphosphate, calcium phosphate monobasic, and calcium pyrophosphate will be used as the test materials. Columns will be prepared using small (14.5 cm length) disposable borosilicate Pasteur-type pipettes. Columns will be plugged with glass wool, then filled with test material to a depth of 2.5 cm. The amount of test material added to each column will be determined by weight difference. Columns will be pre-wetted with 1 ml of distilled water, then 0.5 ml of 1 mg/ml ferrous chloride (FeCl₂) standard (in 0.1N HCl) will be added. Columns will be rinsed with distilled water to a total volume of 10 ml. Ten replicates will be performed for each test material.

Percent Packing Loss. Spent iron binding columns will be dried overnight at 90°C, then cooled in a desiccator. Columns will be weighed to determine the amount of packing solubilized.

Iron Retention. Total iron content of the filtrates will be determined using the Ferrozine assay (Carter, 1971). Briefly, an aliquot of each filtrate at basic pH (~9) will be reduced with an ascorbic acid solution. Ferrozine will be added, and the chromogen allowed to develop in the dark. Total iron will be assayed at 562 nm. Each filtrate will be assayed in duplicate. Iron retention, in mg iron / g packing compound, will be calculated based on a target value of 0.05 mg iron / ml filtrate, the expected concentration where no iron is retained by the column.

Objective 2 – Light and Scanning Electron Microscopy (SEM). Lean ground beef (90%) will be obtained from the USU Meat Lab. Samples will be prepared by adding MM or STP at 0.75% and 1.5% levels to 50 g of meat. Samples will be mixed thoroughly (kneading 25 times), wrapped in plastic film, then placed in resealable sandwich bags and held under refrigeration for three days. Samples will be prepared for light microscopy by dehydrating, embedding in paraffin, sectioning, and re-hydrating. To obtain information on milk mineral solubility (or insolubility), Von Kossa staining (Sheehan and Hrapchak, 1980) will be performed on the re-hydrated sections to test for the presence of undissolved calcium. To obtain information regarding the suspected association of iron with the calcium phosphate of milk mineral, x-ray dispersive SEM will be done on MM samples with and without treatment with ferrous chloride. MM
Objective 3 – Effect of MM on Microbial Load and Enterobacter Levels of Ground Beef.

Preparation of ground beef. USDA select grade beef shoulder dods will be purchased and used for preparation of ground beef within 1 week postmortem. Clods will be first cut into 2.5 cm thick strips. Beef trim will be prepared by coarsely (0.60 cm plate) then finely (0.32 cm plate) grinding the strips. pH of freshly ground beef will be measured, but will not be controlled. MM (1-2%) will be manually mixed with the meat, and re-ground through the fine plate. Portions (130 g each) will be wrapped in oxygen permeable polyvinyl chloride film in a Styrofoam tray, and held for 1, 4, 7, or 14 days at 2°C. Three replicates will be performed.

Microbial load. Total aerobic counts and Enterobacter counts will be taken on beef samples after grinding, and at days 1, 4, 7, and 14 based on AOAC method 990.12 (AOAC 1995). Briefly, 11 g of sample will be stomached in Butterfield’s phosphate diluent. Subsequent dilutions will be plated using Petrifilm™ aerobic count plates and Enterobacter specific plates (3M Corporation, St. Paul, MN), then incubated at 32°C for 48 hours. Colony counts will be taken and results interpreted per the manufacturer’s guidelines. All samples will be plated in duplicate.

Objective 4. Effects of added iron on Myoglobin (Mb) Oxidation in a Model system

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Objective 5. Interaction of Milk Mineral, Phospholipids, & Oxygen Levels on Mb oxidation

Reagent and buffer preparation. FeCl₃ solution will be prepared at a concentration of 0.1 mg/ml in 0.1 N HCl. Bathophenanthroline solution (BPS) will be prepared in a 5% ethanol / 95% hexane mixture. MES buffer solution (0.04 M) will be prepared in distilled water (DI), and adjusted to a final pH of 5.6 using a supersaturated sodium hydroxide solution. Tris buffer (0.1M) will be prepared as MES, with a final pH of 8.0. Residual iron will then removed from buffers using a bathophenanthroline extraction (Schlit 1969). Briefly, 100 ml of buffer will be extracted three times with 10 ml aliquots of BPS, using a separatory funnel. Extracted buffer will be heated to approximately 95°C while being stirred rapidly to remove residual ethanol. Buffer will cooled, and final volume adjusted back to 100 ml with distilled, deionized water (DDI). Iron content of myoglobin and phospholipid. Since myoglobin and lipid cannot be purified of contaminating iron using the bathophenanthroline extraction, their total iron content will be determined and taken into consideration when designing model systems.
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and interpreting their results. Samples will be dry ashed in triplicate, then assayed for iron using the Ferrozine procedure, as outlined previously.

Generation of MbO2 stock solutions. A concentrated solution of horse heart myoglobin (~20 mg/ml) will be prepared in DDI water (Brown and Mebine 1969), and will be reduced by adding a small volume (~100 μl) of a concentrated dithionite solution (Sage et al. 1991). To remove excess dithionite, the myoglobin will be passed through a Sephadex G25 column and eluted with Tris buffer (pH 8.0). Deoxymyoglobin will be converted to MbO2 by bubbling air through the solution with a Pasteur pipette. Conversion to MbO2 will be confirmed spectrophotometrically, based on the presence of the characteristic MbO2 peaks at 545 and 580 nm (Bowen 1949). The concentration of the MbO2 stock solution will be adjusted to ~0.1 mM with MES buffer (pH 5.6). Fresh MbO2 stock will be generated for each experiment and replicate.

Preparation of model system samples to examine the effect of lipids. Lipid-free model systems will be prepared in MES (pH 5.6) using a combination of MbO2, FeCl2, and/or antioxidant to observe the effect of free iron on the conversion of MbO2 to MetMb in the absence of lipid. Lipid-containing model systems will be prepared in MES (pH 5.6) using a combination of MbO2, phospholipid, FeCl2, and/or antioxidant to examine the effect in the presence of lipid.

Systems will be prepared in disposable 3.5 ml spectrophotometer cuvettes, covered with parafilm, inverted 10x to mix, then scanned from 400 to 650nm. Scans will be repeated for each system at 15, 30, 45, and 60 minutes, and at 1 and 2 days. Samples will be held at room temperature (23°C). Five complete replicates will be performed.

Preparation of samples to examine the effect of partial pressure of oxygen. Samples will be prepared as outlined above for lipid-free model systems. To obtain samples with 4 initial oxygen levels (none = 0 mm Hg partial pressure = vacuum; low = 2 mm Hg = 2.6% oxygen; atmospheric = 150 mm Hg = 20% oxygen; high = 600 mm Hg = 80% oxygen), model systems will be flushed with the appropriate gas then sealed with oxygen impermeable caps. Gas cylinders certified to contain the desired oxygen concentration (2.6%, 20%, 80% oxygen, with the remainder as nitrogen) will be used.

Preliminary studies will be conducted to determine the time needed to bubble the gas through the solution in order to obtain the desired oxygen concentration in the headspace and in solution. Actual headspace oxygen concentrations will be measured with a benchtop oxygen headspace analyzer (Illinois Instruments, Ingleside, IL). Spectra will be obtained as outlined above. Five complete replicates will be performed.

Preparation of samples containing varying iron concentrations (35, 180, and 350 μM added iron). Control (0 μM added iron) will consist of equal parts MbO2 and MES. Additional “control” samples will be prepared containing 2 mg/ml MM or STP, to chelate any “free” iron in the MbO2 stock, and to provide for an essentially iron-free system. Representative spectra (400 - 650nm) will be obtained every 5 minutes for 1 hour, for a total of 13 scans per sample.
Objective 6 – Effect of various antioxidants (MM, Trolox, Eugenol, Rosemarinic Acid) on Raw Ground Beef Appearance and Cooked Ground Beef Sensory Acceptability.

Preparation of ground beef. USDA select grade beef shoulder clods will be purchased and used for preparation of ground beef within 1 week postmortem. Clods will be first cut into 2.5 cm thick strips. Beef trim will be prepared by coarsely (0.60 cm plate) then finely (0.32 cm plate) grinding the strips. pH of freshly ground beef will be measured, but will not be controlled. Antioxidants (milk mineral, Trolox (water-soluble vitamin E), eugenol, rosemarinic acid) will be manually mixed with the meat at appropriate levels to be determined in preliminary studies, and re-ground through the fine plate. Four portions (130 g each) for each treatment will be flattened to a thickness <12 mm, to allow for complete oxygenation of the sample interior in the high oxygen atmosphere. The flattened samples will then be flushed and sealed in 80% oxygen MAP and held for 1, 4, 7, or 14 days at 2°C. The gas cylinder containing 80% oxygen and 20% carbon dioxide will be certified to be within ±0.5% of the indicated mixture. Actual oxygen concentration in MAP will be directly measured using an oxygen gas analyzer. MAP samples will be held under refrigeration until analyzed for MbO₂ content, Hunter color, and thiobarbituric acid reactive substances (TBARS) at 1, 4, 7, or 14 days; these analyses will also be conducted on the fresh ground beef the day of preparation. A final portion (1000 g) from each treatment will be placed in a vacuum bag and frozen at -20°C for latter determination of fat and non-heme iron content. Five complete replicates will be performed.

Oxymyoglobin determination. Conversion of MbO₂ to MetMb will be confirmed spectrophotometrically, based on the presence of the characteristic MbO₂ peaks at 545 and 580 nm (Bowen, 1949). Reflectance spectra (400-650 nm) of meat samples will be obtained in duplicate. Due to varying fat and total myoglobin content between meat batches, it may be necessary to normalize reflectance values for statistical analysis. Normalization will be based on the initial total myoglobin concentration, to be determined immediately after the initial grinding step (prior to antioxidant addition). Hunter color measurement. The L*, a* and b* values will be measured using a Hunter Lab Miniscan portable colorimeter (Reston, VA), standardized through the packaging film using a white and black standard tile. Five color measurements will be taken per sample.

TBARS analysis. The thiobarbituric acid reactive substances (TBARS) assay will be performed as described by Buege and Aust (1978). Briefly, duplicate ground beef samples (random 0.5g cores) will be mixed with 2.5 ml of stock solution containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N HCl. The mixture will be heated for 10 min in a boiling water bath (100°C) to develop a pink color, cooled in tap water and then centrifuged (4,300 x g for 10 min). The absorbance of the supernatant will be measured spectrophotometrically at 532 nm.

Microbial load. Total aerobic counts will be taken on beef samples after grinding, and at days 1, 4, 7, and 14 based on AOAC method 990.12 (AOAC 1995). Briefly, 11 g of sample will be stomached in Butterfield’s phosphate diluent. Subsequent dilutions will be plated using Petrifilm™ aerobic count plates (3M Corporation, St. Paul, MN), then incubated at 32°C for 48 hours. Colony counts will be taken and results interpreted as per the manufacturer’s guidelines. All samples will be plated in duplicate.
Sensory Evaluation.
A 6-member panel is currently active for evaluation of various flavors (beef flavor, rancid flavor, rancid odor) in cooked ground beef. All panelists have had previous sensory panel experience with cooked beef products. The panelists were trained in two sessions. In the first session, panelists were familiarized with the 5-point intensity scale and its usage. Panelists were also familiarized with cooked beef flavor (both fresh and rancid samples) in cooked ground beef. For this study, a training session will be held to familiarize the panelists with the study. Cooked ground beef (fresh and rancid, as verified by TBA values) will be served, and panelists will be re-evaluated for their consistency and accuracy of sample identification and scoring.

For cooked ground beef panel evaluation, a set of 3 or 4 samples (6 g each) will be served to each panelist in each session. Cooked (internal temperature of 82°C) ground beef samples will be coded and microwave re-heated for 25 seconds to attain a temperature of 80-85°C immediately before serving. Samples will be evaluated in individual booths under red lights. The serving order will be randomized to avoid positional bias. Panelists will be asked to evaluate samples for intensity of rancid odor, rancid flavor, and beef flavor on a 5-point scale, where 1 = no flavor or odor, 2 = slightly intense, 3 = moderately intense, 4 = very intense, and 5 = extremely intense flavor or odor. Panelists will be also asked to provide additional qualitative comments for each sample. Before evaluating the next sample, ballot instructions will specify that the previous sample be expectorated into cups provided for that purpose. Panelists will be instructed to rinse their mouths with tap water. Unsalted crackers will also be provided to cleanse the palate.

Statistical Analysis

Where appropriate, measured values will be evaluated by analysis of variance using the proc GLM function in SAS version 9.0 (SAS Institute, Inc., Cary, NC). Statistical significance will be identified at the 95% confidence level, and post-hoc means comparisons will be made based on p-values obtained using the Tukey-Kramer adjustment.

k. Data or Results

Funding for this project was received September 1, 2006. Technician Karin Allen has completed objective 1 (iron-binding measurement of MM and other calcium phosphate compounds), objective 2 (microscopy of MM added to ground beef, and scanning electron microscopy of MM particles with regard to iron binding, and objective 4 (Effects of added iron on Myoglobin (Mb) Oxidation in a Model system), as follows:

Objective 1 and 2 Results. MM was compared to sodium tripolyphosphate (STPP), calcium phosphate monobasic (CPM), and calcium pyrophosphate (CPP) to determine iron-binding capacity, sample solubility, and eluate soluble phosphorus after treatment of samples in glass columns with 1 mg/ml
ferrous chloride. Scanning electron microscopy with energy dispersive x-ray analysis was used to localize minerals on iron-treated MM particle surfaces. Histochemical staining for calcium was also performed on raw and cooked ground beef samples with added MM. MM bound more iron per gram \((P < 0.05)\) than the other compounds, and was much less soluble \((P < 0.05)\) than either STPP or CPM. Mineral localization showed an even distribution of calcium, phosphorus, oxygen and iron across the MM particle surface, directly demonstrating iron binding to MM particles. Unlike other common chelating agents, such as STPP and citrate, histochemical staining demonstrated that MM remained insoluble in ground beef, even after cooking. The ability of MM to bind iron and remain insoluble may enhance its antioxidant effect, by removing iron ions from solution. However, MM particles must be small and well distributed in order to adequately bind iron throughout the food system.

Objective 4 Results. A model system was used to study the effect of nonheme iron on myoglobin oxidation at pH 5.6 and pH 7.2 at 23 °C. The addition of ferrous iron significantly \((p < 0.05)\) increased the rate of myoglobin oxidation in the absence of lipid, demonstrating that iron promoted myoglobin oxidation independent of the effect of lipid oxidation. The addition of the type II, iron chelating antioxidants sodium tripolyphosphate (at pH 7.2) or milk mineral (at pH 5.6) negated the effect of added iron, slowing oxidation of myoglobin. A clear concentration dependence was seen for iron-stimulated myoglobin oxidation, based on both spectral and visual evidence. Further investigation is needed to determine the possible role for nonheme ferrous iron on myoglobin oxidation in vivo or in meat.

Conclusion and Next Step:

One new student (Rossarin Tansawat) has just begun working on objective 3 (possible anti-microbial effects of milk mineral (MM). Fish filets (tilapia) were dipped and held for 30 seconds or 24 hr in solutions of 0.9% sodium chloride (control), 2% lactate/diacetate (a widely used anti-microbial solution) or 1% milk mineral. Aerobic plate counts (APC) were conducted on filets after 1, 5 and 9 days storage at 3°C. APC were not different from controls for any treatment when held only 30 sec in the treatment solution. However, APC were 1.5 and 0.5 log lower for samples held 9 days in lactate diacetate and milk mineral, respectively. So, MM had only a small antimicrobial effect in marinated fish filets. Further work will continue on possible antimicrobial effects in model systems and other food systems. New student Rossarin Tansawat will require biosafety level 2 (BL2) training this summer (2007), so that she may initiate the portion of objective 3, regarding possible anti-microbial effects of MM against food pathogens (Listeria, E. coli). This work will be done initially in appropriate media with and without MM, and later in food systems (dairy foods, ground meats). Rossarin and Karin Allen will also this summer initiate work on comparison of MM to other antioxidants for prevention of myoglobin oxidation in model systems, and similar comparisons of MM and other antioxidants in
fresh ground beef, including measurements of pigment and lipid oxidation by chemical measures, and by sensory panel evaluation.

7. Intellectual Property (if applicable)

Please check the applicable box and provide additional explanation, if any.

Yes [ ] No [ x ] Possible [ ]

8. Anticipated Delays or Problems

none

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts
PROJECT PROGRESS REPORT
of
Western Dairy Center

1. Title of Project: Molecular basis of cheese melting in relation to proteolysis

2. Principal investigator: Donald McMahon, Utah State University
   Jeffrey Broadbent, Utah State University
   Craig Oberg, Weber State University

3. Start Date: 1-1-04

4. End Date: 12-31-06

5. Executive Summary:

   We will modify existing methods for monitoring the cleavage of proteins during cheese storage so that we can measure the peptide fragments initially produced from the intact proteins. This method will then be used to examine cheese with different protein breakdown patterns and relate specific protein hydrolysis to how well the cheese melts. This will tell us what parts of the individual proteins need to be split off to reduce the level of interactions between the proteins, thus allowing them to flow past each other when heated.


Please identify the reporting period. This report must contain all research progress accomplished up to this reporting period. Works performed in this reporting period must be clearly identified.

1. Introduction

   m. Objective (s)

      Objective 1. Track the production of large- and medium-sized casein-derived peptides during cheese ripening.

      Objective 2. Correlate changes in cheese melting properties with extent and type of proteolysis.


   n. Method and Materials
Extraction of cheese samples
Cheese samples were extracted in a citrate buffer as described by Kaiser et al. (1992) with some modifications. Cheese samples at 1 week, 2, 4 and 6 months were shredded, and 10 g of each sample were homogenized with 40 ml of 500 mM sodium citrate solution (containing 1% sodium chloride) and 70 ml of deionized water at 40-50°C for 4 min at 260 rpm in a Seward Stomacher 400. The homogenate was then cooled to room temperature and made up to 200 ml with deionized water. An aliquot of this mixture (35 ml) was then centrifuged (Sorvall RC5C; 6000 rpm, 15 min, 4°C) and further dispensed into smaller volumes (1 ml, microcentrifuge tubes) and centrifuged at 13000 rpm for 5 min (Beckman Microfuge Lite). The supernatant (2 ml) was further concentrated (1.12X) using a 3 kDalton Centricon concentrator (Amicon) centrifuged for 2 h at 2500 rpm and 4°C (Sorvall RC5C). Both permeate and retentate were stored at -20°C until analyzed by RP-HPLC.

Reversed-phase HPLC of samples
Permeate and retentate samples were dissolved in 0.1% trifluoroacetic acid (TFA, 1:15) and centrifuged (Beckman Microfuge Lite; 13000 rpm, 5 min). RP-HPLC was performed using an automated Beckman System Gold (Autosampler 507, 168 Detector & 125 Solvent Module) fitted with an analytical Brownlee Aquapore C8 column (Applied Biosystems; RP 300 Å, 7 μm, 2 X 100 mm) and guard column (10 X 4.6 mm). A 25 μl aliquot of the sample was injected and eluted with a gradient of two solvent mixture: 0.1% TFA (Solvent A) and 0.085% acetonitrile (ACN, Solvent B). Solvent B was varied from 40 to 70% over 60 min with a final wash at 80%. The flow rate was maintained at 0.2 ml/min. The eluate was monitored at both 214 and 280 nm with the detector being interfaced with an IBM PC using System Gold software (version 8.10).

Cheesemaking
Using the hypothesis that different coagulants, their levels and different ripening temperatures will produce varying proteolytic patterns and consequent melting properties cheese was made using chymosin and C. parasitica rennet. A split-split plot design was used with the block treatment being the starter and adjunct cultures, split into 3 coagulant levels (chymosin, C. parasitica rennet and a combination of them), further split into 2 ripening temperatures (40 and 55°F). Sampling was carried out at 1 week, 1, 2, 3, 4, 5 and 6 months. The cheese samples were being analyzed for proteolytic patterns using RP-HPLC and CE, soluble nitrogen at pH4.6 and 12% TCA, melt properties using a UW Meltemeter, and TPA with a TPA Analyzer.

Cheese was extracted as previously described in a sodium citrate-sodium chloride extraction buffer and RP-HPLC was carried out as per the method developed.

Cheese Opacity
Cheese opacity was determined by measuring the reflectance L*value using a HunterLab Colorimeter (MiniScan™ XE; Hunter Associates Laboratory, Inc., Reston, VA) using the method of Metzger et al (2000).

Data or Results
Using the above method, a group of hydrophobic peaks were observed (Figure 1). Compounds in the sample that do not have sufficient hydrophobic regions to bind to the reverse-phase column elute very quickly, while hydrophobic compounds have long retention times and required high levels of the acetonitrile buffer to be eluted from the column. During 6 months of aging of cheese diminished in size and retention time (Figure 2), especially when the cheese was aged at 55°F (Figure 3). These peaks represent intact caseins and large peptides derived from them as part of the early hydrolysis of the caseins by the coagulant and bacterial-derived enzymes. When the permeate from a 3 kDalton membrane filter was tested, none of these hydrophobic peaks were present (Figure 4).

see previous report for chromatographs

From the cheesemaking-coagulant study, Fig. 5 is a chromatogram of showing proteins and large hydrophobic peptide present in cheese samples with chymosin used as a coagulant at 1X and 4X levels at 1 week and 1 month of ripening at 40°F. Fig. 6 is for cheese samples with C. parasitica rennet used as a coagulant at 1X and 4X levels at 1 week and 1 month of ripening at 40°F. Fig. 7 is for cheese samples with a combination of chymosin and C. parasitica rennet (1.5:1.0) at 1X and 4X levels at 1 week and 1 month of ripening at 40°F.

Study of the relationship between proteolysis and functional properties including melting, adhesiveness, cohesiveness and elasticity is in progress. Figures 8 and 9 represent RP-HPLC chromatograms of cheese samples made using chymosin (Maxiren®, DSM Ingredients) at 1X and 4X levels at 1 week, 2, 4 and 6 months at 40 and 55°F respectively.

see previous report for chromatographs
Table 1. Areas of peaks assumed to be intact $\alpha_{\text{sl}}$- and $\beta$-casein and $\alpha_{\text{sl}}$(24-199) ($\alpha_{\text{sl}}$-I) at 1 week, 2, 4 & 6 months determined by RP-HPLC.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>1 wk</th>
<th>2 mo</th>
<th>4 mo</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\alpha_{\text{sl}}$</td>
<td>375.8</td>
<td>196.9</td>
</tr>
<tr>
<td>A*</td>
<td>$\alpha_{\text{sl}}$-I**</td>
<td>450.8</td>
<td>126.7</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>888.5</td>
<td>296.1</td>
</tr>
<tr>
<td></td>
<td>$\alpha_{\text{sl}}$</td>
<td>531.7</td>
<td>306.8</td>
</tr>
<tr>
<td>B*</td>
<td>$\alpha_{\text{sl}}$-I</td>
<td>541.1</td>
<td>86.2</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>1141.5</td>
<td>669.9</td>
</tr>
<tr>
<td></td>
<td>$\alpha_{\text{sl}}$</td>
<td>401.3</td>
<td>496.6</td>
</tr>
<tr>
<td>C†</td>
<td>$\alpha_{\text{sl}}$-I</td>
<td>415.8</td>
<td>226.9</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>824.3</td>
<td>718.3</td>
</tr>
<tr>
<td></td>
<td>$\alpha_{\text{sl}}$</td>
<td>489.6</td>
<td>328.5</td>
</tr>
<tr>
<td>D†</td>
<td>$\alpha_{\text{sl}}$-I</td>
<td>537.8</td>
<td>82.9</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>1192.7</td>
<td>970.2</td>
</tr>
</tbody>
</table>

*A, B: cheese with chymosin @ 1X & 4X @ 40°F
**C&D: cheese with chymosin @ 1X & 4X @ 55°F
**Presumptive identification of peak as $\alpha_{\text{sl}}$-I.

Table 2. Total area of peaks assumed to be large hydrophobic peptides and intact proteins from cheeses at 1 week, 2, 4 & 6 months of aging.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>1 wk</th>
<th>2 mo</th>
<th>4 mo</th>
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<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A*</td>
<td>2297.3</td>
<td>885.9</td>
</tr>
<tr>
<td></td>
<td>B*</td>
<td>2747.0</td>
<td>1625.6</td>
</tr>
<tr>
<td></td>
<td>C†</td>
<td>2231.4</td>
<td>2268.8</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>2723.8</td>
<td>2319.0</td>
</tr>
</tbody>
</table>

*A, B: cheese with chymosin @ 1X & 4X @ 40°F
*C&D: cheese with chymosin @ 1X & 4X @ 55°F
Cheeses made using different cultures combinations: Lactococcus lactis starter culture with Lac- Lactococcus lactis and/or a Lactobacillus helveticus adjunct cultures, are being aged. Two storage temperatures, 6°C and 13°C are being used so as to give two different rates of proteolysis and aging.

Cheese Composition. Cheese composition was 35.5±1.0% moisture, 52.5±2.5% FDB, 1.65±0.05% salt, and pH 5.2±0.1.

Meltability. Culture treatments and aging time did have statistically significant effects on the extent of melting that occurred in the cheeses but the effect of aging time was much greater than culture treatment, and there was little numerical difference in melting between the different cheeses. As time increased the initial melt slope increased while the final percent change in height decreased, meaning older cheese melted faster and further than the younger cheese. (See Figure 2 – Initial Melt Slope, Final Melt %) Temperature effected the final percent change in cheese height such that at 15°C the cheese tended to melt faster and further than the cheese stored at 6°C.

Texture Profile Analysis. Culture treatment, aging (time), compression level and temperature had important effects on several of the TPA measurements. Culture treatments caused a slight increase in adhesiveness and a slight decrease in cohesiveness at 25 and 60% compression. Cheeses were harder the younger they were and softened, in general, over time. At 60% compression fracturing increased as the cheese aged, which caused hardness to decrease. This possibly obscured the potential peak force values. When looking at fracturing of the control cheese (60% compression) the first three time points are not different from each other, whereas at 4 m there is a significant jump in fracturing force. Indicating an increase in the amount of fracturing that occurs after 2 m. Due to the fact that when the cheese samples do not fracture a value of 0 is recorded causing the mean values to decrease. Cheeses became more adhesive as they aged, there was no culture significance except at 1 m 60% adhesiveness of adjunct 1 and adjunct 2 tended to be lower than the control. Temperature had the greatest effect on cohesiveness at 25% compression. Adjunct 2 (15°C) showed an obvious drop in cohesiveness as early as 2 m, where adjunct 1&2 showed the greatest decrease after 6 m. All of the 15°C cheeses of 25% compression were lower than the 6°C cheeses.

Opacity
Cheeses made with chymosin had opacity values that decreased with temperature until ~50°C, but then increased with a further increase in temperature. Such a trend, however, was not significantly observed in the cheeses with C. parasitica rennet. This could most likely be due to the fact that most of the b-CN may have been hydrolyzed such that insufficient hydrophobic interactions were occurring to cause the proteins to aggregate together and form light scattering centers that would make the cheese opaque when heated. The lack of a large decrease in opacity also suggests that b-CN is involved in the initial decrease in opacity as well as melting of fat.
Figure 10. Cheese opacity (measured as L* values) as cheese was heated from 10°C to 90°C for cheeses made using chymosin (left) and Sure Curd (right).

p. Discussion

Comparing the peptide profiles in Figs. 5, 6 and 7 there is an obvious difference in the initial hydrolysis of the caseins even within the first week after manufacture, and during the first month of storage. Elevated levels of the coagulant do change the pattern of hydrophobic proteins/peptides present.

At 6 months, all cheese samples had very little intact $\alpha_s$- and $\beta$- caseins. Further, in the case of cheese made with elevated levels of chymosin (Figs 8 and 9), at 1 week, the hydrolysis of $\alpha_s$- casein into $\alpha_{s1}$- casein appears to be fairly rapid due to the excess enzyme (chymosin) available to act on the caseins (primarily $\alpha_s$- casein).

The type of rennet used has a greater effect on meltability of cheese than the use of adjunct cultures.

Another interesting observation is that hydrolysis of beta-casein appears to be related to the opacity of the cheese as it heated. When the amount of beta-casein in the cheese is reduced by using Sure Curd rennet the expected increase in opacity about 50°C was not observed.

q. Conclusion and Next Step
Work on identifying the peaks in the HPLC chromatographs is continuing with preliminary separation of peaks on SDS gels. When this is completed, a map of the change in large hydrophobic peptides during cheese aging can be prepared. Other work includes completing the melt measurements, and nitrogen measurements for TCA-soluble N and pH 4.6 soluble N. Writing of the student's dissertation and manuscripts will commence, and correlation of melting measurements with proteolysis.

7. Intellectual Property (if applicable)

Please check the applicable box and provide additional explanation, if any.

Yes [ ] No [x] Possible [ ]

8. Anticipated Delays or Problems.

There is much writing to do, but it is still expected to be completed by December.

9. Manuscripts/Abstracts Submitted for Publication


10. Published Articles/Abstracts None
PROJECT FINAL REPORT
of the
Western Dairy Center

Title of Project: Designing a low fat cheddar cheese for the school lunch program that has texture and flavor parity with a full fat cheese.

Principal investigator: Donald J. McMahon

Start Date: 1/1/05

End Date: 9/30/06

Executive Summary:


Introduction
Use a combination of technologies previously developed at the Western Dairy Center (and other DMI-funded Centers) to produce low fat cheeses containing only 6% fat that has textural and flavor characteristics with parity to regular cheese.

Hypothesis 1: Cheese textural characteristics in a low fat cheese can be optimized by maintaining the moisture-in-fat-free-substance and the calcium content of the cheese at the same level as in regular cheese.

Objective (s)
Objective 1. Determine the best combinations of low fat cheese make procedure strategies for making a low fat cheddar cheese with acceptable texture.

[Note: The original project also included an objective on cheese flavor but this was deleted (and not funded) when the Low Fat Cheese Strategic Platform project was developed and enacted. The work undertaken in this project was then used to prepare cheesemaking protocols to be used in the Low Fat Cheese Strategic Platform project.]

Method and Materials

Objective 1: The aim of this objective is to test the various cheesemaking parameters to see the effect of combining treatments on the body and texture of low fat cheese. Cheese trials will be conducted by our cheesemaker, Steve Larsen, using about 300 lbs of milk in open vats with cutting and stirring performed manually. This will produce one 20-lb block of cheese. Proximate analysis of the cheeses will be performed, and adjustments made to reach the composition targets. The initial composition targets will be 5.5-6.4% fat, 50.0-52.0% moisture, 1.8-2.0% salt, pH 5.2-5.3. Changes to these targets will be made based on textural analysis of the cheese.

Cheese body and texture was evaluated by our cheese research team after about 7 d to decide on what changes to make to the cheese make, and then after 1 mo of...
storage at 5°C, the cheese was again sampled and tested using texture profile analysis. A sample of each cheese will also be maintained in the aging room for possible future testing.

It was decided to initially test mechanical cheesemaking parameters before investigating addition of ingredients such as lecithin. Each of the following were tested individually using a milled curd make procedure as well as using a stirred curd procedure that included washing the curd with cold water:

- Elevated pasteurization temperature of 82°C for 15 secs.
- Low-pressure homogenization of half of the milk at 800 psi with 120 psi second stage.
- Pre-acidification of milk to pH 6.3 using vinegar

Each strategy was tested in duplicate on separate days using 700 lb of milk split between two vats to make the milled curd and washed curd cheeses. A nonbitter \textit{Lactococcus} starter culture was be used during one replicate, and the \textit{Lactococcus} starter culture in conjunction with the CNRZ32 adjunct was be used in the second replicate. It is not expected that this would influence the body or the texture of the cheese during 1 month of storage.

Following this trial the cheese were tested in all combinations to look for synergistic effects in being able to obtain the desired product composition (moisture, pH, salting, texture)

Finally, a 2x2 factorial experiment with 3 replicates made with a standardized make procedure comparing milk pasteurized at either 163 F or 185 F, and renneted at either pH 6.65 or pH 6.30 was conducted.

d. Data or Results

\textbf{Trial 1.}

The moisture contents during the initial trial were too low:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/10/2005 High Temp Milled</td>
<td>47.05</td>
</tr>
<tr>
<td>11/10/2005 High Temp Washed</td>
<td>48.95</td>
</tr>
<tr>
<td>11/15/2005 Pre-Acid Milled</td>
<td>48.83</td>
</tr>
<tr>
<td>11/15/2005 Pre-Acid Washed</td>
<td>53.13</td>
</tr>
<tr>
<td>11/16/2005 Homogenized Milled</td>
<td>50.26</td>
</tr>
<tr>
<td>11/16/2005 Homogenized Washed</td>
<td>51.36</td>
</tr>
</tbody>
</table>

The initial challenge in making a low fat cheese was that the cheese moisture was too low and the cheese pH would drop too far for the cheese to have an acceptable flavor. Cheese moisture was increased by cutting the curd larger, by lowering the cook temperature, and by cooling the curd after draining the whey. The pH at draining was also raised to compensate for the higher moisture content of the cheese.

These changes increased the moisture content, but the milled curd cheeses were still lower moisture content than the washed curd cheeses:
It was decided that the milled curd process would not produce a low fat cheese with acceptable properties because of difficulty in retaining moisture and a tendency for the pH of the cheese to be too low, i.e., it was necessary to dilute the lactose in the curd to prevent over acidification of the cheese and cheese pH dropping below pH 5.15. High acidity in low fat cheeses was very noticeable during cheese tasting.

**Trial 2**
This involved using combinations of the different strategies.

A comparison was made between adding cold water prior to draining all the whey or after all the whey had been drained. No difference was observed, and adding water and draining the curd was selected as the preferred option as it resulted in less volume of diluted whey, and seemed more practical for large scale cheese making using cheese belts.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>%Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/20/2006 Hom &amp; Pre Acid (Pre Drain)</td>
<td>53.26</td>
<td></td>
</tr>
<tr>
<td>01/20/2006 Homogenized &amp; Pre Acid (Drain)</td>
<td>52.63</td>
<td></td>
</tr>
</tbody>
</table>

Homogenization of the milk was not shown to provide any improvement in cheese properties at this low level of fat (only half the fat was homogenized to prevent interference with renneting of the milk). All of the cheeses in this trial had moisture contents in the range 54% to 59% with the cheeses having both high temperature and pre-acidification treatments being the highest. Better control over pH was also obtained as the trial progressed as conditions of cooking temperature, draining pH, amount of wash water were optimized for making low fat cheese.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>%Moisture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/12/2006 Homogenized &amp; Pre Acid</td>
<td>54.17</td>
<td>5.03</td>
</tr>
<tr>
<td>01/26/2006 Homogenized &amp; Pre Acid</td>
<td>56.04</td>
<td>5.23</td>
</tr>
<tr>
<td>01/12/2006 Homogenized &amp; High Temp</td>
<td>54.65</td>
<td>5.05</td>
</tr>
<tr>
<td>01/26/2006 Homogenized &amp; High Temp</td>
<td>56.73</td>
<td>5.17</td>
</tr>
<tr>
<td>01/13/2006 High Temp &amp; Pre Acid</td>
<td>58.29</td>
<td>5.17</td>
</tr>
<tr>
<td>01/27/2006 High Temp &amp; Pre Acid</td>
<td>56.73</td>
<td>5.22</td>
</tr>
<tr>
<td>01/13/2006 All 3 treatments</td>
<td>57.69</td>
<td>5.18</td>
</tr>
<tr>
<td>01/27/2006 All 3 treatments</td>
<td>59.25</td>
<td>5.42</td>
</tr>
</tbody>
</table>
The final trial was conducted as a 2x2 factorial with treatments being pasteurization at either 165 or 185°F, and renneting at either pH 6.65 or pH 6.30. Two standardized make procedures were used for when the milk was renneted at pH 6.65 or pH 6.30. Thus, differences in cheeses produced by pre-acidification could be related to differences in calcium content of the cheese caused by the pH of renneting, and those produced by temperature of pasteurization could be related to denaturation of whey proteins and their inclusion in the cheese curd (ie., the aim was to have the same final pH, salt and fat content in the cheeses and determine differences in moisture and texture).

<table>
<thead>
<tr>
<th>Cheese Compositio n</th>
<th>Milk Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Moisture</td>
<td>52.5</td>
</tr>
<tr>
<td>% Fat</td>
<td>4.8</td>
</tr>
<tr>
<td>% FDB</td>
<td>10.0</td>
</tr>
<tr>
<td>% MFFS</td>
<td>55.1</td>
</tr>
<tr>
<td>% Salt</td>
<td>1.88</td>
</tr>
<tr>
<td>pH</td>
<td>5.21</td>
</tr>
</tbody>
</table>

Statistical Analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heat Treatment</th>
<th>Pre-acidification</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>0.0003</td>
<td>0.8548</td>
<td>0.4604</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0728</td>
<td>0.5716</td>
<td>0.7756</td>
</tr>
<tr>
<td>Salt</td>
<td>0.0236</td>
<td>0.5549</td>
<td>0.7984</td>
</tr>
<tr>
<td>pH</td>
<td>0.4157</td>
<td>0.5117</td>
<td>0.4621</td>
</tr>
</tbody>
</table>

Effects of Heat Treatment:
- The higher heat treatment results in capture of some whey proteins in the cheese curd, giving a slightly higher yield of cheese. Using a high heat treatment during pasteurization (185°F compared to 165°F) resulting in a cheese with about 3% more moisture ($P=0.0003$).
- Salt contents were slightly higher in the high heat treatment cheeses ($P=0.02$). Because salt was added as a fixed percentage of curd, it appears that less whey (and hence less salt) was expelled during pressing. So if a high heat treatment is used, the rate of salt addition to curd needs to be reduced if based on curd weight (although with the increased yield it may be similar if based upon original milk weight).
- The increased capture of proteins upon higher heat treatment was also apparent in the slight decrease in % fat content of the cheese made from milk that had the higher heat treatment ($P=0.07$). This can be corrected by standardizing the milk to a slightly lower protein-to-fat ratio to account for the denatured whey proteins.
Effects of Pre-Acidifying

Acidifying the milk to pH 6.3 prior to renneting usually increases moisture content of the cheese (because of reduced calcium content and increased hydration of the casein matrix in the cheese). In this case the protocols for making cheese with or without pre-acidification had been designed to produce cheese with similar moisture contents and there was no significant effect of pre-acidification on moisture, fat, salt or pH. This implies that the moisture range needed for a low fat cheese can be reached without pre-acidification by manipulating other cheesemaking procedures.

Melting
No significant differences ($P > 0.3$) in meltability at any of the time points were observed. It was expected that the cheeses made using preacidification would have increased melting but this was not the case. Melting of the cheeses was apparently more dependent on moisture content of the low fat cheeses and since the cheesemaking procedures were developed to give similar moisture contents, the melting properties were similar. There was considerable variation in melting between replicates of the same cheeses which limited our ability to show any significant differences. Even so, within 20 sec all the cheeses had melted to less than 70% of their original height.

Textural observations were that pre-acidifying the milk produced a cheese that appeared to be less rubbery but was slightly more sticky. However, as will be seen below these observations were not verified with the controlled texture profile analysis.
Texture profile analysis was performed using a 2-bite test to 50% compression (strain) and values computed for hardness, springiness, cohesiveness, gumminess, chewiness, resilience and adhesiveness.

- Hardness is the maximum force (stress) recorded during compression (and usually occurs at 50% compression (g)).
- Springiness is a measure of how much the sample returns to its original height after the first compression and is calculated as the ratio of the height of the cheese cylinder after-before the first compression (%).
- Cohesiveness is an indication of how much of the weight bearing structure of the cheese is retained after the first compression and is calculated as the ratio of the force-time area of second versus first compressions (%).
- Gumminess is calculated as hardness divided by cohesiveness
- Chewiness is calculated as gumminess divided by springiness
- Resilience is calculated as the ratio of force-time areas as the probe is being raised after the first compression to that when it is being lowered to 50% strain.

From the statistical analysis (2x2 factorial) it was observed that heat treatment influenced hardness \((P=0.024)\) and cohesiveness \((P=0.0013)\) while pre-acidification only influenced cohesiveness \((P=0.0022)\). The interaction between heat treatment and pre-acidification was also significant for cohesiveness \((P=0.0009)\). All other effects were not significant \((P>0.15)\).

### ANOVA for Texture Profile Analysis using 50% compression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heat Treatment</th>
<th>Pre-acidification</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness</td>
<td>0.0245</td>
<td>0.3788</td>
<td>0.6407</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.3695</td>
<td>0.3064</td>
<td>0.2812</td>
</tr>
<tr>
<td>Cohesiveness</td>
<td>0.0013</td>
<td>0.0022</td>
<td>0.0009</td>
</tr>
<tr>
<td>Gumminess</td>
<td>0.1605</td>
<td>0.6146</td>
<td>0.3644</td>
</tr>
<tr>
<td>Chewiness</td>
<td>0.1677</td>
<td>0.5639</td>
<td>0.3535</td>
</tr>
<tr>
<td>Resilience</td>
<td>0.6560</td>
<td>0.6223</td>
<td>0.8419</td>
</tr>
<tr>
<td>Adhesiveness</td>
<td>0.4908</td>
<td>0.6248</td>
<td>0.3330</td>
</tr>
</tbody>
</table>

When using the normal pasteurization treatment, pre-acidifying the milk resulted in a 38% decrease in cohesiveness (decreased from 0.81 to 0.51), but when using the higher heat treatment (185 °F for 15 seconds) there was a 5% increase in cohesiveness (from 0.79 to 0.83). The most cohesive cheese was that produced using the high heat treatment and pre-acidification, even though it had a higher moisture content (56.6%) than the other pre-acidified cheese (53.2% moisture).

Pre-acidification did not significantly influence any other TPA parameters. Higher heat treatment decreased the hardness of the cheese but this may also have been influenced by these cheeses retaining more moisture.
Mean (standard deviation) of texture Profile Analysis parameters of low fat cheeses

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>High Heat</th>
<th>Pre-acidified</th>
<th>High Heat &amp; Pre-acidified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (g)</td>
<td>5921 (885)</td>
<td>4040 (1488)</td>
<td>6179 (380)</td>
<td>4860 (938)</td>
</tr>
<tr>
<td>Springiness (%)</td>
<td>92 (2.5)</td>
<td>90 (0.7)</td>
<td>74 (20)</td>
<td>91 (2.6)</td>
</tr>
<tr>
<td>Cohesiveness (%)</td>
<td>81 (2.9)</td>
<td>80 (3.3)</td>
<td>73 (16)</td>
<td>51 (0)</td>
</tr>
<tr>
<td>Gumminess</td>
<td>4523 (945)</td>
<td>3193 (1071)</td>
<td>3489 (425)</td>
<td>3631 (1350)</td>
</tr>
<tr>
<td>Chewiness</td>
<td>4175 (984)</td>
<td>2875 (958)</td>
<td>3120 (335)</td>
<td>3320 (1313)</td>
</tr>
<tr>
<td>Resilience (%)</td>
<td>49 (3.7)</td>
<td>44 (4.0)</td>
<td>44 (11)</td>
<td>43 (16)</td>
</tr>
<tr>
<td>Adhesiveness (g.s)</td>
<td>0.55 (0.74)</td>
<td>0.24 (0.14)</td>
<td>0.04 (0.06)</td>
<td>1.68 (2.37)</td>
</tr>
</tbody>
</table>

Flavor
Descriptive sensory flavor analysis was performed at North Carolina State University on two of the replicates when the cheese was 2 to 3 months old. Duplicate replications of four low fat cheeses were received. Each cheese replicate was evaluated in duplicate by 10 highly trained panelists (each panelist evaluated each cheese 4 times) using the Cheddar cheese sensory lexicon. Data were analyzed by analysis of variance with Fisher's least significant difference as a post hoc test.

Sensory profiles of low fat Cheddar cheeses

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Control</th>
<th>High Temp.</th>
<th>Pre-acid.</th>
<th>HTxPreAcid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurization</td>
<td>165°F</td>
<td>185°F</td>
<td>165°F</td>
<td>185°F</td>
</tr>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rennet pH</td>
<td>6.7</td>
<td>6.7</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>Flavor Descriptor</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cooked</td>
<td>3.1a</td>
<td>3.1a</td>
<td>3.1a</td>
<td>3.2a</td>
</tr>
<tr>
<td>Whey</td>
<td>3.3a</td>
<td>3.1ab</td>
<td>3.2ab</td>
<td>3.0b</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>1.2a</td>
<td>0.75b</td>
<td>1.3a</td>
<td>1.2a</td>
</tr>
<tr>
<td>Milkfat</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sulfur</td>
<td>1.1a</td>
<td>1.0ab</td>
<td>1.0ab</td>
<td>0.8b</td>
</tr>
<tr>
<td>Brothy</td>
<td>1.3a</td>
<td>1.4a</td>
<td>1.2a</td>
<td>1.3a</td>
</tr>
<tr>
<td>Sweet</td>
<td>1.8a</td>
<td>1.8a</td>
<td>1.8a</td>
<td>1.9a</td>
</tr>
<tr>
<td>Sour</td>
<td>3.1a</td>
<td>3.0ab</td>
<td>3.1a</td>
<td>2.9b</td>
</tr>
<tr>
<td>Salty</td>
<td>4.2a</td>
<td>4.1a</td>
<td>4.1a</td>
<td>4.2a</td>
</tr>
<tr>
<td>Umami</td>
<td>1.7a</td>
<td>1.7a</td>
<td>1.7a</td>
<td>1.7a</td>
</tr>
</tbody>
</table>

Means in row followed by different letters are different (p<0.05).
Attributes not listed (fruity, free fatty acid, catty, nutty, bitter) were not detected in cheeses. Compared to full fat cheese made with the same cultures, the low fat cheeses had more intense cooked flavor, whey flavor, diacetyl flavor and sulfur flavors. They had less milkfat (lactone) flavor. The cheeses made from milk heated to 185°F were observed to have a slight rosey flavor, which in this analysis was incorporated as part of the brothy flavor (as well as beefy, veggie/mushroom, and other flavors) as so was not differentiated from the other cheeses on total brothy flavor.

e. Discussion
The preferred make procedure for making a low fat cheese includes a washing step to increase moisture content and help prevent over acidification, and a pre-acidification also seems to be beneficial for cheese texture, but did not cause an improvement in meltability. Flavor of the cheese still lacks cheddar cheese flavor, and is probably related to a lack of milkfat flavor.

The following procedure can be used for making low fat cheese

<table>
<thead>
<tr>
<th>Milk Fat Content:</th>
<th>0.55%  (True Protein/Fat = 4.8-5.8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurize:</td>
<td>161 F for 15 sec.</td>
</tr>
<tr>
<td>Preacidify:</td>
<td>Add vinegar to cold milk until milk pH = 6.25 (ca. 4000 ml per 1000 L of cold milk)</td>
</tr>
<tr>
<td>Set Temperature:</td>
<td>Warm the milk to 90 F.</td>
</tr>
<tr>
<td>Add Starter:</td>
<td>125% of recommended level of starter, to obtain a set-to-salt time of ca. 3 h.</td>
</tr>
<tr>
<td>Add Color:</td>
<td>Based on preference</td>
</tr>
<tr>
<td>Calcium:</td>
<td>No calcium added</td>
</tr>
<tr>
<td>Renneting:</td>
<td>40 ml DS chymosin per 1000 lb milk</td>
</tr>
<tr>
<td>Cutting:</td>
<td>½” knives cut in vertical direction only, or large cut in enclosed vats.</td>
</tr>
<tr>
<td>Set to Start Cook:</td>
<td>50 min</td>
</tr>
<tr>
<td>Cooking:</td>
<td>Heat to 96 F over 15 min</td>
</tr>
<tr>
<td>Drain:</td>
<td>When curd pH reaches 6.00</td>
</tr>
<tr>
<td>Set to Drain:</td>
<td>ca. 1 h 40 min</td>
</tr>
<tr>
<td>Washing:</td>
<td>After draining the whey, add cold water at 55-60 F, sufficient to lower curd temperature to 78 F (ca. 10% of original milk volume). Leave wash water on for 10 min then drain.</td>
</tr>
<tr>
<td>Dry stir:</td>
<td>Until curd pH = 5.80</td>
</tr>
<tr>
<td>Set to Salt:</td>
<td>3 h 5 min</td>
</tr>
<tr>
<td>Add salt:</td>
<td>0.26 lb salt per 10 lb of curd, two applications 5 min apart.</td>
</tr>
<tr>
<td>Hoop:</td>
<td>27 lb per 20-lb hoop</td>
</tr>
<tr>
<td>Press:</td>
<td>ca. 8 psi (calculated pressure on cheese) overnight.</td>
</tr>
</tbody>
</table>
Target Composition: 53.0% Moisture, 2.0% Salt 5.5% Fat, pH 5.20-5.25

f. Conclusion

Since this cheese can be made without requiring any special equipment, additives, or cultures, etc., this make-procedure will serve as the baseline low fat cheese (when made using Danisco M701 starter culture and ripened at 8°C) for the DMI Low Fat Cheese Flavor Systematic Platform project that will commence in October 2006.

7. Intellectual Property (if applicable)

Please check the applicable box and provide additional explanation, if any.

Yes [ ] No [X] Possible [ ]

These cheesemaking procedures for making lowfat cheese are intended to be placed in the public domain for use by any interested cheese manufacturers.

9. Manuscripts/Abstracts Submitted for Publication: None

10. Published Articles/Abstracts: None
PROJECT PROGRESS REPORT
of the
Western Dairy Center

1. Title of Project: Low Fat Natural Cheese Strategic Platform Study, Objectives 1 & 4

2. Principal investigator: Donald McMahon

3. Start Date: November 15, 2006

4. End Date: December 31, 2008

5. Executive Summary: Develop sufficient scientific understanding of microbiology, flavor and textural attributes of low fat cheese to allow for commercial manufacture of low fat cheese with high consumer acceptance as a table cheese.

Cheese at three different fat levels will be made for a systematic study of how changing cheese composition when removing fat affects flavor development and texture of cheese. In addition, six low fat cheeses will be made based on the strategies proposed for improving quality of low fat cheese to test their effectiveness.


r. Introduction: For successful manufacture and sales of low fat cheese to occur, the cheese industry needs to better understand the effects of micro-environment and make-procedures of low fat cheese on the microbiology, flavor chemistry, and sensory properties of such cheese (Scientific Recommendations for Product Innovation Research of the Low Fat Cheese Expert Panel Meeting held on March 30th and 31st, 2006). And to do so, the influence of fat removal on cheese properties must be assessed through comparison of full fat, 50% reduced fat and low fat cheese made with uniform make - procedures. Such Uniform procedures for cheese making also allows comparison across researchers studying microbial, enzyme, and sensory aspects (Scientific Recommendations for Product Innovation Research of the Low Fat Cheese Expert Panel Meeting held on March 30th and 31st, 2006). In addition, a group of low fat cheeses will be made using the best available innovative strategies will be included in the study. These will not necessarily allow for developed on the knowledge needed for low fat cheese, but they have the potential to rapidly move the research forward if anyone of these innovative strategies produces a marked improvement over the standardized low fat cheese.

In developing high quality low fat cheeses, it is desirable to have flavor and textural properties as similar to full fat cheese as possible. This implies a complete understanding of the mechanisms responsible for flavor and texture. Determining sensory texture of full and low fat cheeses will establish which specific textural properties (sensory terms) are different among low fat and full fat cheeses. A complete analysis of fracture and rheological properties, in addition to pressure sensitive tack, is needed to know the molecular mechanisms responsible for textural. The instrumental measurement of these properties then
need to be related to human sensory texture measurements to understand consumer perceptions of low fat cheese. To provide a linkage between this project and other cheese research the cheeses will also be tested using texture profile analysis.

s. Objective(s)

Objective 1. Manufacture four (4) standardized cheeses (full fat, reduced fat and low fat) and six (6) innovative “best guess” low fat cheeses, store them for 9 months and distribute samples as needed for flavor, chemical, microbial, textural and functional analyses.

Objective 1.1 Determine the make procedure parameters and composition targets for making the full fat (washed), reduced fat, and low fat cheeses.

Objective 1.2 Manufacture at three locations the four standardized cheeses (made according to Objective 1), store for 9 months of aging, and distribute the cheeses as needed by other investigators.

Objective 1.3 Manufacture at each of three locations, a low fat cheese considered to be most suitable for use as a table cheese, using best-current practices based on past experiences at each institution.

Objective 4. Understanding the molecular mechanisms responsible for textural properties of low fat cheese and their relationship to sensory perceptions and physical measurements of cheese texture.

Objective 4.1 Using rheology to understand the molecular mechanisms responsible for textural properties in low fat cheese.

Objective 4.2 Characterize the changes in textural properties of low fat cheese using texture profile analysis in relation to extent of proteolysis occurring during ripening.

Objective 4.3 Characterize the textural properties of low fat cheese as perceived by sensory testing.

t. Method and Materials

Objective 1.1.
Step 1.1: A well defined Lactococcus lactis starter culture to be decided upon and obtained from a culture supplier.
### Step 1.2: Confirm the composition targets for cheese at each fat level:

<table>
<thead>
<tr>
<th>Cheese</th>
<th>%Fat</th>
<th>%FDB</th>
<th>%Moisture</th>
<th>%Salt</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Fat</td>
<td>31.5-33.5</td>
<td>51.0-52.5</td>
<td>36.0-38.0</td>
<td>1.50-1.70</td>
<td>5.10-5.30</td>
</tr>
<tr>
<td>Reduced Fat</td>
<td>16.0-18.0</td>
<td>32.0-34.0</td>
<td>48.0-50.0</td>
<td>1.70-1.90</td>
<td>5.10-5.30</td>
</tr>
<tr>
<td>Low Fat</td>
<td>5.0-6.0</td>
<td>10.5-13.0</td>
<td>52.0-54.0</td>
<td>1.90-2.10</td>
<td>5.10-5.30</td>
</tr>
</tbody>
</table>

### Step 1.3: Manufacture at Utah State University, full-fat (control) cheddar cheese using the amount of starter culture estimated to produce an approx. 4-hour make time (set to salt) with a standard stirred-curd process so as to be within the target composition. Initial testing of make procedures will be using 300-lb of milk one 22.5 lb block of cheese. Modify this make procedure to use a stirred and washed curd method to produce a cheese with the same target composition. Moisture will be measured by microwave oven, salt by chloride analysis, fat by Babcock, pH by glass electrode, and protein by combustion N.

### Step 1.4: Manufacture at Utah State University using 300-lb milk per vat, reduced fat and low cheeses using the same starter culture (Objective 1) using a stirred/washed curd method with compositions as described above. Modify the make procedure if necessary to reach the target composition.

### Step 1.5: The standardized make procedures to be established and adapted for use at all three institutions based on cheese making equipment at each location.

### Objective 1.2.

### Step 2.1: The four standardized cheeses (full fat-control, full fat-washed, reduced fat, and low fat cheeses) to be manufactured at USU and CPSU, and the standardized low fat cheese to be manufactured at UW, based upon available cheesemaking times, and in coordination with the other investigators. Cheese at USU to be made using 1500-lb horizontal cheese vats, cheese at UW to be made using 600-lb open vats, cheese at CPSU to be made using 1,000-lb Kusel open vats. Cheese making to be scheduled on separate weeks at each institution so that testing for flavor, microbial, texture and functionality can be evenly distributed. Approximate planned times are the weeks beginning:

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Univ Wisconsin</th>
<th>Utah State Univ</th>
<th>Cal Poly SLO</th>
<th>make-up*</th>
</tr>
</thead>
</table>

*If any of the standardized cheeses do not meet the specified composition targets they will need to be remade.

### Step 2.2: After manufacture the cheeses are sampled for proximate analysis to ensure they meet the specified targets, vacuum packaged and stored at 8±1°C. One week after manufacture, the cheese blocks are cut into the required number of 1-lb and 2-lb blocks, vacuumed packaged, the first set of samples shipped to
other investigators as needed, with the remainder returned to 8±1°C storage. Further samples are shipped as needed, one week prior to 6 wk, 3 mo, 6 mo, and 9 mo timepoints.

Objective 1.3.
Step 3.1: In consultation among the expert group, six “best-guess” strategies for potentially improving the quality of low fat cheese to be used as a table cheese are selected based on past research on other reduced fat, low fat and nonfat cheeses. This may include use of adjunct cultures, enzymes or other additives, different make procedures and/or different aging regimes.

Step 3.2: Four of these cheeses to be made at UW, and one each at CPSU and USU using the above time line.

Step 3.3: Each of the “best-guess” low fat cheeses to be stored at its preferred aging temperature, and then analyzed, packaged, and sampled on the same timeline as the standardized cheeses as outlined above.

Objective 4.2:
Texture profile analysis (TPA) will be performed at USU and CPSU so as to provide a basis for relating other cheese research to the low fat cheeses produced during this study. At CPSU, TPA will be measured using a TATX2 texture analyzer (Texture Technologies Corp., NY) with a load of 500 N and a flat head plunger. Each sample will be cut into a cube shape (2 cm × 2 cm × 2 cm) will be subjected to 50 and 70% compression for measurement of textural parameters (hardness, springiness, cohesiveness, and gumminess) as described by Bourne et al. (1978). At USU, TPA will be measured using a TATX+ texture analyzer (Texture Technologies Corp., NY) with a 5-kg load cell and a flat head plunger. Samples will be cut into cylinders (height 2.0 cm, diameter 1.6 cm), obtained using a stainless steel borer and brought to room temperature then subjected to 25 and 60% two-bite compression for measurement of the same textural parameters.

A melt flowability test will be performed at USU on the cheeses using a UW-meltmeter. Cheese samples will be cut into disks (thickness 7 mm, diameter 30 mm), and then heated to 65°C and the change in height measured over 20 s as a constant force of 0.33 N is applied on top of the cheese disk.

u. Data or Results

Objective 1: Manufacturing procedures for the full fat, reduced fat and low fat cheeses were developed and are listed below. A single strain culture M70 from Danisco was selected as the starter culture for use in all of the standardized cheese makes.
Full Fat Cheese - Standard:

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Fat Content:</td>
<td>ca. 3.6% (True Protein/Fat = 0.83)</td>
</tr>
<tr>
<td>Pasteurize:</td>
<td>161 F for 15 sec.</td>
</tr>
<tr>
<td>Starter:</td>
<td>110% of recommended level of M70, set to salt time of ca. 3 h.</td>
</tr>
<tr>
<td>Ripen for 45 min. Setting temp:</td>
<td>88 F.</td>
</tr>
<tr>
<td>Color:</td>
<td>60 ml single strength annatto per 1000 lb milk</td>
</tr>
<tr>
<td>Calcium</td>
<td>No calcium added</td>
</tr>
<tr>
<td>Rennet:</td>
<td>40 ml DS chymosin per 1000 lb milk (adjust level based on rennet activity for 30 min cut)</td>
</tr>
<tr>
<td>Cutting:</td>
<td>standard cutting, healing, stirring for cheddar cheese</td>
</tr>
<tr>
<td>Set to Start Cook:</td>
<td>50 min</td>
</tr>
<tr>
<td>Cooking:</td>
<td>Heat to 98 F over 25 min</td>
</tr>
<tr>
<td>Drain</td>
<td>pH 6.0</td>
</tr>
<tr>
<td>Set to Drain:</td>
<td>ca. 2 h 30 min</td>
</tr>
<tr>
<td>Dry stir:</td>
<td>Until curd pH =5.45</td>
</tr>
<tr>
<td>Set to Salt:</td>
<td>ca. 3 h 10 min</td>
</tr>
<tr>
<td>Add salt:</td>
<td>0.24 lb salt per 10 lb of curd, two applications 5 min apart.</td>
</tr>
<tr>
<td>Hoop:</td>
<td>25.5 lb curd per 20-lb hoop.</td>
</tr>
<tr>
<td>Press:</td>
<td>15 psi (calculated pressure on cheese) overnight.</td>
</tr>
<tr>
<td>Moisture:</td>
<td>36.5 ± 1.0%</td>
</tr>
<tr>
<td>Salt:</td>
<td>1.60 ± 1.0%</td>
</tr>
<tr>
<td>Fat:</td>
<td>32.5 ± 1.0% (FDB: 51.0 – 52.5%)</td>
</tr>
<tr>
<td>pH:</td>
<td>5.20 ± 0.10</td>
</tr>
</tbody>
</table>
### Full Fat Cheese - Washed:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Fat Content</td>
<td>ca. 3.6% (True Protein/Fat = 0.83)</td>
</tr>
<tr>
<td>Pasteurize:</td>
<td>161 F for 15 sec.</td>
</tr>
<tr>
<td>Starter:</td>
<td>125% of recommended level of M70, set to salt</td>
</tr>
<tr>
<td></td>
<td>time of ca. 3 h. Ripen for 45 min. Setting temp: 88</td>
</tr>
<tr>
<td></td>
<td>F.</td>
</tr>
<tr>
<td>Color:</td>
<td>60 ml single strength annatto per 1000 lb milk</td>
</tr>
<tr>
<td>Calcium</td>
<td>No calcium added</td>
</tr>
<tr>
<td>Rennet:</td>
<td>40 ml DS chymosin per 1000 lb milk</td>
</tr>
<tr>
<td>Cutting:</td>
<td>standard cutting, healing, stirring for cheddar</td>
</tr>
<tr>
<td></td>
<td>cheese</td>
</tr>
<tr>
<td>Set to Start Cook:</td>
<td>50 min</td>
</tr>
<tr>
<td>Cooking:</td>
<td>Heat to 98 F over 25 min</td>
</tr>
<tr>
<td>Drain</td>
<td>pH 6.0</td>
</tr>
<tr>
<td>Set to Drain:</td>
<td>ca. 2 h 25 min</td>
</tr>
<tr>
<td>Washing:</td>
<td>After draining whey, add warm water at 95 F (10%</td>
</tr>
<tr>
<td></td>
<td>of original milk volume). Leave wash water on for</td>
</tr>
<tr>
<td></td>
<td>5 min then drain.</td>
</tr>
<tr>
<td>Dry stir:</td>
<td>Until curd pH =5.45</td>
</tr>
<tr>
<td>Set to Salt:</td>
<td>ca. 3 h 10 min</td>
</tr>
<tr>
<td>Add salt:</td>
<td>0.24 lb salt per 10 lb of curd, two applications</td>
</tr>
<tr>
<td></td>
<td>5 min apart.</td>
</tr>
<tr>
<td>Hoop:</td>
<td>25.5 lb curd per 20-lb hoop.</td>
</tr>
<tr>
<td>Press:</td>
<td>15 psi (calculated pressure on cheese) overnight.</td>
</tr>
<tr>
<td>Moisture:</td>
<td>36.5 ± 1.0%</td>
</tr>
<tr>
<td>Salt:</td>
<td>1.60 ± 1.0%</td>
</tr>
<tr>
<td>Fat:</td>
<td>32.5 ± 1.0% (FDB: 51.0 – 52.5%)</td>
</tr>
<tr>
<td>pH:</td>
<td>5.20 ± 0.10</td>
</tr>
</tbody>
</table>
### Reduced Fat Cheese

<table>
<thead>
<tr>
<th>Milk Fat Content:</th>
<th>ca. 1.5% (True Protein/Fat = 1.9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurize:</td>
<td>161 F for 15 sec.</td>
</tr>
<tr>
<td>Starter:</td>
<td>125% of recommended level of M70, set to salt time of ca. 3 h. Ripen for 45 min. Setting temp: 88 F.</td>
</tr>
<tr>
<td>Color:</td>
<td>60 ml single strength annatto per 1000 lb milk</td>
</tr>
<tr>
<td>Calcium:</td>
<td>No calcium added</td>
</tr>
<tr>
<td>Rennet:</td>
<td>40 ml DS chymosin per 1000 lb milk. (adjust level based on rennet activity for 30 min) Add same amount of rennet as used for full fat cheddar</td>
</tr>
<tr>
<td>Cutting:</td>
<td>½” knives cut in vertical direction only</td>
</tr>
<tr>
<td>Set to Start Cook:</td>
<td>60 min</td>
</tr>
<tr>
<td>Cooking:</td>
<td>Heat to 98 F over 20 min</td>
</tr>
<tr>
<td>pH</td>
<td>6.0</td>
</tr>
<tr>
<td>Set to Drain:</td>
<td>ca. 2 h 30 min</td>
</tr>
<tr>
<td>Washing:</td>
<td>After draining add cold water (ca. 55 - 60 F, sufficient to lower curd temperature to 78 F (ca. 10 % of original milk volume. Leave wash water on for 10 min then drain</td>
</tr>
<tr>
<td>Dry stir:</td>
<td>Until curd pH =5.75</td>
</tr>
<tr>
<td>Set to Salt:</td>
<td>3 h 5 min</td>
</tr>
<tr>
<td>Add salt:</td>
<td>0.25 lb salt per 10 lb of curd, two applications 5 min apart.</td>
</tr>
<tr>
<td>Hoop:</td>
<td>26 lb per 20-lb hoop.</td>
</tr>
<tr>
<td>Press:</td>
<td>ca. 8 psi (calculated pressure on cheese) overnight.</td>
</tr>
<tr>
<td>Moisture:</td>
<td>47.0 ± 1.0%</td>
</tr>
<tr>
<td>Salt:</td>
<td>1.80 ± 0.10%</td>
</tr>
<tr>
<td>Fat:</td>
<td>17.0 ± 1.0% (FDB: 30 - 33%)</td>
</tr>
<tr>
<td>pH:</td>
<td>5.20 ± 0.10</td>
</tr>
</tbody>
</table>
### Low Fat Cheese:

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk Fat Content:</td>
<td>0.55% (True Protein/Fat = 4.8-5.8)</td>
</tr>
<tr>
<td>Pasteurize:</td>
<td>161 F for 15 sec.</td>
</tr>
<tr>
<td>Preacidify:</td>
<td>Add vinegar until milk pH = 6.25 ca. 4000 ml per 1000 ml of cold milk then warm the milk to 90 F.</td>
</tr>
<tr>
<td>Starter:</td>
<td>125% of recommended level of M70, set to salt time of ca. 3 h. Ripen for 45 min. Setting temp: 88 F.</td>
</tr>
<tr>
<td>Color:</td>
<td>60 ml single strength annatto per 1000 lb milk</td>
</tr>
<tr>
<td>Calcium</td>
<td>No calcium added</td>
</tr>
<tr>
<td>Rennet:</td>
<td>40 ml DS chymosin per 1000 lb milk. Add same amount of rennet as used for full fat cheddar</td>
</tr>
<tr>
<td>Cutting:</td>
<td>½” knives cut in vertical direction only</td>
</tr>
<tr>
<td>Set to Start Cook:</td>
<td>50 min</td>
</tr>
<tr>
<td>Cooking:</td>
<td>Heat to 96 F over 15 min</td>
</tr>
<tr>
<td>Drain</td>
<td>pH 6.00</td>
</tr>
<tr>
<td>Set to Drain:</td>
<td>ca. 1 h 40 min</td>
</tr>
<tr>
<td>Washing</td>
<td>After draining add cold water at 55-60 F, sufficient to lower curd temperature to 78 F (ca. 10 of original milk volume). Leave wash water on for 10 min then drain.</td>
</tr>
<tr>
<td>Dry stir:</td>
<td>Until curd pH = 5.80</td>
</tr>
<tr>
<td>Set to Salt:</td>
<td>3 h 5 min</td>
</tr>
<tr>
<td>Add salt:</td>
<td>0.26 lb salt per 10 lb of curd, two applications 5 min apart.</td>
</tr>
<tr>
<td>Hoop:</td>
<td>27 lb per 20-lb hoop.</td>
</tr>
<tr>
<td>Press:</td>
<td>ca. 8 psi (calculated pressure on cheese) overnight.</td>
</tr>
<tr>
<td>Moisture:</td>
<td>53.0 ± 1.0%</td>
</tr>
<tr>
<td>Salt:</td>
<td>2.00 ± 0.10%</td>
</tr>
<tr>
<td>Fat:</td>
<td>5.50 ± 0.05%</td>
</tr>
<tr>
<td>pH:</td>
<td>5.20 ± 0.10</td>
</tr>
</tbody>
</table>

For the “best guess” low fat cheese made at Utah, the M70 starter culture was replaced with Chr. Hansens DVS 850, CR319 and LH32 cultures.
Cheeses were made on the following dates with the composition as shown, samples sent to our collaborators for analysis, with the remainder of the cheese in storage at 8°C.

<table>
<thead>
<tr>
<th>Fat Level</th>
<th>Treatment</th>
<th>Rep</th>
<th>Made</th>
<th>Moisture</th>
<th>Fat</th>
<th>pH</th>
<th>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>10/10/06</td>
<td>53.68</td>
<td>5.5</td>
<td>5.10</td>
<td>1.8</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>850/LH32</td>
<td>1</td>
<td>10/11/06</td>
<td>54.3</td>
<td>4.8</td>
<td>5.07</td>
<td>1.92</td>
</tr>
<tr>
<td>RED FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>10/12/06</td>
<td>48.78</td>
<td>15.25</td>
<td>5.15</td>
<td>1.78</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>10/03/06</td>
<td>38.6</td>
<td>31.5</td>
<td>5.22</td>
<td>1.61</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>WASHED</td>
<td>1</td>
<td>10/04/06</td>
<td>38.4</td>
<td>31.75</td>
<td>5.20</td>
<td>1.60</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>11/01/06</td>
<td>53.13</td>
<td>4.5</td>
<td>5.30</td>
<td>1.53</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>850/LH32</td>
<td>2</td>
<td>10/31/06</td>
<td>54.54</td>
<td>4.0</td>
<td>5.21</td>
<td>1.48</td>
</tr>
<tr>
<td>RED FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>11/28/06</td>
<td>47.98</td>
<td>16.5</td>
<td>5.14</td>
<td>1.95</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>11/15/06</td>
<td>37.7</td>
<td>32.0</td>
<td>5.20</td>
<td>1.83</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>WASHED</td>
<td>2</td>
<td>11/14/06</td>
<td>37.7</td>
<td>32.2</td>
<td>5.16</td>
<td>1.77</td>
</tr>
</tbody>
</table>

Cheeses have been sent for testing at 2 weeks, 6 weeks and 3 months.

Objective 4.
Cheese samples were sent for calcium (minerals) and protein (N) analysis.

<table>
<thead>
<tr>
<th>Fat Level</th>
<th>Treatment</th>
<th>Rep</th>
<th>Ca</th>
<th>H2Po4</th>
<th>PROT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOW FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>0.91</td>
<td>1.44</td>
<td>35.0</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>850/LH32</td>
<td>1</td>
<td>0.84</td>
<td>1.50</td>
<td>33.9</td>
</tr>
<tr>
<td>RED FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>0.76</td>
<td>1.60</td>
<td>29.9</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>STANDARD</td>
<td>1</td>
<td>0.69</td>
<td>1.91</td>
<td>24.6</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>WASHED</td>
<td>1</td>
<td>0.71</td>
<td>1.78</td>
<td>24.9</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>0.84</td>
<td>1.38</td>
<td>33.4</td>
</tr>
<tr>
<td>LOW FAT</td>
<td>850/LH32</td>
<td>2</td>
<td>0.89</td>
<td>1.28</td>
<td>31.8</td>
</tr>
<tr>
<td>RED FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>0.79</td>
<td>1.60</td>
<td>29.3</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>STANDARD</td>
<td>2</td>
<td>0.66</td>
<td>1.75</td>
<td>33.4</td>
</tr>
<tr>
<td>FULL FAT</td>
<td>WASHED</td>
<td>2</td>
<td>0.61</td>
<td>1.88</td>
<td>31.8</td>
</tr>
</tbody>
</table>

The differences in textural properties of the cheeses can be seen by the shape of the TPA force versus time curves as shown below. These are an indication of how the cheese responds to increasing strain as it is compressed. Full fat cheddar cheeses typically undergo fracturing during compression which is observed as a linear increase in stress as strain increases, a drop in stress at a specific point (as shown in Fig 1 at arrow 3), or a flattening of the force-time curve as stress is released. Figure 1 is a TPA graph of the full fat standard cheese tested using 60% compression, and shows a fracture strain occurring at 48% compression. Typically, as the cheese ages the fracture occurs at less strain. Such fracturing results in the sample regaining very little of its original height, and the second compression peak being much smaller than the first peak, this is measured as the cheese having a low cohesiveness value.
Figure 1. TPA graph of full fat cheddar cheese tested after 3 months of aging at 8°C.

When the fat content is reduced, the cheese is less likely to fracture and the cheeses having more cohesiveness. This is apparent from the shape of the TPA curves of the reduced fat and low fat cheeses shown in Fig. 2 and Fig. 3 respectively.

Fig. 2. TPA graph of reduced fat cheese tested after 6 wk of storage at 8°C.
Fig. 3. TPA graph of low fat cheese (standard) tested after 2 wk of storage at 8 C.

For the reduced fat and low fat cheeses, the stress induced by compression of the cheese sample continues to increase (exponentially) until the 60% compression is reached. These cheeses retain much of their internal integrity after the compression, and the sample returns to 80% or more of its original height once the strain is released. These cheeses thus have a higher cohesiveness value. This relates to the physical observations about the texture deficiency of low fat cheese in that they are too springy or rubbery, and do not break down in the mouth the same way as a full fat cheese. The interpretation of the TPA data is that to correct this problem there needs to be introduced into the low fat cheese protein matrix, points of interruption to the protein matrix that will allow slippage (fracture) to occur as the cheese is compressed.

7. Intellectual Property (if applicable). Please check the applicable box and provide additional explanation, if any.

Yes [ ]
No [ X ]
Possible [ ]

8. Anticipated Delays or Problems:

Not all the cheeses were analyzed for texture and melting at 2 wks and 6 wks because of a staffing problem and the focus being on making each of the five cheeses within the target specifications. That has been rectified for the 3-month analysis time that will start the first week of January.

No further anticipated delays.

9. Manuscripts/Abstracts Submitted for Publication:
The following abstract was submitted for the DMI Symposium at the 2007 ADSA meeting.

**Improving the Quality of Low Fat Cheese**  
**D.J. McMahon**

With the renewed interest in low fat foods, a DMI-funded collaborative project was undertaken to systematically study the differences between full fat and low fat cheddar cheese, with the aim of providing a basis for improving the flavor and texture of low fat cheeses. A method that included pre-acidification of milk to pH 6.25 and washing curd with cold water was developed to make a low fat cheese with 52 to 54% moisture and pH 5.15 to 5.25. A 50%-reduced fat cheese and a full fat Cheddar cheese were made without preacidification but with curd washing. A full fat Cheddar cheese without curd washing was also made. These cheeses were aged at 8 C and tested at 2 wk, 3 and 6 mo of aging. This presentation will present findings from analysis of these cheeses comprising sensory flavor, flavor chemistry, rheology, sensory texture, melting, bacterial microflora, and cheese biochemistry.

10. Published Articles/Abstracts: None
Title of Project: Low Fat Natural Cheese Strategic Platform Study. Objective 3.

Principal investigator: Jeff Broadbent

Start Date: November 15, 2006

End Date: December 31, 2008

Executive Summary: The important chemical attributes of cheese that influence microbial activity as well as the starter and nonstarter lactic acid bacteria populations will be studied in full fat, reduced fat, low fat and nonfat cheeses.


Introduction: There is a lack on information and scientific understanding related to the flavor defects that are prominent in lowfat cheese. A key to understanding why flavor development in low fat cheese is so different to that which occurs in full fat cheese (for which there is a large knowledge base accessible by scientists and the cheese industry) is to determine the differences in microbial populations and microbial activities between cheeses with different fat levels. This project addresses the research target of better understanding the effects of micro-environment and make-procedures of lowfat cheese on the microbiology and consequent flavor chemistry and flavor sensory properties of such cheese (Scientific Recommendations for Product Innovation Research of the Low Fat Cheese Expert Panel Meeting held on March 30th and 31st, 2006).

Objective (s)

Objective 3. Characterize important microbiological and chemical attributes in full fat, reduced fat, and low fat Cheddar cheeses made with a single, defined Lactococcus lactis starter culture.

Objective 3.1. Measure the starter and nonstarter lactic acid bacterial populations in the cheeses and how they change during 9-mon storage of the cheese.

Method and Materials

Objective 3.1:

Cheese made at each of the three institutions are being analyzed at USU for starter and NSLAB populations on wk 2, wk 6, 3 mo, 6 mo and 9 mo.
Lactococcal starter are enumerated on Ellikers agar incubated at 30°C, and NSLAB populations will be enumerated on Rogosa SL agar incubated at 37°C. In addition, cheese extracts will be plated on M17 agar and incubated at 45°C to determine the presence of adventitious streptococci.

Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA fragments will be performed at Utah State University on all cheeses using procedures already established in the Broadbent lab. Briefly, the variable V3 region of the 16S rDNA in cell pellets or DNA extracted from cheese is amplified by PCR using primers that are complementary to conserved regions of the eubacterial 16S rRNA gene. The fragments are then separated in a Denaturing Gradient Gel Electrophoresis System (CBS Scientific Company, Solana Beach, CA, USA) using an 8% (wt/vol) acrylamide gel (in 1X TAE: 40 mM Tris acetate [pH 7.4], 20 mM sodium acetate, 1mM Na2-EDTA) with a chemical gradient from 25 to 60% denaturant. After electrophoresis, the gels are stained with SYBR Gold solution (Molecular Probes Inc., Carlsbad, CA, USA) then photographed using a UV transilluminator). Bands of interest are excised and placed in separate tubes, then DNA is eluted from the gel by the procedure outlined by Koo and Jaykus (2000). DNA eluted from the excised DGGE bands is re-amplified by PCR with the original primer pair, purified, then cloned into *E. coli* and sequenced. Sequences are compared against the nucleotide database to determine the species from which each band was derived.

### y. Data or Results

Under the strategic platform project, the following cheeses were all made with the same culture, standardized make procedures, and standardized compositions, to investigate the effect of fat content on flavor, texture, microbiology:

- **Cheese A.** Full fat cheddar made using stirred curd method
- **Cheese B.** Full fat cheddar with the same proximate composition as Cheese A but made using stirred curd method with a water washing step.
- **Cheese C.** 50% reduced fat cheddar made using stirred curd method with washing step
- **Cheese D.** Low fat cheddar made using stirred curd method with washing step plus use of preacidification of milk prior to renneting.

Cheeses made at USU include duplicate vats of cheese A (made Oct. 3, Nov. 15), B (Oct. 4 and Nov. 14), C (Oct. 12 and Nov. 21) and cheese D (Oct. 10 and Nov. 1); UW cheese include duplicate samples of cheese A (made Feb. 2), B (Nov. 15) and cheese D (Oct. 10 and Oct. 31); Cal-Poly products include cheese A (Nov. 21 and Feb. 6), B (Nov. 21 and Feb. 6), C (Oct. 26 and Nov. 15), and D (Oct. 26 and Nov. 14).

Microbiological sampling for starters and NSLAB has been performed on all A, B, C, and D cheeses manufactured to date at the assigned sampling intervals (2wk, 6 wk, 3, 6, 9 mo). We also collected cells from plates incubated at 10, 30, or 45°C for later DGGE analysis, and have also extracted DNA samples from these cheeses for DGGE. We still have a lot of sampling to do, but looking at micro data thus far suggests that initial NSLAB levels are generally much lower in A&B cheese versus C&D in product made at all 3 sites. In
addition, starter die-off (autolysis?) seems to occur at a more rapid rate in A&B cheese versus C&D in product made at all 3 sites

7. Intellectual Property (if applicable). Please check the applicable box and provide additional explanation, if any.

   Yes [ ]        No [ X ]        Possible [ ]

8. Anticipated Delays or Problems

   None

9. Manuscripts/Abstracts Submitted for Publication

   None

10. Published Articles/Abstracts

    None
PROJECT PROGRESS REPORT
of the
Western Dairy Center

1. Title of Project: **Formulation of extrusion-textured whey products low in carbohydrates**

2. Principal investigator: **Marie Walsh**

3. Start Date: 1-1-05

4. End Date: 6/30/07

5. Executive Summary:

We are concluding the statistical analysis on the textured whey extrudates (TWP) produced as described below. We have had to repeat several analyses including the total soluble protein and functional performance ratio. We have also included new controls of commercial TVP and TSP and will compare these samples to ours.

The textured why extrudates (TWP) produced at the three fiber levels as described below are being analyzed for solids lost, water holding capacity, functional performance ratio, water soluble protein, total soluble protein, and water soluble carbohydrate. The data will be analyzed by ANOVA to complete this study.

Previous summary: Textured whey extrudates as a meat analog (TWP) consisting of 50.4% whey protein with fiber levels of 0%, 7%, 15%, and 22% (w/w) were extruded in triplicate producing 30 TWP samples. The three different fibers used were (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC all from J.Rettenmaier USA LP, Schoolcraft, MI) The chemical analysis of these samples is currently being conducted and the data will be analyzed by ANOVA.

Previous Summary: Each extrudate produced at three different fiber levels (18, 36, and 48% total dietary fiber (TDF) for three fiber types ((Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC all from J.Rettenmaier USA LP, Schoolcraft, MI) has been analyzed for physical and chemical properties. The
physical and chemical characteristics of the extrudates were found to be greatly affected by combined interaction of the fiber type and level of fiber in the extrudate. As the amount of fiber in the extrudate increased, moisture content increased \( (p < 0.0001) \) which caused a decrease in expansion ration \( (p < 0.0001) \), air cell size \( (p < 0.0001) \), and water solubility index \( (p = 0.0013) \) and increased extrudate density \( (p < 0.0001) \), breaking force \( (p < 0.0001) \) and water absorption index \( (p < 0.0001) \). Extrudates with 18% TDF were comparable to extrudates with 0% TDF (control) for each of the three fibers used. Extrudates with 48% were significantly different than the control (0% TDF). It is concluded that 48% fiber addition under the aforementioned extrusion conditions is too high for product requirements of low extrudate density and high expansion ratio. Extrudates containing TDF as high as 36% may be acceptable for some products, such as chips, crackers, and other snack foods.

For the textured whey protein as a meat analog (TWP) three fiber types were chosen (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC and all from J. Rettenmaier USA LP, Schoolcraft, MI) from the 4 stated below to complete the second half of this project. Each fiber was blended with WPC 80, fiber and starch to yield compositions of 50.4% protein with fiber levels of 0%, 7%, 15%, and 22% \( (w/w) \) and extruded in triplicate producing 30 TWP samples. Each of these TWP samples is now being analyzed for physical and chemical properties and data will be analyzed by ANOVA.

Previous Summary: Research conducted for this reporting period included extruding blends of three different fibers (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC all from J. Rettenmaier USA LP, Schoolcraft, MI) at 3 levels in triplicate yielding 18, 36, and 48% total dietary fiber in extrusion-expanded whey protein products containing 32% protein. Each extrudate produced (control and treatments totaling 28 samples) was analyzed for breaking strength, density, air cell diameter, expansion ratio, moisture, water adsorption index, water solubility index, water soluble protein, total soluble protein and water soluble carbohydrate. This data is currently being analyzed by ANOVA.

In addition, four fibers (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, Vitacel Apple fiber AF 401, and Vitacel Powdered Cellulose L601 FCC and all from
J. Rettenmaier US/A LP, Schoolcraft, MI) were blended with WPC 80 and normal cornstarch to yielding blends of 60% WPC 80 (final protein concentration of 48%), 30% cornstarch, and 10% fiber. These blends were extruded using extrusion parameters to produce a fibrous-textured product. The extrudates will be evaluated with respect to ease of extrusion and solubility and three fibers will be selected to complete objective 1 of this project.

Previous Summary. Research conducted for this reporting period has focused on screening 12 different commercially fibers in an extruded-expanded puffed product consisting of approximately 30% whey protein.


Please identify the reporting period. This report must contain all research progress accomplished up to this reporting period. Works performed in this reporting period must be clearly identified.

Introduction

Our current research involves the texturization of whey protein concentrate and cornstarch blends to produce different types of extruded whey protein products (fibrous texture for use as a meat extender or meat analog and a puffed snack product). We would like to investigate the production of extrusion-textured whey-based products that will fit into the current high protein/low carbohydrate consumer market by replacing the cornstarch in our formulation with a high fiber polysaccharide such as oat bran, Benefiber (Novartis) or a non-caloric fiber such as carboxymethylcellulose.

Objective (s)

Objective 1: Investigate the substitution of cornstarch with 3 different polysaccharides (oat bran, Benefiber, and carboxymethylcellulose) at four levels (0, 30, 60, and 100% wt/wt) in extrusion-textured whey protein (TWP). TWP samples will be analyzed for chemical (water holding capacity, soluble protein, soluble carbohydrate) and physical (extrudate breaking strength and diameter) characteristics and compared to our TWP standard.

Objective 2: Investigate the substitution of cornstarch with 3 different polysaccharides (oat bran, Benefiber, and carboxymethylcellulose) at four levels (0, 30, 60, and 100% wt/wt) in extrusion-expanded whey protein snacks. Expanded samples will be analyzed for chemical (water holding capacity, soluble protein, soluble carbohydrate) and physical (extrudate breaking, air cell diameter, expansion ratio and diameter) characteristics and compared to our expanded snack standard.
Method and Materials

Project overview.

For this project, initially 12 different fibers were evaluated using extruder parameters set to produce an expanded extrudate (Objective 2). The fibers were blended with WPC 80 and normal cornstarch (Argo, Memphis, TN) to yield compositions of approximately 30% whey protein, 15 to 30% dietary fiber, and the remainder cornstarch. Three fibers were selected from the 12 different fibers based on ease of extrusion, commercial availability and radial expansion to continue with objective 2. Four fibers were selected from the 12 tested will be blended with WPC 80, normal cornstarch, and extruded under conditions to produce a fibrous-textured product. Three fibers from the four tested will be used to complete objective 1.

Whey protein extrusion

To produce an extruded-expanded sample, fibers were extruded in random order employing a bench-top scale APV Baker MP-19TC twin-screw extruder (APV Baker, Inc., Grand Rapids, MI). Dry feed was added to the extruder and mixed with liquid feed (0.1 M NaOH) in the barrel (Hale, 2000). Liquid feed rate was held constant at 5.6 g / min. Die temperature and temperature zones along the barrel and were controlled and monitored with CAL3200 Autotune temperature controllers (Cal Controllers, Inc., Libertyville, IL). The four barrel temperature zones 25, 25,115, 135°C were set for all extrusion runs. Die temperature or temperature of extrudate exiting the die was set for 145°C. Other controllable extrusion conditions of dry feed rate, and screw speed, were initially set (dry feed rate 500 rpm, and screw speed 200rpm) with some minor variations due to extrusion optimalization of each dietary fiber sample. Optimized extrusion was obtained with absence of product surging indicating a state of equilibrium and minimal elastic recoil of expansion of exiting extrudate. Torque and pressure was measured using an NRC120 Safeguard Meter (Anders Electronics, London, UK) and an EPR3 3M-6M561 pressure transducer (Dynisco Instruments, Franklin, MA), respectively. The exit die was conical with a 2.5 mm diameter.

The composition of the 12 blends used to screen the fiber samples is given in Table 1. Three of the fibers will be used to produce blends containing 18, 36, and 48% TDF and 32% protein.

The extruded puffed product was produced containing 32% total protein and 50% normal cornstarch and 10 % BAKA-SNAK (National Starch and Chemical,
Bridgewater, NJ) as the standard control. Sufficient BAKA-SNAK was used to help with "puffing" of the product and incorporated at a constant 10% (w/w) for each blend (Allen, 2004). Three different fibers selected were separately blended with the cornstarch, BAKA-SNAK and WPC80 to form final % total dietary fiber (%TDF) levels of 16, 32 and 48% (wt/wt). Adjustments were made in formulations to account for different % TDF content of each fiber sample. Fiber content levels of extrudates were based on estimated 30, 60, and 80% replacement of combined cornstarch and BAKA SNA.K volume with each fiber type selected. The dry mixed blend had a final total protein content of 32% from WPC80. Each extrudate produced (control and treatments totaling 28 samples) will be analyzed for breaking strength, density, air cell diameter, expansion ratio, moisture, water adsorption index, water solubility index, water soluble protein, total soluble protein and water soluble carbohydrate. The samples will be produced and analyzed in triplicate.

Extrusion of fibrous-textured whey protein was performed based on procedures outlined by Hale et al. (2001) and Taylor et al. (2001). The standard extrudate will be extruded on an APV Baker MPF 19 twin screw extruder (Grand Rapids, MI) from a dry mix containing 66% WPC 80 (Grande Ingredients, WI) and 33% cornstarch (Argo, Memphis, TN). Sodium hydroxide (0.2M) will be the liquid source and fed into the barrel at a rate of 8 g/min while the dry mix will be fed at a rate of 9 g/min. Extruder screws and paddles will promote laminar flow. Extrudates exiting the extruder barrel and cooling die at a minimum of 160 C will be collected and dried overnight before sealing in airtight plastic bags.

Three fibers selected will separately be blended with the cornstarch to form fiber: starch ratios of approximately 30, 60 and 100% (wt/wt). The starch:fiber blends will be used to produce the fibrous-textured samples. The samples will be analyzed for water holding capacity, soluble protein, soluble carbohydrate, breaking strength and diameter and compared to each other and the controls using Analysis of Variance. The samples will be produced and analyzed in triplicate.

Extrudate Analysis.

Physical tests. Average Air Cell Diameter. Samples were embedded in melted household wax (Parowax, Roswell, Geo.) and allowed to cool. Embedded extrudates were cut lengthwise as close to the center as possible with a razor to expose the
longitudinal cross section. Images of the cross section were taken using a stand-
mounted digital camera (Nikon Coolpix 5700). Camera settings included a focal length
of 15.7 mm, a Fine picture setting, F3.6 and a 2560 x 1920 pixel resolution. Paper
squares with known areas of 1, 0.5 and 0.25 cm² area were placed along-side the
extrudate and photographed with every extrudate cross sectional image.

Twelve, clear air cells from the images of the cross sections were randomly selected
and analyzed using Adobe PhotoShop (Adobe Systems Inc., San Jose, Calif.). Air cell
surface area was outlined with the Magnetic Lasso generating a total pixel count within
the outlined circumference. Air cell size was calculated from the pixel count using a
standard curve made from the pixel count of known areas of the squares photographed
earlier with the extrudates.

Breaking Strength and Expansion Ratio. A Salter 235 shear device with a Warner-
Bratzler shear cell (GR Electric Manufacturing, Manhattan, Kan.) was used to provide
shear values for breaking strength determination of the extrudates. Ten extrudates
were randomly selected from each starch: fiber ratio and sheared. Extrudate length and
diameters at point of shear where measured with calipers. Sheared, measured
extrudate fragments were weighed. Breaking strength force was calculated using the
following equation:

\[
\text{Force (Pa) } = (9.7865 \text{ N/kg}) \times 1000 \times \frac{\pi \text{ (extrudate diameter (mm)/2)}^2}{\text{breaking strength (kg)}}
\]

The ratio of cross sectional area of each extrudate (CS) to the area of the die exit was
used for expansion ratio calculation. Ten values for obtained for randomly selected
extrudate samples.

Extrudate Density. A gravimetric displacement method was not used to determined
extrudate density for concern of media entering the extrudate’s air cells and the effect
on volume measurements (Allen, 2004). Instead, a simple mathematical formula was
used:

\[
\text{Density (g/cm}^3\text{) } = \frac{\text{segment weight (g)}}{\text{Segment length (mm) \pi (d_{ave}(mm)/2)^2}}
\]

where \(d_{ave}\) is the average of the initial and final diameters of a given extrudate segment
taken at point of shear. Length, and weight measurements of each extrudate sample
used for breaking strength determination where used for density calculation.
Chemical tests. Moisture Determination. Four 20 second samples randomly collected during extrusion and weighed for product flow determination were used for moisture determination. The first and third samples were immediately dried overnight for at least 16 hours at 70 °C in a drying oven while the second and third samples were then dried 24 hours later under same conditions. Pans were allowed to cool and were weighed. Moisture content was calculated as a percent of the weight difference before and after drying.

Water Adsorption Index (WAI) and Water Solubility Index (WSI). The water adsorption index (WAI) and water solubility index (WSI) were determined using minor modifications to procedures as described by Jin et al. (1995). Samples were ground in an Osterizer Galazie blender for 30 seconds. The finely ground sample was then sifted through a #16 sieve and through a #60 sieve. Particles that passed through both sieves were used for WAI and WSI determination. Into a tarred centrifuge tube, approximately 0.5 g of ground sample was weighed and 5.0 ml of distilled water was added. The mixture was sealed, immediately inverted and allowed to hydrate for 15 minutes. The sealed tube was inverted every 5 minutes to ensure proper mixing. Samples were centrifuged for 15 minutes at 1000 x g using a Sorvall RC-5B fixed angle rotor (DuPont Instruments, Wilmington, Del.). The resultant supernatant was decanted into a pre-weighed aluminum dish, allowed to dry overnight in a drying oven (70 °C) and then re-weighed. (Centrifuge tubes were reweighed after supernatant removal to determine the sediment weight. WAI and WSI values were calculated as shown by Onwulata et al. (1998). WAI values were reported as grams of water absorbed per 100 grams sample. However, WSI values are reported as grams of sample solubilized per 100 grams sample. Analysis was done in triplicate for each sample.

Water Soluble and Total Soluble Protein and Soluble Carbohydrate. Extrudate samples were prepared as described for WAI and WSI except sample portions that passed through a #16 sieve and retained in a #60 sieve were used for analysis. For each ground sample, two portions of approximately 0.2 g were added into a 15-ml centrifuge tube. Into one tube a 10-ml solution of 1-% w/v sodium dodecyl sulfate and 1-% beta-mercaptoethanol (SDS/BME) was added; into the other, 10-ml of distilled water was added. The sealed tubes were rocked overnight on a laboratory rocker (Rocking Platform 200, VWR Scientific, Bristol, Conn.) on a rock setting of 4. The samples were centrifuged for 15 min at 5000 x g, filtered through Whatman 4 glass fiber filters, and...
analyzed for water soluble and total soluble protein using a modified Lowry protein assay (Pierce, Rockford, Ill.) with bovine serum albumin as standard (Taylor et al., 2006). SDS/BME filtrates were diluted 1:11, distilled water filtrates were not. Each sample filtrate was analyzed in triplicate. Values for distilled water filtrates were used for water soluble protein determination and SDS/BME filtrates provided values for total soluble protein determination. The distilled water filtrates were diluted 1:20 and water soluble carbohydrate analysis was performed using a phenol/sulfuric acid method or the Dubois Assay (Dubois et al., 1956) with glucose used as standard. Each sample filtrate was analyzed in triplicate. Sample filtrates of SDS/BME solution were not used for analysis due to interference with the colorimetric glucose assay (Allen, 2004).

Data or Results
The twelve different fibers screened for suitability in an extruded product are listed below at the compositions given in table 1.

a. Vitacel Orange fiber OF400 (J.Rettenmaier USA LP, Schoolcraft, MI)
b. Vitacel Apple fiber AF 401 (J.Rettenmaier USA LP, Schoolcraft, MI)
c. Vitacel Oat fiber HF 600 (J.Rettenmaier USA LP, Schoolcraft, MI)
d. Vitacel Oat fiber HF 401 (J.Rettenmaier USA LP, Schoolcraft, MI)
e. Vitacel Wheat Fiber WF 600 (J.Rettenmaier USA LP, Schoolcraft, MI)
f. Vitacel Powdered Cellulose L601 FCC (J.Rettenmaier USA LP Schoolcraft, MI)
g. Oatvantage Oat fiber (Nuture Advanced Oat Technologies, Devon, PA)
h. Cargill Corn fiber Maizewise 60 (Cargill, Indianapolis, IN)
i. Cargill Corn fiber Maizewise 80 (Cargill, Indianapolis, IN)
j. Fibersol-2 (ADM Specialty Ingredients Division, Decatur, IL)
k. Litesse (Danisco Sweeteners, Ardsley, New York)
l. Oat Fiber X (Roman Meal Milling Company, Tacoma, WA)
### Table 1. Composition of dry blends used for extrusion.

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Protein</th>
<th>Fiber Sample</th>
<th>Cornstarch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange fiber OF400</td>
<td>42%</td>
<td>15.5%</td>
<td>20%</td>
</tr>
<tr>
<td>Apple fiber AF 401</td>
<td>38%</td>
<td>15%</td>
<td>24%</td>
</tr>
<tr>
<td>Oat fiber HF 600</td>
<td>38%</td>
<td>24%</td>
<td>37%</td>
</tr>
<tr>
<td>Oat fiber HF 401</td>
<td>44%</td>
<td>28%</td>
<td>13%</td>
</tr>
<tr>
<td>Wheat Fiber WF 600</td>
<td>39%</td>
<td>24%</td>
<td>25%</td>
</tr>
<tr>
<td>Powdered Cellulose L601 FCC</td>
<td>37%</td>
<td>20%</td>
<td>33%</td>
</tr>
<tr>
<td>Oatvantage Oat fiber</td>
<td>38%</td>
<td>36.5%</td>
<td>16%</td>
</tr>
<tr>
<td>Cargill Corn fiber Maizewize 60</td>
<td>36%</td>
<td>18%</td>
<td>23%</td>
</tr>
<tr>
<td>Cargill Corn fiber Maizewize 80</td>
<td>32%</td>
<td>40%</td>
<td>16%</td>
</tr>
<tr>
<td>Fibersol-2</td>
<td>40%</td>
<td>40%</td>
<td>10%</td>
</tr>
<tr>
<td>Litesse</td>
<td>40%</td>
<td>38%</td>
<td>8.3%</td>
</tr>
<tr>
<td>Oat Fiber X</td>
<td>26%</td>
<td>55%</td>
<td>11%</td>
</tr>
</tbody>
</table>

Three fiber sources were not successfully extruded: Oat fiber X, Litesse, and Fibersol-2 fiber sources. Unsuccessful extrusion was determined by seizing of extruder before extrusion parameters could be obtained and or inability to consistently form an extrudate. Apparent radial expansion was best seen in the extrudates extruded from Vitacel Oat fiber HF600, Vitacel Apple fiber AF401, Cargill Corn fiber Maizewize 60, Vitacel Powdered Cellulose L601 FCC, and Vitacel Wheat fiber HF600. For this experiment, samples with similar %TDF content were desired. Vitacel Apple fiber AFF401 and Cargill Corn fiber Maizewize 60 only contained 60% TDF. Vitacel Powdered Cellulose L601 FCC was considered 100% TDF, and Vitacel Oat fiber HF600 and Vitacel Wheat fiber WF600 had 96 and 97% TDF, respectively. Cargill Corn fiber Maizewize 60 was in the prototype stages and sufficient quantities of fiber sample could not be obtained. Based on the observations stated above, Vitacel Powdered Cellulose L601 FCC, Vitacel Oat fiber HF600 and Vitacel Wheat Fiber WF600 were then selected to complete objective 2.

**Extrudate Physical Parameters**

Extrudate characteristics exhibited highly statistical differences based on the amount of fiber in the extrudate (18, 36, 48%) and to some degree by the type of fiber (powdered cellulose, wheat fiber and oat fiber). Generally, as the amount of fiber in the
extrudate increase, negative effects on physical parameters were observed. These findings were consistent with previous research (Onwulata et al., 2000; Lue et al., 1991; Rinaldi et al., 2000). Expansion ratio was strongly influenced by fiber level (p < 0.0001) in the extrudate and the fiber level/fiber type interaction (p < 0.0001), while air cell size was significantly influenced (p < 0.0001) only by fiber level.

Expansion ratio (Fig. 1) and air cell size (Fig. 2) both decreased with an increase of the amount of fiber present in the extrudate. Powdered cellulose had the lowest expansion and wheat fiber had the highest expansion ratio with 18% fiber. However, as fiber increased to 36 or 48%, powdered cellulose had the highest expansion ratio. There was no difference between air cell size of the extrudates at all TDF levels. The 48% TDF level was significantly different from the other fiber levels for both expansion ratio and air cell size, except for wheat fiber. No expansion was observed at the 48% TDF level, nor were there any air cells present. There was more of a layered effect observed in the extrudate interior for all samples. Generally, as the amount of fiber increased in the extrudates, regardless of fiber type, extrudate air cells were smaller and more numerous. Fiber particle size has been shown to reduce air cell size and reduce extrudate expansion (Moore et al., 1990; Lue et al., 1991; Huber, 2001). Powdered cellulose may have more air cells present than the other fiber types and less unexpanded material despite no differences in air cell size between fiber types. However, expansion ratio and air cell size is also influenced by moisture content of the extrudate, extrudate temperature, pressure during extrusion and the water absorption of the fiber and starch in the extrudate. These factors will be discussed below. All fiber types were significantly less than the control (0% TDF) for both expansion ratio and air cell size at every fiber level.

Expansion ratio is usually attributed to the degree of starch gelatinization. As stated previously, dietary fibers binds water more tightly than starch (Harper, 1981; Gomez and Aguilera, 1984; Bhattacharya and Hanna, 1987; Moraru and Kokini, 2003). Therefore, as more water is structurally bound by fiber, less water may become available for starch gelatinization and moisture flash-off as the extrudate leaves the die (Onwulata et al., 1998; Lue et al, 1991). This would result in decreased extrudate expansion because less steam would be available to act as a leavening agent (Camire, 1991). Decreased expansion may be attributed to insufficient amount of starch in the extrudate and not just the presence of dietary fiber.
Extrudate density (Fig. 3) and breaking force (Fig. 4) where both strongly influenced by fiber type, fiber level and the fiber type/fiber level interactions with p < 0.0001 for all parameters. The same trends were observed with both density and breaking strength. Each fiber type at 48% TDF was significantly different than the other fiber levels. Each fiber type had an increase in extrudate density and breaking force with an increase in the amount of fiber in the extrudate. Thus, as breaking strength and density increased, extrudate expansion ratio and air cell size decreased. Powdered cellulose had the lowest density and breaking strength. Wheat usually had the highest density and breaking strength at all fiber levels. There was no difference between samples with added fiber and the control for breaking force at 18% TDF. Powdered cellulose was not significantly different from the control at 18% TDF for density.

Despite no differences between fiber types with air cell size at 36 and 48% TDF, and expansion ratio at 48% fiber levels, there were statistical differences between fiber types with density and breaking strength at 36 and 48% TDF levels. It is therefore possible other parameters are affecting the expansion ratio and air cell formation in the extrudates. The water absorption ability of the fiber types and extrudate moisture content may affect the air cell formation and expansion ratio of the extrudates.

**Extrudate Chemical Parameters**

Moisture content of extrudates was strongly influenced (p < 0.0001) by the level of fiber incorporated in the extrudate. Both WAI (p < 0.0001, p = 0.0028, p < 0.0001) and WSI (P < 0.0001, p = 0.0371, p = 0.0013) were strongly influenced by the fiber level, fiber type, and fiber level/fiber type interactions, respectively.

Generally, moisture content (Fig. 5) and WAI (Fig. 6) increased with increasing fiber level of the extrudate. With moisture content, there was no difference between fiber types at each fiber level. The 48% TDF level was statistically different from the other fiber levels. Oat fiber at 18% TDF level was the only fiber type to not be statistically different from the control. The significant increase of moisture content at 48% TDF level can be attributed to increase fiber leading to more water being bound during extrusion (Moraaru and Kokir, 2003; Onwulata et al., 1998). Thus, as more water is structurally bound by fiber, less water may become available for moisture flash-off as the extrudate leaves the die (Onwulata et al., 1998; Lue et al., 1991), allowing for extrudates with higher moisture content.
The WAI is the amount of water an extrudate can absorb. WAI is usually dependent on the starch present in the extrudates. It was found that WAI generally exhibited an increase with an increase in fiber. There were no differences between fiber types at 18% TDF. However, at the 36% and 48% TDF levels, powdered cellulose was statistically different from the other fiber types. Powdered cellulose had no differences in WAI at all fiber levels. Both oat and wheat fibers were not statistically different from each other at all fiber levels. Oat and wheat fibers absorbed the most water, thus having the highest WAI. Powdered cellulose at 48% TDF was significantly different from the control and oat and wheat fibers.

Unlike moisture content and WAI, the ability of extrudates to be solubilized by water (WSI) was decreased with an increase of fiber (Fig. 7). This is consistent with the findings of Jin et al. (1995) who reported an increase of fiber in extrudates from 20% up to 40% caused an increase in WAI and a decrease in WSI. All fibers had significantly less WSI values than the control at all fiber levels. Generally, there was no difference in WSI between fiber types at each fiber level. Oat and wheat extrudates were more likely to absorb water than dissolve in water with an increase of fiber. At each fiber level, regardless of fiber type, WSI and WAI values were statistically different.

The total percentage of protein in extrudates resolubilized after extrusion (total soluble protein) was influenced by the level of fiber \( p = 0.0278 \) and the type of fiber used \( p = 0.0278 \). Total soluble protein (Fig. 8) generally increased as the fiber level increased. However, powdered cellulose at the 48% TDF was higher in total soluble protein from the control and all levels of oat fiber. Generally, extrudates had >45% of the protein resolubilized after extrusion. This is indicative of the protein’s involvement in some form of covalent bonding or cross-linking with other protein molecules, or with starch and/or fiber components. It was expected that 100% of the protein from the extrudates would be resolubilized after extrusion. As mentioned in the methods for chemical tests, the protein of ground extrudate samples was solubilized by the addition of SDS, thereby, denaturing the proteins. Any proteins involved with disulfide bonds were cleaved by the addition of BME and thus were able to be denatured and resolubilized after extrusion.

Water soluble protein \( p < 0.0001, p = 0.004, p < 0.0001 \) and water soluble carbohydrate \( p = 0.0004, p < 0.0001, p < 0.0001 \) were strongly influenced by fiber level, fiber type and fiber level/fiber type interaction, respectively. The percentage of protein
solute in water after extrusion (water soluble protein) was generally not significantly different between fiber types at any fiber level (Fig. 9). Water soluble protein for the control and oat at 48% TDF were significantly higher than all other fiber levels and fiber types.

Different trends were observed for water soluble carbohydrate after extrusion (Fig. 10). The control was only significantly different to the powdered cellulose at 48% TDF and the oat fiber at 18% TDF. No trend was observed for powdered cellulose and wheat fiber with an increase in fiber. Powdered cellulose was significantly higher than the wheat fiber at every fiber level. Oat fiber had a significant decrease in water soluble carbohydrate at the 36% TDF level.

Water soluble carbohydrate was affected by both fiber content and fiber type. Hemicellulose, cellulose, and lignin have increased solubility through hydrolysis, dextrinization and or thermal degradation into low molecular weight fragments during extrusion (Fornal et al., 1987; Huber 1991; Lue et al., 1991; Camire and Flint, 1996; Lukesova et al., 1996; Gualberto et al., 1997). The open structure and low stability of the glucosidic bonds between pentose and hexose sugar units allow for easy hydrolysis of hemicellulose. Thus, soluble carbohydrate will be shifted upward about 4-5% (Huber, 1991). This will account for the water soluble carbohydrate values above 100% for powdered cellulose and oat fiber. It is difficult to interpret the changes in water soluble carbohydrate because the fractions of dietary fiber (i.e. cellulose, hemicellulose, lignin) that comprise the fiber types are not known. The processing conditions of the fibers can affect functional properties of the fibers such as solubility. Wheat fiber had less water soluble carbohydrate than the control and the other fiber types but followed the same trend as the other fiber types between 36 and 47% TDF. The interactions of protein, starch and fiber, regardless of the fiber type or fiber level, are ambiguous and further analysis is needed. The fragmentation of fiber will affect analysis of WSI and WAI values. As stated before, the increase in WAI and decrease in WSI may reflect more of the nature of the fiber in the extrudates than the starch.
Fig. 1. Expansion ratio of extrudates (◇, Powdered Cellulose; □ Wheat; ▲ Oat). Control (0% TDF), not shown, has a mean of 13.49 and letter sharing of ‘e’. Points are means of all three extrusion blocks. Means sharing letter are not different at $p > 0.05$.

Fig. 2. Air cell size of extrudates (◇, Powdered Cellulose; □ Wheat; ▲ Oat). Control (0% TDF), not shown, has a mean of 0.22195 and letter sharing of ‘c’. Points are means of all three extrusion blocks. Means sharing letter are not different at $p > 0.05$. 
Fig. 3. Extrudate density (●, Powdered Cellulose; ◦, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 0.0225 and letter sharing of 'a'. Points are means of all three extrusion blocks. Means sharing letter are not different at \( p > 0.05 \).

Fig. 4. Breaking force of extrudates (●, Powdered Cellulose; ◦, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 16.68 and letter sharing of 'a'. Points are means of all three extrusion blocks. Means sharing letter are not different at \( p > 0.05 \).
Fig. 5. Moisture content of extrudates (♀, Powdered Cellulose; ■, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 9.7325 and letter sharing of 'a'. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.

Fig. 6. WAI of extrudates (♀, Powdered Cellulose; ■, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 707.33 and letter sharing of 'c'. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.
Fig. 7. WSI of extrudates (♦, Powdered Cellulose; ■, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 55.64 and letter sharing of ‘f’. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.

Fig. 8. Total soluble protein of extrudates (♦, Powdered Cellulose; ■, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 47.16 and letter sharing of ‘a’. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.
Fig. 9. Water soluble protein of extrudates (♦, Powdered Cellulose; □, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 4.25 and letter sharing of ‘e’. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.

Fig. 10. Water soluble carbohydrate of extrudates (♦, Powdered Cellulose; □, Wheat; ▲, Oat). Control (0% TDF), not shown, has a mean of 98.02 and letter sharing of ‘a,b,c,d’. Points are means of all three extrusion blocks. Means sharing letter are not different at p > 0.05.
In addition, based on screening the 12 different fibers, four fiber types (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, Vitacel Apple fiber AF 401, and Vitacel Powdered Cellulose L601 FCC, and all from J.Rettenmaier USA LP, Schoolcraft, MI) were blended with WPC 80 and normal cornstarch to yielding blends with 60% WPC 80 (final protein concentration of 48%), 30% cornstarch and 10% fiber. These blends were extruded using extrusion parameters to produce a fibrous-textured product (TWP). The extrudates were evaluated with respect to ease of extrusion and solubility and three fibers were selected (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC, and all from J.Rettenmaier USA LP, Schoolcraft, MI) to complete objective 1 of this project. Each fiber was blended with WPC 80, fiber and starch to yield compositions of 50.4% protein with fiber levels of 0%, 7%, 15%, and 22% (w/w) and extruded in triplicate producing 30 TWP samples. Each of these TWP samples is now being analyzed for physical and chemical properties and data will be analyzed by ANOVA.

z. Discussion

We have successfully evaluated 12 different fiber types for the production of an extruded-expanded product and selected three fibers to complete objective 2. The extrudates have been analyzed for physical and chemical properties and the data analyzed by ANOVA. The level of fiber had a greater impact than the type of fiber added on extrudate characteristics. Generally, as the amount of fiber increased, moisture content increased leading to decreased expansion ratio, WSI, and air cell size and an increase in total soluble protein, density, WAI, and breaking strength. It is unclear whether the increased amount of fiber affected chemical and physical parameters more so than the decrease in starch. We believe that dietary fiber can be incorporated into an extruded snack product. Extrudates with 18% TDF were comparable to extrudates with 0% TDF (control). Extrudates with 48% were significantly different than the control (0% TDF). It is concluded that 48% fiber addition under the aforementioned extrusion conditions is too high for product requirements of low extrudate density and high expansion ratio. Extrudates containing TDF as high as
36% may be acceptable for some products, such as chips, crackers, and other snack foods.

We have also evaluated 4 different fiber types for the production of a fibrous-textured extruded product and have narrowed the list to 3 different fibers (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC and all from J.Rettenmaier USA LP, Schoolcraft, MI) to continue with objective 1. Each fiber was blended with WPC 80, fiber and starch to yield compositions of 50.4 % protein with fiber levels of 0%, 7%, 15%, and 22% (w/w) and extruded in triplicate producing 30 TWP samples. Each of these TWP samples is now being analyzed for physical and chemical properties and data will be analyzed by ANOVA.

Conclusion and Next Step
We complete the statistical analysis of the data obtained for objective 2 and continue to generate data for objective 1.

References


7. Intellectual Property (if applicable)

Please check the applicable box and provide additional explanation, if any.

Yes [ x ]  No [ ]  Possible [ ]

A patent application was filed to the US patent office on August 19, 2003 entitled Textured whey protein product and method (Walsh and Carpenter authors) which covers the research described in this report.

8. Anticipated Delays or Problems
Western Dairy Center

none

9. Manuscripts/Abstracts Submitted for Publication
none

10. Published Articles/Abstracts
None
1. Title of Project: Synthesis, characterization, and bioactivity of lactose laurylestes

2. Principal investigator: Marie Walsh

3. Start Date: August 30, 2006

4. End Date: January 31, 2009

5. Executive Summary:

We have initiated the research and have investigated the synthesis of lactose esters using the enzyme Lipozyme TL IM from Novozyme and a lactose laurate ratio of 1:1. We are still optimizing the HPLC separation for quantitation of the products and we should have this worked out by mid summer.

I have been recruiting a graduate student to work on this project. I have also hired a laboratory technician to begin procuring supplies essential to starting this project. We have no data to date.

Previous summary: Carbohydrate esters are biodegradable and nontoxic and are currently used in the food and personal care industries. They have a variety of documented activities including antimicrobial, insecticidal, emulsification and foaming properties. The antimicrobial activity of carbohydrate esters has been demonstrated recently against food spoilage bacteria and bacteria which are involved in the formation of dental caries. Over 30 different carbohydrate esters have been screened with respect to antibacterial activity, yet there have been no publications to date on the synthesis, characterization and bioactivity of lactose esters.


aa. Introduction. Carbohydrate esters are biodegradable and nontoxic and are currently used in the food and personal care industries. They have a variety of documented activities including antimicrobial, insecticidal, emulsification and foaming properties. The antimicrobial activity of carbohydrate esters has been demonstrated recently against food spoilage bacteria and bacteria which are involved in the formation of dental caries. Over 30 different carbohydrate esters have been screened with respect to antibacterial activity, yet there have been no publications to date on the synthesis, characterization and bioactivity of lactose esters.

bb. Objective (s)

Objective 1. Determine the optimum conditions for the synthesis of lactose laurylestes
Objective 2. Investigate the antimicrobial properties of lactose lauryl esters

Objective 3. Data analysis and manuscript preparation

c. Method and Materials

Lactose will be purchased from Glanbia Nutrionals (Monroe WI). Vinyl laurate and lauric acid will be purchased from (Sigma-Aldrich Fluka, St. Louis MO). Immobilized enzymes (Lipozyme TL IM from Thermomyces lanuginosus and Novozyme 435 from Candida antartica B), which are Novozyme AS products will be purchased from Sigma-Aldrich (St. Louis MO). Lipase PS-C from Pseudomonas cepacia will be purchased from Amano Pharmaceuticals (Elgin IL). Molecular sieves (3 A, 8-12 mesh), tributyrin, sodium azide, Penicillin G, Polymyxin B, and reagents (acetone, methanol, ethanol, ethyl methylketone, acetonitrile, buffer salts) will be purchased from Sigma Chemical (St. Louis MO).

Microorganisms to be used for assaying the microbial inhibitory activity of the lactose lauryl esters are listed in Table 4. Microorganisms will be purchased from ATCC and the media and growth temperature listed for culturing the organisms will be purchased from Invitrogen-Gibco (Carlsbad, CA).

Table 4. Microorganisms to be used to test the microbial inhibitory activity of lactose lauryl esters.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Biosafety level</th>
<th>Media and ATCC Media Reference Number</th>
<th>ATCC #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus sobrinus</td>
<td>1</td>
<td>ATCC 44: Brain heart infusion, 37 C</td>
<td>27351</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>1</td>
<td>ATCC 1169: Glucose tetrazolium medium, 37 C</td>
<td>31341</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>1</td>
<td>ATCC 18: Trypticase soy agar, 37 C</td>
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</tr>
<tr>
<td>E. coli K12</td>
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<td>ATCC 1065: LB medium, 37 C</td>
<td>35695</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
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<td>ATCC 416: Lactobacillus MRS broth, 37 C</td>
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</tr>
<tr>
<td>Pseudomonas fluorescence</td>
<td>2</td>
<td>ATCC 368: Blood agar base, 37</td>
<td>25006</td>
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</tbody>
</table>

Objective 1. Determine the optimum conditions for the synthesis of lactose lauryl esters
Statistical Analysis for Objective 1
Response surface methodology (RSM) will be used to determine the optimum conditions for lactose lauryl ester synthesis based on literature conditions for the synthesis of sucrose, maltose and glucose esters of lactic acid and palmitic acid. When the goal is to determine treatment values for optimal responses (maxima or minima) RSM is a valuable tool. Although factorial-treatment structures can be used for these kinds of experiments, RSM is preferred when treatment factors are varied across a continuous range of values. The treatments (factors) that influence the production of sucrose, glucose and maltose esters include the type of solvent (generally acetone, ethyl methylketone or ethanol), temperature (from 40-60 C), the concentration of reactants (molar ratios of sugar:fatty acids from 1:1 to 1:5) the type of lipase (non-specific or sn-1,3 specific lipase) and the time (24-60 hrs). A response surface design (central composite design with orthogonal blocking) with 4 significant factors is shown in Table 1. Each condition will be analyzed in triplicate. Analysis of variance, regression and canonical analysis for the nature of the response variables will be done using SAS Design of Experiments
Table 1. Response Surface Central Composite Design with 4 Factors

<table>
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<tr>
<th>Run Number</th>
<th>Block</th>
<th>Solvent</th>
<th>Temp (C)</th>
<th>Molar ratio</th>
<th>Lipase type</th>
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</table>

(Cary NC). Response surface graphs will also be generated with SAS. The optimum treatment conditions predicted by RSM will be used to produce lactose
lauryl esters to confirm the RSM outputs at various times (0, 8, 16, 32, 48 hrs) to
define optimum conditions for synthesis. For example, a possible outcome from
the RSM listed in Table 5 would be the use of acetone at 55 C with Lipase PSC at
a molar ratio of 1:2 (note, RSM uses linear regression to determine the optimum
conditions). These treatments would then be used at various times to determine
the best possible time resulting in maximum lactose lauryl monoester synthesis.

Lipase Activity
Lipase activity will be determined to ensure the same units of enzyme activity
are used for each of the three immobilized enzymes. Lipase activity will be
determined using the tributyrin assay according to Ferrer et al. (1999) and Nam
and Walsh (2005). Briefly, the reaction mixture (10 mL) will contain tributyrin
(68 mM), 0.1 M NaCl, 0.1 M CaCl2, 1 mM Tris-HCl (pH 7) and 3% acetonitrile.
Aliquots (vol amount) will be added to the reaction mixture and allowed to react
at 25 C for 30 min while shaking on an orbital shaker (100 rpm). The reaction
will be titrated with 1 M NaOH to determine the amount of fatty acids liberated.
Activity will be expressed as microkat (amount of enzyme that liberates 1
micromol of fatty acid per minute), which is equal to 60 U.

Lactose Lauryl Ester Synthesis
Lactose lauryl esters will be synthesized according to Ferrer et al. (2005) by
transesterification of lactose with vinyl laurate at the concentrations listed below
(Table 2) in various solvents (acetone, ethyl methylketone or ethanol) in a 5 ml
volume.

<table>
<thead>
<tr>
<th>Lactose (M)</th>
<th>Laurate (M)</th>
<th>Lactose Laurate (M ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>0.03</td>
<td>1:1</td>
</tr>
<tr>
<td>0.03</td>
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<td>1:3</td>
</tr>
<tr>
<td>0.03</td>
<td>0.15</td>
<td>1:5</td>
</tr>
</tbody>
</table>

Immobilized lipases (Lipozyme TL IM from Novozyme, Novozyme 435 from
Novozyme or Lipase PS from Amano Pharmaceuticals) will be used as
biocatalysts (1060 units as determined as described above). Reactions will be
performed at a set temperature of 40, 50 or 60 C on an orbital shaker (100 rpm) in
the presence of 3 A molecular sieves (100 mg/ml).

Products will be monitored by HPLC using a Nucleosil 100-C18 reverse phase
column (250 mm x 4.6 mm Beckman Cultor, Fullerton CA) maintained at 40 C on
a Beckman System Gold HPLC with a 1255 solvent module (Beckman Cultor,
Fullerton CA). The mobile phase will consist of a 90:10 (v/v) solution of
methanol:water at a 1.1 ml/min flow rate. Detection will not be via refraction
index as described by Ferrer et al. (2005) but instead we will use an evaporative
light scattering detector (Altech ELSD 800, Deerfield IL) which is more sensitive
and stable than refractive index detectors. Chromatography standards for
identification and quantitation will include lactose, lauric acid, and vinyl laurate.
The products will be quantified and collected after HPLC separation for future characterization by $^1$H NMR.

Characterization by $^1$H NMR will be done as described by Ferrer et al. (1999) by the USU Center for Integrated Biosystems (Logan, UT). Briefly, $^1$H NMR spectra will be recorded on a Varian INOVA (300 MHz) spectrometer (Palo Alto, CA) at 30 C for samples.

Objective 1 will be completed in 15 months

Objective 2. Investigate the antimicrobial properties of lactose lauryl esters

The minimum inhibitory concentration (MIC) for each of the organisms will be determined by the microbroth dilution method as described by the National Committee for Clinical Laboratory Standards (Woods and Washington, 1995). The microorganisms will be grown overnight in their respective optimal growth media at the appropriate temperature (Table 4) from stock cultures stored in liquid nitrogen. Each culture will be sub-cultured twice, harvested in mid log phase (approximate based on dividing time for each organism) and washed with saline. Plate counts (using optimum growth media with agar as listed in Table 4) and OD600 measurements will be taken. Graphs of OD600 vs plate counts will be made for each organism. New stock cultures will be grown overnight in their respective optimal growth media at the appropriate temperature and sub-cultured twice, harvested in mid log phase and resuspended in optimal growth media to $10^8$ CFU/ml (as determined by OD600 measurements of the same culture) containing lactose laurylester at concentrations of 0, 2, 6, 8, 10, 20, and 50 micrograms/ml in a total volume of 500 microliters in 48-well microplates (Corning NY). The plates will be incubated in optimal growth conditions for the respective organism and monitored for an increase in OD600 after 12, 24 and 48 hours using a Perkin-Elmer (HTS 7000) plate reader (Downers Grove IL). A positive control for inhibition of growth using Polymyxin B at 1000 micrograms/ml for Gram-negative organisms and Penicillin G at 1000 micrograms/ml for the Gram-positive organisms will be included in each microplate. Negative controls of organisms without lactose lauryl esters of each organism will also be included. The least concentration at which there is no increase in OD600 after 48 hours will be reported as the MIC. Each MIC will be determined in two replicates with triplicates test per replicate. The triplicates will be averaged for each replicate reported.

The rate of antimicrobial action for lactose lauryl esters will also be determined at the MIC value as well as at 0 and 2xMIC. Presuming the method of antimicrobial action involves the formation of pores in the microbial cell wall, the rate of uptake of propidium iodide (Fluopure grade, Molecular Probes Inc Eugene OR) will be used as described by Haughland (2002). Briefly, all cultures will be grown overnight in their respective optimal growth media and temperature from liquid nitrogen stock cultures. Each culture will be sub-cultured twice, harvested in mid log phase, washed with saline and adjusted to an OD600 of 0.25 (as determined from experiment described above on OD600 vs plate counts) in saline. Propidium iodide, with an excitation wavelength of 535 and an emission wavelength of 617 will be added to the culture suspensions at final
concentrations of 0, MIC and 2x MIC. The increase in fluorescence (RFU) will be measured with a Shimadzu RF 1501 spectrophotofluorometer (Columbia MD) at 15 s intervals for approximately 120 min. The rate of propidium iodide entering the pores in the cell walls will be determined in two replicates with triplicates test per replicate. The triplicates will be averaged for each replicate reported. The rate of antimicrobial action (pore formation) will be expressed as the inhibition rate (IR) using the following equation and the OriginPro Ver 7.0 (Natick MA) program. IR = ((LogRFU/time)-C)/Time (when dLogRFU/dt>0

Objective 2 will be completed after objective 1 in 9 months.

dd. Data or Results

7. Intellectual Property (if applicable). Please check the applicable box and provide additional explanation, if any.

Yes [ ]  No [ X ]  Possible [ ]

8. Anticipated Delays or Problems:

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts
PUBLICATIONS AND PRESENTATIONS

Abby Tyler: Senior design report, Department of Biological Engineering, 2006.


Chen, Dong, Ming-Xiang Liang, Daryll DeWald, Bart Weimer, Michael D. Peel, Bruce Bugbee, Jacob Michaelson, Elizabeth Davis, Yajun Wu. 2006. Identification of drought response genes from two alfalfa cultivars using Medicago truncatula microarrays. (submitted) Plant Molecular Biology.

Dhruv, H., M. Draper, D. Britt, Role of lactose in modifying gel transition temperature and morphology of self-assembled hydrogels, Chemistry of Materials, Accepted for publication, 2005.


Hopwood, W., 2006. M.S. thesis titled, Using Lactose and Lactose-Based Compounds for Controlling Erosion in Furrow Irrigation


Lee, W, S. Clark and B. G. Swanson. 2006. Low fat processed cheese food containing


Torres, J.A. 2006. Alta presión hidrostática (APH): Una nueva tecnología de alto valor agregado a frutos tropicales y subtropicales. In Propiedades Físicoquímicas y Sistemas de Procesado de Productos Hortofrutícolas en el Desarrollo Agroalimentario, Ibayqué, Colombia


Vazquez-Landaverde, P. A., G. Velazquez, J. A. Torres, and M. C. Qian. 2005. Quantitative determination of thermally derived volatile compounds in milk using solid-


Vázquez-Landaverde, P. A., J. A. Torres, and M. C. Qian. 2006. Inhibition of off-flavor formation in milk subjected to high hydrostatic pressure. Under revision to be submitted to Journal of Agricultural and Food Chemistry.


Vázquez-Landaverde, P.A., Qian, M.C., Torres, J.A. 2006. Kinetics of the formation of off-flavor compounds in milk under high hydrostatic pressure combined with moderate heating. Journal of Agricultural and Food Chemistry. SUBMITTED.

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