2010

2009 Annual Report

Various Authors

RESEARCH PROJECTS AND PAPERS

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Iron-binding by milk mineral: a possible antioxidant and anti-microbial mechanism

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ABSTACT

Study 1 (JFS 72:C78-C83). MM was compared to sodium tripolyphosphate (STPP), calcium phosphate monobasic (CIPM), and calcium pyrophosphate (CPP) to determine iron-binding capacity, sample solubility, and eluate soluble phosphorus after treating samples with a ferrous chloride standard. 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 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.

Study 2 (Meat Sci In Press). Effects of Type I antioxidants eugenol and rosmarinic acid were compared to those of Type II antioxidants milk mineral (MM), sodium tripolyphosphpate (STPP), and phytate in raw ground beef held for 14 days at 4 C in oxygen-permeable polyvinylchloride. Meat color stability was measured as % oxymyoglobin, Hunter a* value, chroma, and hue angle. By day 14, STPP-treated patties lost more red color ($P < 0.05$ for a* and hue angle) and had higher thiobarbituric acid values than other treatments. By day 14, MM was as effective as eugenol and rosmarinate at preventing oxymyoglobin oxidation (72, 76, and 71% retained, respectively) and red color loss as measured by a* (9.2, 9.4, and 10.9), hue angle (58.4, 56.2, and 53.5), and chroma (17, 17, and 18), but was unable to inhibit microbial growth as effectively as the spice-derived antioxidants. Although MM was an effective chelator of ionic iron (study 1 above), MM did not inhibit bacterial growth in ground beef. Likewise, MM did not inhibit growth of *Listeria, E. coli*, or *Psuedomonas viridescens* in meat-based media. Although these bacteria require iron for growth, it appears that they are capable of utilizing heme iron. Thus, chelation of nonheme iron by MM was not inhibitory to growth in meat systems containing heme iron.

BACKGROUND

To receive acceptance of MM as a food ingredient, further studies are needed on antioxidant mechanism, possible anti-microbial effects, and sensory evaluation of products with added MM. It is proposed that iron binding is the mechanism by which MM prevents lipid oxidation (rancidity) and meat pigment oxidation (browning) in meat products. Iron binding may also have anti-microbial effects in raw meat products. Since antioxidants have known health benefits, demonstration of antioxidant effects of MM will create a positive health image for all dairy products. Demand for MM and related dairy products (nonfat dry milk) will increase as a result of a better understanding of MM properties.

MM iron-binding capacity will be measured as the ability of MM particles to absorb iron from a solution of ferrous chloride. Iron remaining in solution will be measured by the ferrozine colorimetric assay. Iron-binding of MM will be compared to that of various calcium phosphate salts. It is hypothesized that the large MM particles with numerous phosphate groups will bind iron more effectively

than the water-soluble calcium phosphate compounds. Iron binding to MM particles will also be examined by scanning electron microscopy, using energy dispersive X-ray spectrometry. Minerals (calcium, phosphate, iron) have different x-ray absorption properties that can be used to determine the position of each element in the MM particle. It is hypothesized that the bulk I the MM particle is calcium phosphate. Iron-binding to MM particles may result in detection of iron atoms on the surface of MM particles. Some many bacteria require iron, addition of MM to raw ground beef may inhibit microbial growth. To test this possibility, total plate count and Enterobacter numbers will be monitored in ground beef with and without MM, using dried media on petri film, available from 3M Corp (St. Paul, MN).

Recent work here has shown that MM also prevents browning of raw ground beef during storage. This may be due to anti-microbial effects of MM, or to iron-binding to prevent iron-catalyzed myoglobin (Mb) oxidation to brown metmyoglobin (metMb). Preliminary work here has shown that iron indeed stimulates Mb (Sigma Chemical, St. Louis, MO), and 35-350 μM iron. MM counteracts the effects of iron addition. This work will be replicated in order to compare treatment means by analysis of variance. Further work will also be done to determine the level of MM needed to prevent oxidation in presence or absence of phospholipids, and at high (80%) or low (1-2%) oxygen levels, as might exist in different meat packaging methods. MM from two commercial sources (Glanbia Twin Falls, ID and First District Assn, Litchfield, MN) will be compared in the myoglobin model system. Finally, MM will be compared with recognized antioxidants (Trolox, eugenol, rosemarinic acid) for their ability to prevent lipid oxidation and rancid flavor development in cooked ground beef, as measured by thiobarbituric acid (TBA) assay and trained panel sensory measurement, respectively. PI Cornforth will supervise all laboratory experiments and be responsible for data analysis, preparation of reports, and submission of results for publication in appropriate journals.

RESEARCH PLAN

Objective 1. Measure iron-binding capacity of milk mineral (MM) by column chromatography.

Hyopthesis: 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.

Hyopthesis: 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 oxidation. This is new, and somewhat controversial. While iron is known to stimulate lipid oxidation, the possibility that low levels of iron directly stimulate myoglobin oxidation has not been examined.

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.

MATERIALS AND METHODS

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 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 ($FeCl_2$) 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 dessicator. 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 will be prepared in PI's lab and shipped to the SEM Lab, Materials Science and Engineering Dept, University of Utah (Salt Lake City, UT) for x-ray dispersive SEM mineral localization photography.

Objective 3: Effect of MM on Microbial Load and Enterobacter Levels of Ground Beef.

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. The 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.

Preparation of samples for examination of concentration dependence.

Samples will be prepared containing varying iron concentrations (35, 180, and 350 μM added iron). Control (0 μM added iron) will consist of equal parts MbO_2 and MES. Additional "control" samples will be prepared containing 2 mg/ml MM or STP, to chelate any "free" iron in the MbO_2 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 5. Interaction of Milk Mineral, Phospholipids, and Oxygen Levels on Mb oxidation.

Reagent and buffer preparation.

$FeCl_2$ 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 sepratory 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 and interpreting their results. Samples will be dry ashed in triplicate, then assayed for iron using the Ferrozine procedure, as outlined previously.

Generation of MbO_2 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 MbO_2 by bubbling air through the solution with a Pasteur pipette. Conversion to MbO_2 will be confirmed spectrophotometrically, based on the presence of the characteristic MbO_2 peaks at 545 and 580 nm (Bowen 1949). The concentration of the MbO_2 stock solution will be adjusted to ~0.1 mM with MES buffer (pH 5.6). Fresh MbO_2 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 MbO_2, $FeCl_2$, and/or antioxidant to observe the effect of free iron on the conversion of MbO_2 to MetMb in the absence of lipid. Lipid-containing model systems will be prepared in MES (pH 5.6) using a combination of MbO_2, phospholipid, $FeCl_2$, 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 clarify role of hydrogen peroxide.

Samples will be prepared as outlined above for lipid-free model systems, using a combination of MbO_2, $FeCl_2$, catalase, and/or antioxidant to examine the potential effect of hydrogen peroxide on the conversion of MbO_2 to MetMb. Thymol-free catalase will be added at a level of 3mmol per mol heme (Watkins et al. 1985, Brantley 1993). After preparation, samples will be handled and spectra obtained as outlined above. Five complete replicates will be performed.

Preparation of samples to examine the effect of partial pressure of oxygen.

Samples will be prepared as outlined for lipid-free model systems. To obtain samples with four 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 for examination of concentration dependence.

Samples will be prepared containing varying iron concentrations (35, 180, and 350 μM added iron). Control (0 μM added iron) will consist of equal parts MbO_2 and MES. Additional "control" samples will be prepared containing 2 mg/ml MM or STP, to chelate any "free" iron in the MbO_2 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 MbO_2 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 (100 g) from each treatment will be placed in a vacuum bag and frozen at –20° C for later determination of fat and non-heme iron content. Five complete replicates will be performed.

Oxymyoglobin determination.

Conversion of MbO_2 to MetMb will be confirmed spectrophotometrically, based on the presence of the characteristic MbO_2 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.

TBA 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.

RESULTS AND DISCUSSION

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).

Objectives 1 and 2.

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 3.

Q4 2008.

Rossarin Tansawat conducted control studies on the stability of MM or STP after autoclave heating, to better

understand previous results. She found that when solutions of 0.5% STP in distilled water or tryptic soy broth (TSB) were autoclaved, orthophosphate concentrations increased to 3.79 mg Pi per ml in TSB but remained at zero for STP in water solution. Thus, there was some effect of TSB solution to degrade STP to orthophosphate (Pi) during autoclave heating. When MM (0.75%) was added to water, measurable Pi was found even at room temp (1.65 mg Pi/ml), but after autoclave heating, Pi levels increased to 2.89 mg Pi/ml. When MM was added to TSB solution to a level of 0.75% MM, Pi levels were 1.43 mg Pi/ml, and increased to 4.02 mg Pi/ml after autoclave heating. Again, there was some effect of TSB solution to increase Pi levels after heating. The degradation of STP to Pi in heated TSB does not appear to be an acid hydrolysis effect, since TSB solutions were buffered to pH 7.3. We postulate that microbial phosphatases were present and active in the warm-up time prior to reaching autoclave temperatures, and that STP was degraded during this period. The increase in Pi after heating MM solutions was probably a heat solubilization of MM phosphates, although the possibility that some enzymatic degradation of MM was occurring cannot be ruled out. Rossarin also conducted an experiment to measure aerobic plate count (APC) and inorganic orthophosphate phosphate (Pi) in ground beef patties formulated with 0.5% STP or 0.75% MM, and incubated at 2or 22 ° C for 2 days. There was almost no increase in APC or Pi for 2 days at °C. However, both APC and Pi increased significantly in patties held at 22°C. MM was more stable (Pi increase was less) for MM vs STP patties. There was a high correlation (r = 0.77) between aerobic plate count and Pi levels of ground beef patties at 22°C. Thus, there is good indication that MM is more stable than STP added to fresh ground beef patties, particularly at higher temperatures (22°C) conducive to rapid bacterial growth. It also appears that microbial phosphatases are responsible for at least some of the degradation of STP that occurs when this compound is added to ground beef patties. Next steps include publication of this data in the MS thesis of Rossarin Tansawat and submission of a manuscript on this topic to an appropriate journal.

Q1 2009.

Polyphosphate decomposition to inorganic monophosphate (Pi) was measured after addition of sodium tripolyphosphate (STP) or milk mineral (MM) to ground beef patties. The Pi level as % of added STP increased from 5.9% initially to 29.0% after 2 days at 22°C. The Pi level as % of added MM did not increase (21.7% Pi initially, versus 20.3% after 2 days at 22°C). Thus, STP was susceptible to decomposition to Pi when added to ground beef, but MM was not degraded. However, there was a significant amount of MM phosphate that was readily released as Pi when MM was initially added to ground beef. Similar results were observed when polyphosphates were added to ground beef and held at 2°C, rather than 22°C for 2 days. The Pi level as % of added STP increased from 5.9% initially to 35.1% after 2 days at 2°C. The Pi level as % of added MM increased only slightly (21.7% Pi initially, and 34.0% after 2 days at 22°C).

MM released more Pi than STP when added to tryptic soy broth, then autoclaved at 121°C for 15 min. For MM, Pi as % of total added MM polyphosphate was 39.3% on day zero, but did not change after 2 days at 22°C (40.3%). STP decomposition was lower (22.1% and 24.9% after 0 or 2 days at 22°C, respectively. Similar results were found at 2°C. STP decomposition was 23.6 and 28.2% (as Pi) after 0 or 2 days at 2°C, respectively, compared to 44.0 and 46.7% for MM after 0 or 2 days at 2°C, respectively.

Q2 2009.

Rossarin Tansawat presented 2 posters on her work at national meetings.

At IFT in Anaheim, she presented data comparing stability of MM and STP added to ground beef (STP was more prone to hydrolysis to Pi).

At RMC in Rogers, AR, she presented data showing that MM was not inhibitory to Listeria innocua, E. coli DH5-α, or Pseudomonas fluorescens in media, nor to aerobic plate count (APC) in ground beef. It appears that in spite of the ability of MM to bind ionic iron, there is sufficient heme iron and other available iron (perhaps existing as iron-amino acid chelates) in media and meat to support growth of iron-dependent bacteria. Rossarin is currently working on a manuscript summarizing this data for submission to the Journal of Food Science.

Objective 4.

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.

Objective 6.

Daren Cornforth and Karin Allen delivered an invited presentation at the Reciprocal Meat Conference (RMC)

in Rogers, AR summarizing the effects of MM to prevent both lipid oxidation (rancidity) and myoglobin oxidation (browning) in fresh ground beef, with particular application for ground beef in 80% oxygen – modified atmosphere packaging.

Q3 2009.

Effects of Type I antioxidants eugenol and rosmarinic acid were compared to those of Type II antioxidants milk mineral (MM), sodium tripolyphosphpate (STPP), and phytate in raw ground beef held for 14 days at 4°C in oxygen-permeable polyvinylchloride. Meat color stability was measured as % oxymyoglobin, Hunter a* value, chroma, and hue angle. Significant correlations ($P < 0.0001$) were observed between all color measurement methods. By day 14, STPP-treated patties lost more red color ($P < 0.05$ for a* and hue angle) and had higher thiobarbituric acid values than other treatments. By day 14, MM was as effective as eugenol and rosmarinate at preventing oxymyoglobin oxidation (72, 76, and 71% retained, respectively) and red color loss as measured by a* (9.2, 9.4, and 10.9), hue angle (58.4, 56.2, and 53.5), and chroma (17, 17, and 18) but was unable to inhibit microbial growth as effectively as the spice-derived antioxidants. [This work is currently under peer review for publication in "Meat Science", authored by Karin Allen and Daren Cornforth].

Q4 2009. (Final Report).

The paper described above (Q3 2009) is still under peer review for publication in "Meat Science", authored by Karin Allen and Daren Cornforth.

The final paper from this project is currently in preparation for submission to "Meat Science". This is the work by Rossarin Tansawat, described above (Objective 3, for Q4 2008 and Q1 2009). In summary, she found that milk mineral added to ground beef or to microbial cultures was not inhibitory to Salmonella or E. coli species, even though these organisms are known to require iron for growth and MM is known to be an efficient iron-chelating compound (Allen K, Cornforth DP. 2007. Antioxidant mechanism of milk mineral – High affinity iron binding. J Food Sci. 72:C78-C83). The explanation for this apparently incongruent result is that meat or media used to grow these pathogens contained sufficient non-ionic iron (heme iron or protein-bound iron) to meet the growth needs of these organisms, even if all ionic iron was chelated to added milk mineral. This paper will further describe Rossarin's results demonstrating that MM added to minced beef was more resistant to enzymatic degradation than sodium tripolyphosphate (STP; the most commonly used antioxidant in enhancement-injected fresh beef products).

CONCLUSIONS

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 anti-microbial effect in marinated fish filets.

A similar experiment was conducted in ground beef. In trial 1, APC of control ground beef after 4 days storage at 2°C was 2.3 x 106. In comparison, samples with 1% MM had lower APC of 1.5 x 105 (about a l-log reduction with MM treatment). Samples with 3% lactate/diacetate, a widely used anti-microbial in meat products, had APC of 4.1 x 105. After 7 days storage, APC of all treatments was 106 – 107, and there were no significant differences among treatments. In a second ground beef trial with low initial microbial load (101 APC/g), there were no significant differences in APC among treatments at any storage time (1, 4, 7 days).

Further work will continue on possible antimicrobial effects in model systems and other food systems. New student Rossarin Tansawat will initiate the portion of objective 3 regarding possible anti-microbial effects of MM against food pathogens (Listeria, E. coli). She will initially inoculate test organisms (Listeria inocula, E. coli K-12, Psuedomonas viridescens) into culture media with and without 1% MM. It will be necessary to determine the iron level of media before addition of MM. It will also be necessary to conduct preliminary experiments with inoculated media with variable levels of MM (0.1 – 2%) to determine the minimum levels of MM needed for possible anti-microbial effects.

Rossarin Tansawat has completed the USU lab safety training course and also completed training with Ms. Becky Thompson, Lab Manager, USU NFS Microbiology Labs, on procedures for culturing, transferring, and plating the microorganisms of interest in this study. She has initiated tests of MM effects on growth of Listeria inocula, E. coli DH5α, and Psuedomonas fluorsecens in Brain Heart Infusion broth, Tryptic Soy broth, and Nutrient broth, respectively. Each broth was chosen as the best media for growth of each organism, respectively. Each organism was inoculated into appropriate broth to obtain a vigorously growing culture, which was serially diluted and plated onto 3M aerobic plate count petrifilm. Vigorous cultures have been obtained for Listeria inocula and E. coli DH5α but

not Pseudomonas fluorescens. We have placed an order for a new frozen culture of Pseudomonas fluorescens from American Type Culture Collection (ATCC; Manassas, VA). In the meantime, experiments will continue with Listeria inocula and E. coli DH5α to compare organism growth in control media, compared to media containing 0, 0.75. and 1.5% milk mineral (MM).

Karin Allen has begun experiments on objective 6: Effect of various antioxidants (MM, Trolox, Eugenol, Rosemarinic Acid) on raw ground beef appearance and cooked ground beef sensory acceptability. She has found that rosmarinic acid is a very fast reductant of ferric iron to the ferrous form aqueous solutions. In a model system containing myoglobin (Mb), iron (Fe), and various antioxidants, myoglobin remaining after 60 min was as follows:

Mb + Fe + Rosmarinic acid – 9.9% Mb remaining (a).

Mb + Fe + Eugenol – 37.3 % Mb remaining (b).

Mb + Fe – 53.8% c

Mb + Fe + Phytate – 60.4% d

Mb only – 63.3% d

Mb + Fe + sodium tripolyphosphate (STP) – 67.7% Mb remaining (d).

This result is quite interesting regarding the mechanism of Mb oxidation. The type 1 antioxidants (hydrogen donors: rosmarinic acid and eugenol) act to rapidly reduce ferric iron to ferrous. The ferrous iron in turn forms a putative complex with oxygen that facilitates the transfer of an electron from the heme iron of Mb to oxygen, forming superoxide and Met Mb (brown pigment formation). The type 2 antioxidants (iron chelators: Phytate and STP) are much more effective at preserving Mb and preventing formation of metMb. So, this experiment supports the hypothesis that soluble ferrous iron greatly stimulates Mb oxidation in aerobic environments. Also, this result again demonstrates the powerful antioxidant effects of MM to prevent brown color formation in fresh meats.

Karin has completed one additional experiment comparing the effect of antioxidants on myoglobin oxidation as affected by oxygen concentration in the solution. She has constructed an apparatus to obtain oxygen concentrations of zero (under nitrogen), 2 % oxygen, 20% oxygen (atmospheric), and 80% oxygen. To obtain these oxygen concentrations, she purchased gas cylinders of 100% nitrogen, 2% oxygen + 98% nitrogen, compressed air (20% oxygen), and 80% oxygen + 20% nitrogen. The apparatus consists of tubing connecting the gas cylinders with appropriate regulators to glass jars, fitted with glass inlet and out tubes and stop cocks, so that the jar may be flushed with the desired gas. The sample cuvettes containing myoglobin + antioxidant solutions are placed in the jar, the lid is tightened, the air is replaced by flushing with the desired gas, and then the inlet and outlet stopcocks are closed to to maintain the desired atmosphere for up to 24 hrs. She has completed one run with myoglobin + rosemarinic acid in both 80% oxygen and 2% oxygen. She verified with an oxygen probe analyzer that the desired oxygen atmosphere was obtained.. She found that in presence of myoglobin + iron + rosemarinic acid, myoglobin oxidation proceeded rapidly, at both 2% or 80% oxygen. Thus, 2% oxygen level was not limiting to myoglobin oxidation in presence of ferrous iron.. She is currently conducting further experiments to evaluate possible interactions of oxygen level, with or without added iron, and with or without various antioxidants, on myoglobin oxidation rate (measurements taken at 15 min, 60 min, 2 hr, 4 hr, 8 hr, and 24 hr in the appropriate atmosphere).

Q4 2007.

Karin Allen has completed her final experiment (described above). In summary, she evaluated the interaction effects of oxygen level (2.6, 20, 80 % oxygen) on rate of myoglobin oxidation to metmyoglobin (browning) in a control solution (200 micromolar myoglobin, pH 5.6), a myoglobin solution with 2 micrograms/ml added ferrous chloride, or myoglobin + iron + 0.3% sodium tripolyphosphate (STP – an iron-binding compound)., for 15, 30, 45, and 60 min at 20°C. Desired oxygen levels were obtained by flushing (bubbling) the solution with the desired oxygen level. The 3 oxygen levels were purchased as gas cylinders from PraxAir (Salt Lake City, UT), certified to contain oxygen at the desired levels. The remaimder of the gas in each cylinder was nitrogen.

Karin repeated each treatment interaction 5 times, and she is currently conducting statistical analysis of the results. It was apparent that iron addition stimullated myoglobin oxidation at 2.6% and 20% oxygen, but at 80% oxygen, metmyoglobin formation was lower. Sodium tripolyphosphate additon also lowered metmyoglobin formation at 2.6 and 20% oxygen, but at 80% oxygen, there was no combined effect of STP and high oxygeen to lower metmyoglobin levels. High oxygen levels alone were about as effective as high oxygen + STP to prevent metmyoglobin formation in the model system during the 1 hr incubation period. These results are importtant in understanding the factors (iron, oxygen levels) affecting browning in fresh meats packaged by various modified atmosphere packaging methods.

Rossarin Tansawat has recently completed one replication of a study with lean ground beef packaged in

high (80%) oxygen modified atmosphere packaging, to compare the stability of added STP or milk mineral (MM). Inorganic phosphate levels increased after 2-3 days in meat + 0.3% STP, compared to meat + 0.75% MM. This result indicated that phosphatase enzymes from either the meat or from bacterial growth in meat were degrading the added STP. This provided partial explanation for the increased antioxidant effects of MM, compared to STP, when added to ground meats. If the STP is degraded to inorganic orthophosphate, it would have less affinity for soluble iron. Thus, at longer storage times, meat + STP would have higher free iron, and thus more lipid oxidation, compared to meat + MM. The added MM appeared to be less susceptible to degradation by phosphatase enzymes, compared to added STP.

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Internal structure analysis.

See Southwest Dairy Foods Research Center report.

Mechanical testing.

See Southwest Dairy Foods Research Center report.

Sensory analysis.

See Southwest Dairy Foods Research Center report.

Objective 2.

Microparticles.

Obtain Sephadex G-50 carbohydrate beads of three different sizes: medium (100 to 300 μm), fine (40 to 160 μm), and superfine (20 to 80 μm).

Cheese manufacture.

Make a control full fat (33%) control reduced fat cheese (16.5%), control low fat (6%) and corresponding microparticulate (MP) cheeses using the Sephadex G-50 beads.

- Manufacture cheddar cheese using 10 L of skim milk standardized with cream so that the resultant cheese contains 33% fat and 37% moisture.
- Manufacture cheddar cheese using 10 L of skim milk to which is added 370 g of hydrated medium, fine or superfine Sephadex G-50 beads. Make adjustments to the make procedure to obtain cheddar cheese with approx. 65% moisture [Assume 90% yield into cheese so that beads occupy about 33% of the volume of the final cheese, and that the beads consist of 86% moisture.]
- Manufacture 50% reduced fat cheddar cheese using 10 L of skim milk standardized with cream so that the resultant cheese contains 16.5% fat and 46% moisture.
- Manufacture 50% reduced fat cheddar cheese using 10 L of skim milk to which is added 185 g of hydrated medium, fine or superfine Sephadex G-50 beads. Make adjustments to the make procedure to obtain cheese with approx. 60% moisture
- Manufacture low fat cheddar cheese using 10 L of skim milk standardized with cream so that the resultant cheese contains 6% fat and 52% moisture.
- Manufacture low fat cheddar cheese using 10 L of reduced fat milk to which is added 65 g of hydrated medium, fine or superfine Sephadex G-50 beads. Make adjustments to the make procedure to obtain cheese with approx. 57% moisture
- Make 2 replicates of each cheese.

Chemical analysis.

Measure pH by glass electrode, salt by chloride analysis, moisture by vacuum oven, fat by Babcock, protein by combustion N, calcium and other minerals by ICP spectroscopy at Utah State University, and measure water activity.

Storage.

The cheeses will stored at 6°C and physical testing performed when the cheese is 1 mo, and 4 mo old.

Mechanical testing.

See Southwest Dairy Foods Research Center report.

RESULTS AND DISCUSSION

Objective 1.

Cheese make procedures were developed for making cheeses with fat contents ranging from 3% to 33% that all had comparable protein matrix composition, i.e., only the fat volume in the cheese was different. The first replicate of cheeses were manufactured at Utah State University with target fat contents of 3, 8, 13, 18, 23, 28 and 33% (Table 2). As shown below the proximate composition obtained at day 5 ($\pm$ 1) was close to the target composition, except for the salt levels and pH. These were higher than planned in the first few cheeses made, and so the remainder were made in a similar fashion with salt-in-water contents of about 4.7%, which is more typical of regular full fat cheddar cheese anyhow, and pH of the second set of cheeses was within target (Table 3). The cheeses were being stored at 6°C and sent for rheological and texture studies at North Carolina State University.

Confocal microscope images obtained at the Western Dairy Center show distribution of fat in the curd particles as shown in Figure 1. The low fat cheeses show smaller fat globules. As fat % increases, fat globules appear to increase in size.

See Southeast Dairy Foods Research Center report for results on rheological and sensory testing.

Objective 2.

See Southeast Dairy Foods Research Center report for results on rheological and sensory testing.

Table 2. Proximate analysis data for Rep 1 cheeses.

Make Date	% Fat		% Moisture		% Salt		% MNFS		% Salt in H2O		pH
	Target	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Actual*
3/11/08	3.0	3.0	52.80	53.03	2.10	2.56	54.43	54.67	3.98	4.83	5.40
3/25/08	8.0	8.5	50.10	49.60	2.00	2.09	54.46	54.21	3.99	4.21	5.30
3/4/08	13.0	15.5	47.40	46.82	1.90	2.20	54.48	55.41	4.01	4.70	5.58
3/5/08	18.0	20.3	44.60	45.35	1.80	2.03	54.39	56.90	4.04	4.48	5.52
3/18/08	23.0	23.0	41.90	43.39	1.70	2.04	54.42	56.35	4.06	4.70	5.37
3/19/08	28.0	28.8	39.20	40.04	1.60	1.96	54.44	56.20	4.08	4.90	5.46
3/26/08	33.0	33.0	36.50	37.45	1.45	1.85	54.44	55.90	3.97	4.94	5.46

Table 3. Proximate analysis data for Rep 2 cheeses.

Make Date	% Fat		% Moisture		% Salt		% MNFS		% Salt in H2O		pH
	Target	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Target*	*Actual*	*Actual*
3/11/08	3.0	2.5	52.80	53.42	2.10	2.13	54.43	54.79	3.98	3.99	5.29
3/25/08	8.0	11.0	50.10	47.18	2.00	2.09	54.46	53.01	3.99	4.44	5.23
3/4/08	13.0	15.0	47.40	46.48	1.90	2.17	54.48	54.68	4.01	4.68	5.42
3/5/08	18.0	20.0	44.60	43.73	1.80	1.87	54.39	54.66	4.04	4.27	5.30
3/18/08	23.0	24.0	41.90	41.24	1.70	1.87	54.42	54.27	4.06	4.53	5.27
3/19/08	28.0	29.0	39.20	39.66	1.60	2.07	54.44	55.86	4.08	5.21	5.29
3/26/08	33.0	33.0	36.50	37.94	1.45	1.75	54.48	56.63	3.97	4.60	5.19

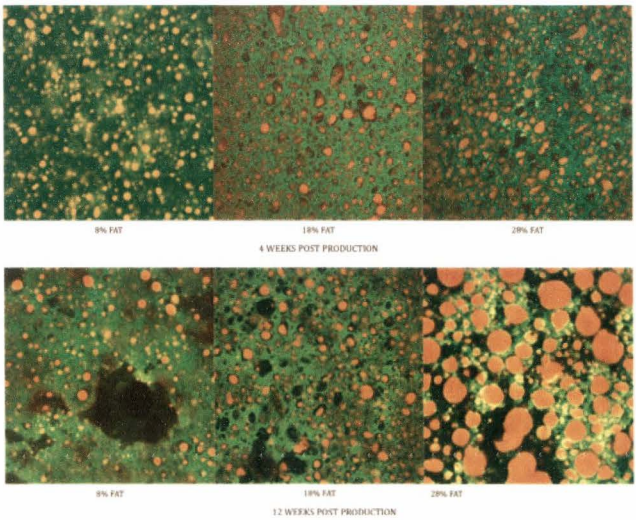

Figure 1. Confocal microscope images showing distribution of fat (orange) and protein (green).

Table 4. Proximate analysis of cheeses.

Make Date	Experiment	Rep	% Moisture	% Fat	% Salt	pH
1/6/09	Seph_Low Fat _SF	Rep 1 LF	58.26	1.0	2.18	4.88
1/6/09	Seph_Low Fat _Fine	Rep 1 LF	56.76	1.0	2.10	4.95
1/6/09	Seph_Low Fat _Med	Rep 1 LF	56.43	1.0	2.12	4.99
1/6/09	Seph_Low Fat _ Crtl	Rep 1 LF	53.04	5.5	2.10	4.96
1/21/09	Seph_Reduced Fat _SF	Rep 1 RF	54.65	6.0	1.86	5.00
1/21/09	Seph_Reduced Fat _Fine	Rep 1 RF	57.17	5.5	2.08	4.95
1/21/09	Seph_Reduced Fat _Med	Rep 1 RF	52.65	8.0	1.81	5.05
1/21/09	Seph_Reduced Fat _ Crtl	Rep 1 RF	48.13	15.5	1.92	5.07
2/10/09	Seph_Full Fat SF	Rep 1 FF	58.5	3.0	2.26	4.81
2/10/09	Seph_Full Fat _Fine	Rep 1 FF	55.64	3.5	2.50	4.95
2/10/09	Seph_Full Fat _Med	Rep 1 FF	53.7	3.0	2.20	4.79
2/10/09	Seph_Full Fat _Crtl	Rep 1 FF	40.8	30.0	2.40	5.23
4/17/09	Seph Low Fat _SF	Rep 2 LF	59.5	2.0	1.82	5.16
4/17/09	Seph_Low Fat _Fine	Rep 2 LF	57.9	2.5	1.70	5.16
4/17/09	Seph_Low Fat _Med	Rep 2 LF	56.39	2.0	1.72	5.16
4/17/09	Seph_Low Fat _Crtl	Rep 2 LF	54.13	7.0	1.68	5.08
4/30/09	Seph_Reduced Fat _SF	Rep 2 RF	60.02	2.0	2.22	5.22
4/30/09	Seph_Reduced Fat _Fine	Rep 2 RF	58.44	2.0	2.18	5.25
4/30/09	Seph Reduced Fat _Med	Rep 2 RF	56.98	3.0	2.10	5.24
4/30/09	Seph Reduced Fat Crtl	Rep 2 RF	50.9	16.5	1.92	5.25
6/2/09	Seph_Full Fat SF	Rep 2 FF	58.94	1.5	2.30	5.23
6/2/09	Seph_Full Fat _Fine	Rep 2 FF	55.31	2.0	2.08	5.16
6/2/09	Seph_Full Fat _Med	Rep 2 FF	54.04	2.5	2.12	5.30
6/2/09	Seph_Full Fat _Crtl	Rep 2 FF	41.51	28.5	1.72	5.15

CONCLUSIONS

Objective 1.

When the moisture to protein ratio is held constant, the main differences seen are due to relative amounts of the gel and fat phases. Aging causes a gradual decrease in G' and this is most logically associated with changes in the gel network but not the fat. Both the protein network and fat are affected by temperature but it appears that the major effect is due to fat.

Objective 2.

See Southeast Dairy Foods Research Center report for results on rheological and sensory testing.

NEXT STEPS

Objective 1.

Write manuscript for publication.

Objective 2.

Analysis of data and manuscript writing.

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Synthesis, characterization, and bioactivity of lactose lauryl esters

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ABSTACT

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 that 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.

BACKGROUND

Review of literature.

Justification.

Carbohydrate fatty acid esters are biodegradable, nontoxic and have broad applications in the food industry as well as other fields including cosmetics, detergents, oral-care products, agriculture, and pharmaceuticals. Their antimicrobial, insecticidal, and food functional properties have been reported. The specific properties of carbohydrate esters depends on the characteristics of the fatty acid esterified (generally carbon length), sugar moiety, and number of fatty acids esterified. Sucrose esters are tasteless, odorless, and are digested by pancreatic lipases to their components which are metabolized in the normal way. However they are resistant to degradation in the saliva due to the absence of lipases. Sucrose esters are stable at pH values between 4 and 8 and up to 180 C (Devulapalle et al., 2004). The most commonly produced sugar esters include sucrose, sorbitan and akyl polyglycosides (Table 1) which are gaining increased attention due to advantages with regard to performance, health of consumers, and environmental compatibility.

As reviewed by Hill and Rhode in 1999, only a few carbohydrates fit the criteria of price for economical production of carbohydrate esters (Table 2). As of 1999, these included sucrose, glucose, and sorbitol. Interestingly, the current price of lactose is less that the above listed carbohydrates. Therefore, the production of lactose esters would be at least as economical as the currently produced carbohydrate esters.

Activity.

The activity of carbohydrate esters depends on the type of carbohydrate uses as well as the type of fatty acid esterified (carbohydrate chain length) and the degree of esterification. For example, carbohydrate esters containing multiple long chain fatty acids (C12-C18) are commonly used in the food industry as emulsifiers (i.e. available from Degussa Food Ingredients, Danisco, Mitsubishi-Kagaku Foods Corporation) (Tual et al., 2006). The degree of esterification influences the hydrophilic-hydrophobic balance (HLB) that determines its usefulness in either a water in oil or oil in water emulsion.

In addition to the use of carbohydrate esters as emulsifiers, there has been significant research on the use of carbohydrate esters as antimicrobial or insecticidal agents. Recent research by Puterka et al. (2003) tested the activity of a variety of sugar esters on the insecticidal activity against a range of arthropod species. Overall, most of the carbohydrate esters examined had superior insecticidal activity compared to insecticidal soap. Specifically, sucrose octanote high in monoester content had the highest activity against the range of arthropod pests at concentrations of 1200-2400 ppm. Sorbitol octanote and xylitol decanote

Table 1. Production and use of common carbohydrate esters (Hill and Rhode, 1999).

Name	Manufacturers	Applications	Production (world tons annually)
Sorbitan esters	Akeros, Dai-ichi Kogyo Seiyaku, Henkel, Kao, ICI, PPG, SEPPIC	Pharmaceuticals, personal care, food, coatings	20,000
Sucrose esters	Croda, Dai-ichi Kogyo, Seiyaku, Mitsubishi	Food, personal care, pharmaceuticals	4,000
Alkyl polyglycosides	BASF, Henkel, ICI, Union Carabide	Personal care, detergents, agrochemicals	80,000

Table 2. Availability of carbohydrates as raw materials.

Carbohydrate	Price per Kg
Sucrose[1]	0.8
Glucose[1]	0.55-1.21
Sorbitol[1]	0.80-1.70
Lactose[2]	0.14

1 Hill and Rhode, 1999.
2 Glanbia Nutritionals, personal communication, May 2006.

also showed insecticidal activity. In addition to the research of Puterka et al. (2003), other investigators have also shown carbohydrate esters have insecticidal activity to soft bodied arthropods including mites, aphids, whitefly and psyllids (Chortyk et al., 1996; Chortyk, 2003; Puterka and Severson, 1995; Liu et al., 1996; Liu and Stansly, 1995).

The antimicrobial activity of carbohydrate esters (6-O-lauroylsucrose and 6'-O-lauroylmaltose, fructose laurate, and galactose laurate) has been demonstrated recently against the food spoilage bacteria *Bacillus* sp, *Bacillus stearothermophilus*, *Lactobacillus* (Ferrer et al., 2005) and two bacteria involved in formation of dental caries formation, *Streptococcus mutans* (Wantanabe et al., 2000) and *Streptococcus sobrinus* (Devulapalle et al., 2004). Ferrer et al. (2005) studied the effect of sugar (glucose, sucrose, or maltose), length of fatty acid (lauric or palmitic) and degree of esterification (mono or diester) on antimicrobial properties. They synthesized 6 different carbohydrate esters including 6-O-lauroylsucrose, 6'-O-lauroylmaltose, 6'-O-palmitoylmaltose, lauroylsucrose, and lauroylsucrose and tested their antimicrobial activity against a variety of organisms including *Bacilus* sp., *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Escherichia coli*, *Pichia jadinii*, *Bacillus stearothermophilus* and *Lactobacillus plantarum*. 6-O-lauroylsucrose and 6'-O-lauroylmaltose were successful at concentrations of 0.25 to 5 mg/ml against *Bacillus* sp., *E. Coli*, *P. jadinii*, *B. stearothermophilus* and *L. plantarum*. For reference, sucrose esters are used as emulsifiers in foods at concentrations as high at 10 mg/ml (Ferrer et al., 2005).

Watanabe et al. (2000) synthesized 23 different carbohydrate esters from various sugars and fatty acids and evaluated their bacteriostatic activity against the cariogenic bacterium *Streptococcus mutans*. Galactose and fructose laurate showed the highest bacteriostatic activity. Generally, the carbohydrate esters containing lauric acid as the fatty acid were significantly more active than those containing capric, butyric, caproic, myristic or palmitic acids at concentrations as low as 0.01%.

Devulapalle et al. (2004) investigated the antimicrobial activity of lauroylsucrose, lauroylmalttose and lauroylmaltotriose against the cariogenic bacterium *Streptococcus sobrinus*. No bacterial growth was detected in the liquid medium supplemented with lauroylmaltose ((0.5 mg/ml) or with lauroylmaltose (1 mg/ml) or lauroylsucrose (2 mg/ml). Other researchers have also demonstrated the antimicrobial activity of carbohydrate esters (Marshalll et al., 1994; Bergsson et al., 2001; Kato et al., 1997).

In general with respect to antimicrobial activity, Gram-positive bacteria are more susceptible to carbohydrate esters than Gram-negative bacteria and fungi are more susceptible to carbohydrate esters than yeasts (Marshall, 1994). The mechanism of antimicrobial action of these carbohydrate esters has not been elucidated. It is possible that sugar esters disrupt the cell membrane, thereby altering its permeability and causing a selective leakage of glycollytic intermediates. In *B. subtilis* cells, there is a change in morphology and induced autolysis resulting in cell death (Ferrer et al., 2005) in the presence of carbohydrate esters.

Considering that lauric carbohydrate esters showed the highest antimicrobial activity, we are proposing to esterify lauric acid to lactose.

Synthesis.

Various sugar esters have been synthesized from sucrose, maltose, leucrose, moltotriose with acyl chains ranging form 2 to 18 carbons. There are two methods of esterifying fatty acids to carbohydrates, one is chemical and the other is enzymatic using lipases.

The chemical process results in a high energy cost, chemical waste, undesirable by-products and heterogeneous esters (mixture of compounds differing in the degree of esterification on the position of the acyl group on the sugar moiety). The chemical process is done under basic conditions and results disadvantages including low selectivity and yields and colored derivatives as side-products. The enzyme-catalyzed process is more selective and does not result in off products (Ferrer et al. 2000) and can result in esters that can be used as ingredients in foods.

By controlling the degree of esterification (enzyme type, solvent composition, ratio of reactants, and temperature) it is possible to modify their properties. The factors listed (enzyme type, solvent composition, ratio of reactants, and temperature) are interrelated with respect the degree of esterification. Therefore, a response surface statistical design is described in the methods section.

Lipases (EC 3.1.1.3) catalyze the hydrolysis of ester linkages in acylglycerols in aqueous environments at the oil-water interface. Under nonaqueous environments, lipases can catalyze the synthesis or interesterification of triacylglycerols. The optimum water levels for lipase-catalyzed reactions vary from 0.042 (%v/v) to as high as 50 (%v/v) depending on whether net esterification or net hydrolysis is sought (Balcao et al., 1996). The interesterification reactions can be divided into three different processes: 1) acidolysis is the exchange of fatty acids between acylglycerols and free fatty acids, 2) transesterification is the exchange of fatty acids between two acylglycerols, and 3) glycerolysis is a reaction between free fatty acids or acylglycerols and glycerol. These activities are used to produce trans-free and structured triacylglycerols. Lipases can also catalyze the transesterification reaction between free fatty acids and a hydroxyl group on sugars to form sugar esters. Recently, the use of the immobilized form of the lipase is preferred since immobilization improves the lipase stability and activity and the enzyme can be reused. The number of fatty acids esterified depends the type of solvent, the ratio of fatty acids to sucrose molecules and the type of enzyme used.

Plou et al. (2002) screened a variety of immobilized lipases for the acylation of di- and tri-saccharides with laurate and found four lipases which were capable of catalyzing this reaction efficiently. These lipases were from *Thermomyces lanuginosus*, *Candida antarctica* and *Pseudomonas*. Each lipase showed a different specificity based on the hydroxyl groups in the sugars. For example, with *C. antarctica* lipase and sucrose, both the 6C-OH and 6'C-OH groups were acetylated but with lipases from *T. lanuginosus* and *Pseudomonas* only the 6C-OH were acetylated. With maltose, each of the enzymes listed above was specific only for the 6C-OH. In general, the chemical reactivity of sucrose hydroxy groups follows the order of 6C-HO>6'C-OH>1'COH>secondary-OHs. The ratio of sucrose to fatty acids in these experiments was 1:1 and the solvent included dimethylsulfoxide. The authors also noted that increasing the concentration of dimethylsulfoxide resulted in the formation of diesters, not monoesters. The chemical, physical, and antimicrobial properties of carbohydrate esters changes with the degree of esterification as noted above.

Immobilized lipases are available commercially from a variety of vendors and vary in specificity from Sn-1,3 or nonspecific (Table 3). The commercial use of immobilized enzymes includes the production of trans-free fats, cocoa butter equivalents, and diacylglycerols (Enova™ Oil) (Archer Daniels Midland, Quincy IL) (reviewed in Walsh, 2006).

In order to synthesize carbohydrate esters it is necessary to find a medium that maintains the carbohydrate and the fatty acid soluble in the presence of the lipase. High aqueous systems favor ester hydrolysis, which limits sugar ester formation due to equilibrium. Common solvent systems for carbohydrate ester synthesis include dimethylsulfoxide, tert-butanol, propane, butane, hexane, ethyl methylketone, acetone, ethanol and propanol. Solvents permitted in the synthesis of a food ingredient include acetone, ethyl methylketone and ethanol (Gulati et al., 2003; Plou et al., 2002). For example, the yield of sorbitol monostearate in propanol and ethanol was lower than in other solvents (hexane, butanol) but it was still possible to achieve a 70% conversion. It is also possible to synthesize carbohydrate esters in aqueous environment although the yields are generally much lower than in a nonaqueous environment (Dossat et al., 2002). Therefore the methods section of this proposal describes the comparison of acetone, ethyl methylketone and ethanol for lactose lauryl ester synthesis.

It is common to include molecular sieves (10% w/v) for the removal of water produced during the reaction (Gulati et al., 2003) to maintain a low water activity (<0.5 a_w). The low water activity shifts the equilibrium towards ester synthesis and prevents ester hydrolysis (Ducret et al., 1995; Ferrer et al., 1999). For example, Watanabe et al. (2000) esterified various fatty acids to carbohydrates using *P. cepacia* lipase in acetone at 60C for 48 hrs in the presence of molecular sieves.

In general, the monoesters of carbohydrates were shown to have greater antimicrobial activity compared to di- or tri-esters. This is a function of both the lipase used and the ratio of the carbohydrate to the fatty acid. Generally, molar ratios of 1:1 to 1:5 (carbohyrate:fatty acid)

have been used to determine the optimum concentration of fatty acid resulting in monoester formation (Gulati et al., 2002; Ferrer et al., 1999; Reyes-Durate et al., 2005). For example, the synthesis of sorbitol monostearate was optimum at a sorbitol:stearic acid molar ratio of 1:4 at times of 12 h to 48 hours using a lipase from *Pseudomonas* (Gulati et al., 2002). Ferrer at al. (2005) used 2-methyl-2-butanol as a reaction medium when synthesizing antimicrobial carbohydrate esters (6-O-larrylsucrose and 6'-O-laruoylmaltose) with lipases from *T. lanuginosus* and *C. antarctica* at a sugar:fatty acid ratio of 1:5 at 40 C for at least 2 hrs.

In addition to the choice of solvent, ratio of reactants, and type of lipase, temperatures of esterification reactions vary from 40 to 60 C (Ferrer et al, 1999, Plou et al., 2002, Gulati et al., 2003) and the reaction times vary from 8 h to 7 days (Ferrer et al., 1999; Piao and Adachi, 2004).

Due to the interrelationship of the factors (solvent, enzyme, temperature, ratio of reactants) the methods section describes a response surface statistical design.

Preliminary findings or research efforts by the principal investigator, or others

The PI has no preliminary findings in this area of research but many examples on the synthesis of various carbohydrate esters has been presented in this literature review.

RESEARCH PLAN

Objective 1.

Determine the optimum conditions for the synthesis of lactose lauryl esters.

Objective 2.

Investigate the antimicrobial properties of lactose lauryl esters.

Objective 3.

Data analysis and manuscript preparation.

MATERIALS AND METHODS

Lactose will be purchased from Glanbia Nutritionals (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).

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 5. 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 (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

Table 3. Characteristics of some commercially available immobilized enzymes (Walsh, 2006).

Name	Supplier	Organism	Specificity	Matrix
Lipozyme TL IM	Novozyme A/S	*Thermomyces lanuginose*, TLL-1	Sn-1,3 specific	Silica granules
Lipozyme RM IM	Novozyme	*Rhizomucor miehei*, RML	Sn-1,3 specific	Macroporous ion exchange resin
Novozyme 435	Novozyme	*Candida antartica* lipase B	nonspecific	Macroporous acrylic resin
Lipase PS-C	Amano	*Pseudomonas cepacia* lipase, PCL	nonspecific	Ceramic particles
Lipase AK-C	Amano	*Burkholderia cepacia* lipase (formerly *Pseudomonas fluorescens* lipase PFL)	Sn-1,3 specific	Ceramic particles

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

Bacteria	Biosafety level	Media and ATCC Media Reference Number	ATCC #
Streptococcus sobrinus	1	ATCC 44: Brain heart infusion, 37 C	27351
Streptococcus mutans	1	ATCC 1169: Glucose tetrazolium medium, 37 C	31341
Bacillus subtilis	1	ATCC 18: Trypticase soy agar, 37 C	39088
E. coli K12	1	ATCC 1065: LB medium, 37 C	35695
Lactobacillus plantarum	1	ATCC 416: Lactobacillus MRS broth, 37 C	14431
Pseudomonas fluorescence	2	ATCC 368: Blood agar base, 37	25006

Table 5. Response surface central composite design with four factors.

Run Number	Block	Solvent	Temp (C)	Molar ratio Lactose:Fatty Acid	Lipase type
1	1	Acetone	40	1:1	Lipase PSC
2	1	Acetone	40	1:5	Lipozyme
3	1	Acetone	60	1:1	Lipozyme
4	1	Acetone	60	1:5	Lipase PSC
5	1	Ethanol	40	1:1	Lipozyme
6	1	Ethanol	40	1:5	Lipase PSC
7	1	Ethanol	60	1:1	Lipase PSC
8	1	Ethanol	60	1:5	Lipozyme
9	1	EMK	50	1:3	Novozyme
10	1	EMK	50	1:3	Novozyme
11	2	Acetone	40	1:1	Lipozyme
12	2	Acetone	40	1:5	Lipase PSC
13	2	Acetone	60	1:1	Lipase PSC
14	2	Acetone	60	1:5	Lipozyme
15	2	Ethanol	40	1:1	Lipase PSC
16	2	Ethanol	40	1:5	Lipozyme
17	2	Ethanol	60	1:1	Lipozyme
18	2	Ethanol	60	1:5	Lipase PSC
19	2	EMK	50	1:3	Novozyme
20	2	EMK	50	1:3	Novozyme
21	3	Water	50	1:3	Novozyme
22	3	Water	50	1:3	Novozyme
23	3	EMK	30	1:3	Novozyme
24	3	EMK	70	1:3	Novozyme
25	3	EMK	50	0	Novozyme
26	3	EMK	50	1:7	Novozyme
27	3	EMK	50	1:3	None
28	3	EMK	50	1:3	None
29	3	EMK	50	1:3	Novozyme
30	3	EMK	50	1:3	Novozyme

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 6) in various solvents (acetone, ethyl methylketone or ethanol) in a 5 ml volume.

Table 6. Concentrations of reactions.

Lactose (M)	Laurate (M)	Lactose Laurate (M ratio)
0.03	0.03	1:1
0.03	0.09	1:3
0.03	0.15	1:5

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 10^5 CFU/ml (as determined by OD600 measurements of the same culture) containing lactose lauryl ester 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 off 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 (Fluropure 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.

Objective 3. Data analysis and manuscript preparation.

The data generated in objectives 1 and 2 will be analyzed and a manuscript will be prepared.

RESULTS AND DISCUSSION

HPLC chromatograms of reactions A-C did not show peaks other than the sugar or fatty acid unless molecular sieves were added. With the addition of molecular sieves, multiple peaks were observed in chromatograms. We decided to run reactions from the literature with sucrose and vinyl laurate as a standard (Table 1, Batch D, Figure 1) followed by TLC to identify the peaks in the chromatograms. Figure 1a is the sucrose:vinyl laurate chromatogram with the peaks identified by TLC. There were two major peaks for sucrose esters. The same reaction conditions were used with lactose in place of sucrose (Table 1, Batches D and E) and the chromatogram is shown in Figure 1b. Two minor peaks with elution times similar to sucrose esters are shown. In addition, a TLC with various reactions from reaction batches D and E is shown in Figure 1c. The brown bands at the height of the arrow contain both lipid and carbohydrate moieties. These fractions will be collected for NMR analysis for composition.

Our previous experiments on ester synthesis using DMSO as a solvent were successful, but the DMSO fouled our HPLC equipment. Therefore, we changed to a factorial design with a 4x4x2 design (solvent type, enzyme type, and sugar type) to determine the influence of enzyme type and solvent type on monoester formation.

Initially we determined the solubility of lactose and sucrose (as a control) in each of the four solvents. The following figure shows that lactose is more soluble in EMK, acetone, and acetonitrile than in sucrose, but both are well below a 0.2% solubility compared to water. The sugars show the highest solubility in 2M2B but still at a very low solubility level (<0.6% of water). Previous researchers have hypothesized that the sugars continue to become solubilized in the organic solution as esters are formed. Based on the date described below, we believe that to be the case since we have calculated 10% monoester efficiency, which is greater than the theoretical efficiency of 0.1% as determined by the solubility.

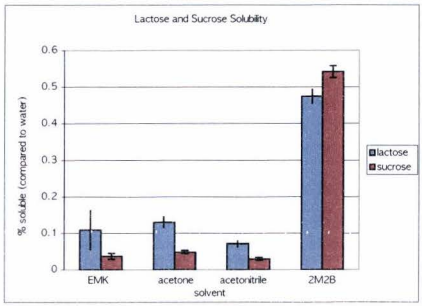

Figure 1. Lactose and sucrose solubility.

The following chromatogram shows the profile of a typical reaction using the enzyme from Thermomyces. This enzyme is by far more active than the others; the full factorial is still being analyzed. Peak 1 is lactose and peak 3 is lauric acid. Peak 2 (presumably lactose monoester) was collected using a preparative HPLC column from a 30 ml reaction using acetone and Thermomyces lipase. We were able to quantitate the amount of monoester using a lauric acid standard curve. The total amount purified was 42 mg (10% synthesis efficiency). We will be using 10 mg for NMR, and the remainder will be used to initiate antimicrobial studies.

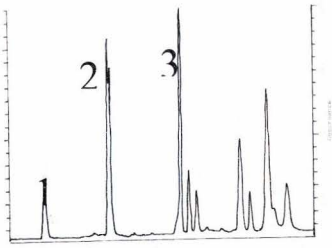

Figure 2. Profile of a typical reaction using the enzyme from Thermomyces.

Q1 2008.

Objective 1.

We have investigated the effect of enzyme type (T. lanuginosus, C. Antarctica) with acetone, 2M2B, and EMK on the synthesis of lactose and sucrose lauryl esters. We

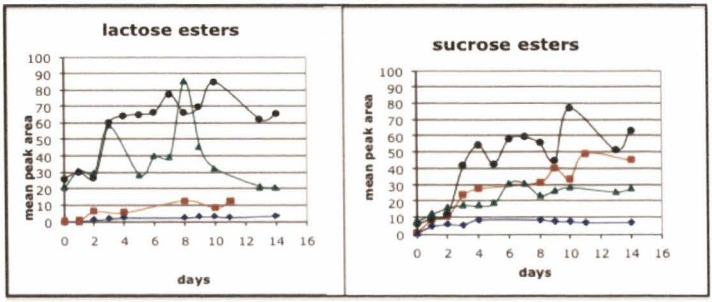

Figure 3.

are in the process of investigating the influence of Lipozyme and Amano Lipase with acetone, 2M2B, and EMK for ester synthesis. The results obtained to date are given in the figures above.

As shown in Figure 3, the Thermomyces enzyme (black and green symbols) is more active at ester production than the Candida enzyme. Within the enzymes, the solvents seem to be similar (statistical analysis will be conducted after the experiment has been replicated). We also will convert the y axis to gram quantities based on a standard curve.

Q2 2008.

We have completed the study that investigated the influence of enzyme and solvent type on the yield of lactose and sucrose monoester synthesis. With respect to lactose monolaurate, Table 1 shows that the Pseudomonas, Mucor Miehi, and Thermomyces enzymes are more effective than the Candida Antarctica enzyme. Pseudomonas and Mucor Miehei and more effective in the solvent 2M2B, while the Thermomyces enzyme is more effective in acetone. Considering the price per mg of enzyme, the most cost effective is Mucor Miehei since this cost about $4.48/g with an ester yield of 77%. The rate should also be considered. Again, the lipase from Mucor Miehei is faster with a rate of 3 mg ester/ml/day with the thermomyces and Pseudomonas lipases coming in second and third, resectively.

Therefore, the most effective lipase for lactose monolaurate synthesis based on yield, rate, and cost is the Mucor Miehei lipase.

With respect to sucrose monolaurate synthesis, the highest yield was only 51% using Pseudomonas in 2M2M. This is similar to literature values. I believe that the higher yield for lactose ester synthesis is due to the slightly higher solublility of lactose compared to sucrose in the MEK and acetone.

Synthesis of lactose and sucrose monolaurate was influenced by enzyme and solvent type. The cost of each enzyme is also given in the column with the enzyme. (Table 7.)

Since we have shown that there are only three viable lipases in two different solvents that are effective for lactose monolaurate synthesis, we have designed the following response surface experiment to determine the optimum conditions of temperature and reactant concentrations to maximize the yield.

The following response surface design is being conducted with Thermomyces in acetone, Mucor Miehei in 2M2B, and Pseudomonas in 2M2B. We are completing the reactions and will have the results of this data for the next report. (Table 8.)

New response surface results.

The RSM analysis was conducted for synthesis of LML using MM in 2M2B because this combination resulted in a high yield, the fastest rate, and the enzyme is more economical than the others. The experimental design and concentration of LML synthesized at each design point are given in Table 3. Among the various treatments, the highest yields were obtained with runs 4, 6 and 7, while runs 2 and 10 showed the lowest yields.

ANOVA results revealed that all three variables and the interactions of temperature x temperature and ratio x ratio exhibited statistically significant effects ($p < 0.05$) on the yield of LML. The estimate response model equation, without the insignificant variables, was used to estimate the enzymatic synthesis of LML with MM and is as follows:

$$Y = -353.78 + 5.81\ X1 + 6.9\ X2 + 101.13\ X3 - 00.11\ X2X2 - 13.50\ X3X3 \quad (1)$$

where Y is the response factor in peak area and X1, X2, and X3 are the independent factors of temperature, enzyme concentration (mg/ml), and ratio of lactose to vinyl laurate.

Table 7. 3 mL reaction volume. 0.04 mol/L (44.16 mg) sugar, 0.13 mol/L (87.10 mg) vinyl laurate, 22.58 mg/mL enzyme, 22.58 mg/mL molecular sieves. 55°C

		Lactose		Sucrose	
Enzyme	***Solvent***	***% Yield Ester***	***Rate of Reaction (mg/mL/day)***	***% Yield Ester***	***Rate of Reaction (mg/mL/day)***
Thermomyces $37.50/g	2M2B	52.3 ± 6.42	0.459	48.4 ± 4.36	0.564
	acetone	63.5 ± 0.13	0.674	19.5 ± 0.01	0.279
	MEK	17.7 ± 0.02	0.208	2.4 ± 0.27	0.033
Mucor Miehei $4.48/g	2M2B	77.2 ± 3.02	3.046	10.6 ± 0.01	0.093
	acetone	47.9 ± 3.31	1.568	1.2 ± 0.59	0.034
	MEK	18.0 ± 2.77	0.155	0	n/a
Pseudomonas $10.70/g	2M2B	86.8 ± 2.34	0.920	51.3 ± 0.03	0.551
	acetone	28.7 ± 0.34	0.415	25.32 ± 1.39	0.213
	MEK	9.0 ± 0.11	0.099	1.9 ± 0.48	0.015
Candida Antarctica $11.70/g	2M2B	32.2 ± 2.52	0.354	31.0 ± 0.01	0.333
	acetone	2.0 ± 0.23	0.019	5.4 ± 0.01	0.153
	MEK	0.9 ± 0.22	0.011	0.7 ± 0.17	0.011

Table 8. Response surface design to maximize lactose monolaurate synthesis.

Reaction	Temperature	Amount Enzyme (mg)	Vinyl laurate: lactose ratio	MM results in peak area	PC results in peak area
1	40	30	5.82	118.3	33.48
2	40	30	0.17	0.09	16.84
3	25	10	4.14	23.26	1.83
4	55	10	4.14	229.92	33.54
5	25	50	4.14	45.67	6.68
6	55	50	4.14	228.5	109.54
7	61.2	30	1.58	89.80	148.51
8	18.7	30	1.58	18.67	2.922
9	40	58.3	1.58	43.51	60.18
10	40	1.7	1.58	9.15	0
11	40	30	3	149.79	23.04

The coefficient of determination (R^2) was 0.95 indicating that the model was suitable to represent the factors.

Canonical analysis of the three variables determined that the most critical factor was temperature, with the concentration of enzyme being the second most influential factor on the yield. Figure 5 shows the effect of ratio, temperature, and enzyme concentration on the amount of LML synthesized. The stationary point for maximum yield was determined to be a saddle point, therefore there was no unique optimum. This can be seen in Figure 5, where there is a narrow range of ratios (3.7-3.8) at 61°C that gives maximum lactose monoester yield. Figure 5 also shows the influence of temperature on yield is linear, with increasing yields with an increase in temperature, while the influence of ratio and enzyme concentration have narrow optimum values.

The ridge maximum analysis was conducted as described by Chang et al., 2008, which determines the optimum reaction conditions with the maximum predicted yield. The conditions of 61°C 32 mg/ml of enzyme and a lactose:vinyl laurate ratio of 1:3.8 was predicted to yield 28 mg/ml LML. Our experimental results were in agreement with a concentration of 27.8 mg/ml obtained with conditions listed above. Therefore RSM was successful in determining the optimum conditions for LML synthesis in 2M2B with MM.

NMR results.

In the results, the carbon spectra for the reaction products show a shift of about 4.0 ppm from either the C6 or C6' carbon when compared with the standard. Because these peaks are so close together it was hard to tell which one shifted. But, using the spectra for the α/β mix of lactose, the C1 peak was determined because it contained a resonance for both α and β. This same peak was identified

in the α lactose, and the corresponding proton peaks were assigned using a 2-dimensional HETCOR spectrum. When the proton spectra for the standard, and a proton spectra from one of the products was compared, a shift containing the hydrogens corresponding to the C6' carbon was seen. The observed shift meant that it was the C6' carbon which moved about 4.0 ppm. The C1 peak on the other hand, appeared to only have shifted about 0.6 ppm. The shift from the C6' carbon indicates that esterification has occurred at this site.

When a carbon is esterified, the carbon spectrum will of course show a shift downfield for that carbon. In addition, the neighboring carbon will show an upfield shift (8). This phenomenon was observed in the carbon spectra with the 6' carbon shifting downfield and the 5' carbon shifting upfield.

Q1 2009.

Objective 2.

We investigated the antimicrobial activities of lactose lauryl esters at a concentrations of 0.1%, 0.01%, 0.005%, and 0.001% against the organism organisms in microtiter format using media as the control: S. typhimurium, K.pneumoniae, E.coli 0157H7, S.suis, L.monocytogenes, E.fecalis, and S.aureus. The experiment was replicated 3 times with triplicates for each experiment.

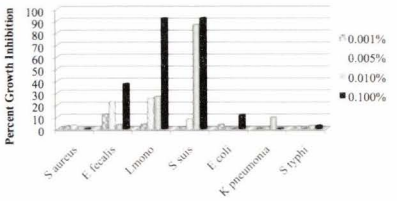

Figure 4.

There is significant growth inhibition (>90%) of Streptococcus suis (Gram-positive bacterium and an important pathogen of pigs) and Listeria monocytogenes (Gram-positive bacterium) at concentrations of 0.1%. There is a concentration dependent inhibition of both of these bacteria. The use level of sucrose esters in food products is up to 1%. Staphylococcus aureus is also a Gram-positive bacterium, but its growth was not inhibited by lactose monolaurate. There is also approximately 40% growth inhibition of E. feacalis.

Q3 2009.

We completed the response surface analysis (RSA) for two enzymes, MM and PC. We decided not to include TL in the analysis. Reactions in TL show a maximum yield in acetone, unfortunately the boiling point for acetone is 56.6°C and many of our samples evaporated under these conditions. Also, looking at monoester yields and the cost of the enzymes, MM and PC are more economical than TL. The response surface graphs are listed below the table and show the optimum conditions for MM and PC.

ANOVA results of the two RSA reactions revealed that all three variables exhibited statistically significant effects on the yield of lactose monolaurate. Canonical analysis of the three variables determined that the most critical factor was temperature, with the ratio of the substrates being the second most influential factor on the yield. In addition, the stationary point for maximum yield was determined to be a saddle point for PC, therefore, there was no unique optimum, but the canonical analysis of MM showed a maximum value.

The equation for MM was determined to be at a temperature of 60°C, an enzyme amount of 30 mg/ml with a lactose:laurate ratio of 3. We confirmed this equation by setting-up this reaction, and we were able to obtain a yield of 85%. We are completing the RSA with PC to determine the optimum conditions for maximum synthesis. (Figure 5.)

Q4 2009.

We used the same methodology to investigate the inhibitory effects of lactose monolaurate against five clinical strains of Listeria monocytogenes listed in Table 9.

Each of these strains was inhibited with 1% lactose monolaurate as observed with the original strain. The plate counts indicated that the starting colony forming units (cfu) were at 10^5 and the final cfu were the same. For the control (strains without lactose monolaurate), the initial cfu were again 10^5 while the final cfu were 10^9. Therefore, lactose monolaurate is effective at inhibiting the growth of multiple strains of this organism at a concentration of 1%.

CONCLUSIONS

The antimicrobial (or bacterial static) activity of lactose lauryl esters against S. typhimurium, K.pneumoniae, E.coli (general), S.suis, L.monocytogenes, E.fecalis, B. subtilis spores, S. sobrinus, and M. smegmatis at a concentration of 50 microg/ml was demonstrated.

The optimum conditions for lactose lauryl synthesis is still being conducted, but preliminary research to date has identified the enzyme from Thermomyces as being more effective than the Candida enzyme.

Q3 2009.

We conducted Response Surface Analysis for Muucor miehie (MM) and Pseudomonas cepacia (PC) since these

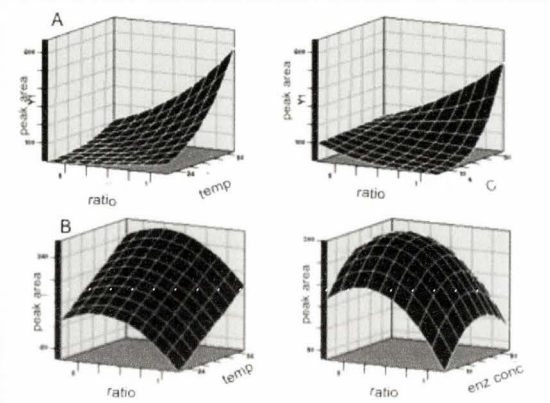

Figure 5. Response surface graphs to optimize the conditions (enzyme amount, temperature and lactose:laurate ratio). A. Pseudomonas cepacia B. Mucor miehei

Table 9.

FSL J1-177; ribotype DUP-1051D; lineage I; serotype 1/2b	Isolated from human sporadic case
FSL C1-056; ribotype DUP-1030A; lineage II; serotype 1/2a	Isolated from human sporadic case
FSL N3-013; ribotype DUP-1042B; lineage I; serotype 4b	Food isolate associated with human listeriosis epidemic in the UK (1988-1990)
FSL R2-499; ribotype DUP-1053A; lineage II; serotype 1/2a	Human isolate associated with US outbreak linked to sliced turkey (2000)
FLS N1-227; ribotype DUP-1044A; lineage I; serotype 4b	Food isolate associated with US outbreak (1998-1999)

two enzymes were determined to be the most cost effective with respect to lactose monoester synthesis. The conditions for maximum monoester synthesis using MM resulted in an 85% yield.

Current results show that synthesized lactose monolaurate is effective at concentrations of 1% at inhibiting the growth of 6 strains of Listeria monolaurate.

Q4 2007.

We have purified mg amount of lactose monoester with a new solvent/enzyme system that will be used for NMR and antimicrobial studies.

Previous conclusions.

We believe we have synthesized lactose lauryl esters using 2M2B:DMSO and a lipase from Candida antarctica. This will be confirmed with NMR analysis. We have also developed methods for HPLC and TLC analysis of sugar:ester synthesis.

NEXT STEPS

NMR analysis of potential lactose vinyl laurate esters.

Initiate antimicrobial studies.

Continue with optimizing the synthesis of lactose monoesters for food use.

Complete NMR analysis to confirm lactose lauryl ester structure and position of esterified lauric acid.

Complete the response surface analysis with PC to determine the optimum conditions for synthesis

Replicate antimicrobial analysis.

We are replicating the antimicrobial analysis with the monolaurate produced from Thermomyces. We will also replicate the antimicrobial experiment with the monolaurate

synthesized with Mucor Miehei in 2M2B since this is the most effective enzyme. We have used concentrations of 0.1 and 0.01, and 0.005 mg/ml of ester in bacterial growth media for the replicates. We are currently analyzing the data. We prepared the lactose esters in 50% ethanol and 10% DMSO for the antimicrobial activities.

We will complete the microbial inhibitory studies by adding a concentration of 1% lactose monolaurate. We will also be calculating the growth inhibition with plate counts to correlate the optical density values used to determine the growth inhibition with colony forming unit values.

We have completed the objectives of this experiment, and we will continue with producing another peer-reviewed manuscript based on the microbial inhibition.

REFERENCES

Inventions or Patents

An invention disclosure has been submitted to Utah State University Technology Commercialization Office, March 31, 2008.

Manuscripts and Abstracts

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Publications

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Development of a descriptive taste panel for dairy application

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Funded by: Dairy Management Inc., January 2008 - December 2009

ABSTACT

A descriptive sensory panel was developed in the Dairy Technology Innovation Lab at the Western Dairy Center. Panelists were selected and trained to evaluate the flavor of dairy products. Techniques and standards were used mainly from existing bibliography. The descriptive panel began eveluating products in May 2009. The panel receives ongoing training, and replacement panelists were trained and added as needed.

RESEARCH PLAN

Objective 1.

Development of a descriptive sensory panel for dairy products.

MATERIALS AND METHODS

A schematic diagram of the panel selection and training process is given in Figure 1.

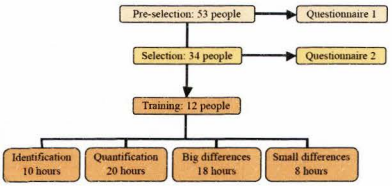

Figure 1. Process during the pre-selection, selection, and training of panelists.

Pre-selection.

Panelists will be recruited from the local community and asked to complete Questionnaire 1. Participants will be sellected from this pool according to their responses and availability. Subjects with health conditions or allergies to dairy products or other foods will be discarded from the pool.

Selection.

Subjects chosen from the pre-selection will then be asked to identify and rank the intensity of tastes and odors (Questionnaire 2). Aqueous solutions of the following compounds will be used for taste identification: caffeine (0.07%) for bitterness, sucrose (2%) for sweetness, sodium chloride (0.2%) for salty, monosodium glutamate (1%) for umami, and citric acid (0.07%) for sourness. The concentrations of compounds used for intensity ranking is listed in Table 1.

Table 1. Attributes used for intensity ranking exercise.

Taste	Concentration (%)
Bitter (caffeine)	0.035
	0.07
	0.14
Sweet (sucrose)	1.00
	2.00
	4.00
Salty (sodium chloride)	0.10
	0.20
	0.40
Sour (citric acid)	0.035
	0.07
	0.14

The following compounds will be used for odor recognition: butyric acid for free fatty acids, diacetyl for buttery, pineapple for pineappley/fruity, omega-3 fatty acids for fishy, wheat germ for nutty, hexanal for grassy, and eucalyptus oil for floral.

Panelists with the best scores will be selected based on the following criteria:

1. One point awarded for each taste that was correctly identified.
2. One to five points awarded for recognition of odors.
3. An ANOVA test will be used to eliminate the judges that are significantly different from the group in intensity ranking.

Training.

One hour training sessions will be conducted four times a week until training is complete. Flavor attributes, representative compounds, and concentrations are listed in Table 2. Panelists will first be trained to recognize these flavor attributes, then trained in quantification of the attributes. Milk will be used as the carrier. When available, references and scales from the Spectrum Method (rating of intensity using a 15 cm scale) will be used. Mixtures of attributes will also be used for training. The intensity of each attribute will standardized to a specific rating in the scale as described in the Spectrum Method. An example of the scale used is presented in Table 3.

Panelists were presented with ten Ricotta cheese samples, in a random order, and asked to evaluate them for some of the attributes found in dairy products: bitter, brothy, salty, sour, sulfur, sweet, and umami flavor attributes. Two of the samples presented to each panelist were control samples, and the remaining eight samples were spiked with the attributes of interest and prepared as follows:

Bitter: 1 g caffeine + 425 g Ricotta

Brothy: 40 g low-sodium chicken broth + 415 g Ricotta

Salty: 5.5 g salt + 425 g Ricotta

Sour: 4.35 g 88% lactic acid + 425 g Ricotta

Sulfur: (0.47 ppm H_2S) Formulated sample by adding 0.0225 g saturated hydrogen sulfide water (0.4g H_2S/100ml at 25 °C) to 100 ml skim milk. Then, added 1 ml of that milk to 415 g Ricotta.

Sweet: 25 g sugar + 380 g Ricotta

Umami: (0.1% MSG) 0.5 g monosodium glutamate + 425 g Ricotta

Following the criteria stated above, the best 13 panelisists were chosen. Most of the training has been completed, aand the panel has begun training with cheese. Training shouuld be completed by the end of April, and the panel will be ready to evaluate cheese from Western Dairy Cennter projects and industry

Panel Evaluation.

Panelists identified and quantified 18 attributes in commercial cheeses. The cheeses used were: Precioous Mozzarella Whole Milk, Cache Valley Sharp Chedddar, Gossner Unsalted Mild Cheddar, Aggiano, Gossner Saltted Mild Cheddar, Cracker Barrel Vermont Sharp-White, Agggie Extra Sharp Cheddar, Cache Valley Mozzarella Skim, aand Aged Cheddar Cheese. Each cheese was evaluated for all 18 flavor attributes on a scale of 0 to 15. The attributtes quantified were: bitter, brothy, cooked, fruity, fatty accid, metallic, nutty, oxidized, pineapely, rancid, rosy/floproal, salty, sour, sulfur, sweet, umami, whey, and buttery. Tabble 4 shows the types of cheeses tasted and the abbreviatioons used in the results section.

The panel and individual panelist performance wwas analyzed with "PanelCheck" software. PanelCheck is free software that provides graphical interpretation of descriptive panels performance. The panelists nammes were deleted from the Figures (Figures 1-3) to maintatain confidentiality.

Table 4. Cheeses used for the panel evaluation.

Cheese	Abbrev.
Precious Mozzarella Whole Milk	WMozz
Cache Valley Sharp	Sharp
Gossner Unsalted Mild Cheddar	NS Mild
Aggiano	Aggiano
Gossner Salted Mild Cheddar	S Mild
Cracker Barrel Vermont Sharp-White	White
Aggie Extra Sharp Cheddar	E.Sharp
Cache Valley Mozzarella Skim	SMozz
Aged Cheddar Cheese	Aged

RESULTS AND DISCUSSION

Q2 2008.

After a lengthy advertising and interview period, we hired a descriptive panel coordinator. This person will continue training and maintenance of the panel, maintatain the sensory laboratory, work with industry and researchhers to conduct necessary tests, perform statistical analysis, a and write reports.

Table 2. Scaling references for training. References marked with an (S) mean that they correspond to the Spectrum Method.

Attribute	Reference Set(s)
Sour	0.05, 0.08, 0.15, and 0.20% citric acid in water (S) or 0.035, 0.07, and 0.014% lactic acid in milk or water
Fishy	2, 5, or 7 drops fish oil supplement/ 900 ml skim
Oxidized	0.2 ml of 1% copper sulfate solution in 900 ml of non-homogenized milk (stored at 5 °C for 1 week). Stock solution and following dilutions, prepared with fresh pasteurized non-homogenized milk: 1/3 oxidized, 2/3 oxidized, and unoxidized.
Bitter	0.05, 0.08, 0.15, and 0.20% caffeine in water (S) or 0.02, 0.05, and 0.08% caffeine in water or milk
Salty	0.2, 0.35, 0.5, and 0.7% NaCl in water (S) or 0.1, 0.2, 0.4, and 0.75% NaCl in water or milk
Sweet	2, 5, 10, and 16% sucrose in water (S)
Lactone/Fatty Acid	skim, 1/3 whole, 2/3 whole, whole milk
Cooked	Cooked on stove top at 80-90 °C for 45 min and presented this to panelists along with the dilutions: skim, 1/4 cooked (3/4 uncooked skim), 1/2 cooked, 3/4 cooked; also given in dilutions of 1/3, rather than 1/4
Umami	0.1, 0.25, or 0.75% monosodium glutamate (MSG) in skim milk
Nutty	0.5 ml, 2 ml, and 5 ml hazelnut extract in 950 ml skim
Whey	(skim milk:whey) 6:1, 3:1, 1:1
Buttery	2g, 10g, 20g melted butter in warmed milk (~350ml total) ultra turraxed for up to 3 minutes until blended well enough to have the butter droplets remain dispersed in the milk. Stirring immediately before presentation reduces visual difference between the samples. Must present immediately.
Fruity	2, 5, 10, and 20 g fruit juices blend in 1000 ml skim milk (Blend consists of ½ 100% apple juice frozen concentrate and ½ 100% peach/white grape juice frozen concentrate.)
Rosy/Floral	0.01505 g 2-Phenethylamine to 1000 ml (or 1050-75 g) skim (stock solition), a 1/2 dilution of this, and 1/10 dilution
Sulfur	Stock solution: 4 x 10^{-7} g H_2S. The references are formulated from this as follows: 25% stock in water/skim, 37% stock in water/skim, and 50% stock in water/skim. Water dilutions are stable; whereas milk one's must be stored for less than two days in order to avoid degradation and loss of the attribute to other flavors typically found in UHT milk (i.e. cabbagey).
Brothy	2, 10, and 20 g organic low sodium chicken broth in 1000 ml skim
Pineappley	2, 10, and 20 g 100% pineapple juice frozen concentrate
Metallic	0.04, 0.08, and 0.16 g ferrous sulfate/1000 ml skim.

Table 3. Scale used to train the panelists. Adapted from the Spectrum Method.

NAME:						DATE:									
Sample:															
Attribute:															
Intensity:	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Comments:															

During the last three months, the descriptive panel met once or twice a week to practice (maintenance) the sensory skills developed during training. The panel performance as a whole and the individual performance were evaluated to assure that panelists are able to correctly identify and rate flavor attributes in dairy products. Panelists were evaluated for their ability to identify and rate all 19 flavor attributes in skim milk and in the following cheeses: Aggie Squeaky Cheese Curd; store-bought mild, medium, and sharp Cheddar cheeses; Cracker Barrel-Vermont White Cheddar, store-bought Monteray and Colby Jack cheeses, Precious Mozzarella, Cache Valley Mozzarella, aged Aggiano, week-old USU Feta, and year-old USU Feta. Panelists received additional training as needed.

Ricotta controls.

The reproducibility of panelist ratings between the two controls was evaluated. Differences between replicates were not significant, with a p-value of 0.6060. As would be expected, there was a significant interaction between the panelist and flavor attribute variables, with a p-value of 0.0058. When a mean comparison was performed, no significant differences were found between the panelists' ratings for each attribute. Though insignificant, there is room for the group, a couple panelists in particular, to improve their ability to rate the intensity of brothy and sweet attributes in this food system.

Spiked Ricotta Samples.

Bitter Sample.

Judge ratings were not significantly different from one another ($p = 0.1389$). Sample was rated significantly more bitter than all other samples ($p \leq 0.0001$). Bitter sample had an lsmean of 8.4 with all other samples having means < 1.0.

Brothy Sample.

Judge ratings were not significantly different from one another ($p = 0.3201$). Sample was rated significantly more brothy than all other samples ($p \leq 0.0001$). Brothy sample had an lsmean of 7.35 with all other samples having means < 3.0.

Salty Sample.

Judge ratings were not significantly different from one another ($p = 0.4358$). Samples were rated significantly more salty than all other samples ($p \leq 0.0001$). Salty sample had an lsmean of 6.95 with all other samples having means ≤ 1.5.

Sour Sample.

Judge ratings were significantly different from one another ($p \leq 0.0001$). One of the judges rated samples significantly lower than other judges (lsmean 0.3794)), and two other judges rated samples significantly higher than other judges with lsmeans of 3.179 and 2.779, respectively. Even though no problems were found during the control testing, the tasting with real samples show that the group could be further trained in rating sourness in ricotta cheeses. Samples were also significantly different from one another ($p \leq 0.0001$). Sour sample had an lsmean of 7.55 with all other samples having means < 1.8.

Sulfur Sample.

Unfortunately, judge ratings were significantly different from one another ($p = 0.0109$), and sample ratings were not ($p = 0.9539$). Only three or four judges could taste any sulfur flavor in this sample. One judge could significantly taste more sulfur in this sample ($p < 0.05$). All samples, except sweet (lsmean 0.3), had lsmeans between 0.9 and 1.5. The amount of sulfur added did not significantly increase the intensity of sulfur flavor in the spiked samples. Therefore, the performance of these judges on this attribute could not be evaluated. Changes to this reference will be made in the future.

Sweet Sample.

Judge ratings were significantly different from one another ($p \leq 0.0001$). Three judges rated samples significantly less sweet when compared with the rest of the group, with lsmeans of 0.798, 0.998, and 0.07798, respectively. The lsmeans for all other panelists ranged from 1.89 to 3.49. Evidenced by these results, the group could use some more work on this attribute, as well. Samples were also significantly different from one another ($p \leq 0.0001$). Sweet sample had an lsmean of 9.0 with all other samples having means < 2.1.

Umami Sample.

Judge ratings were significantly different from one another ($p \leq 0.0001$). This difference was created by a single panelist who rated the umami flavor significantly higher than the rest of the panelists. While the rest of the group had an lsmean rating for umami no greater than 1.025, this panelist had an lsmean of 2.925. This panelist could use a little help with toning down umami ratings in order to rate consistently with the rest of the group. Samples were also significantly different from one another ($p \leq 0.0001$).. Sour sample had an lsmean of 3.6 with all other samples having means < 0.9.

Table 5. Summary of results.

Attribute	Significance between judges	Significance between samples	Attribute LSmean	Attribute LSmean in other samples	Comments
Bitter	0.1389	0.0001	8.4	<1.0	Good identification and rating
Brothy	0.3201	0.0001	7.3	<3.0	Good identification and rating
Salty	0.4358	0.0001	6.9	<1.5	Good identification and rating
Sour	0.0001	0.0001	7.5	<1.8	Judges need more training
Sulfur	0.0109	0.9539	0.3-1.5	0.3-1.5	Attribute concentration too low
Sweet	0.0001	0.0001	9.0	<2.1	Judges need more training
Umami	0.0001	0.0001	3.6	<0.9	Judges need more training

Q3 2008.

Line Plots.

In the line plots, the score for each panelist, represented by colored circles, is plotted for each taste attribute. The average score of the panel for each attribute is indicated by the blue line. Samples of line plots for selected cheeses are shown in Figure 2.

Correlation Plots.

With correlation plots, the score of each panelist is plotted against the panel average for each flavor attribute. The score of the individual panelist being examined is represented by a colored circle. The score of the other panelists is represented by an open circle. A perfect correlation between an individual panelist's score and the panel average is represented by a dashed blue line. Selected plots for individual flavor attributes are shown in Figure 3.

CONCLUSIONS

The panelists are performing well, and additional panelists are being trained to keep the panel size above 12 individuals.

The panel continues to evaluate samples for WDC research projects, as well as for industry.

NEXT STEPS

Panelists will continue with training and evaluation.

Panels will be conducted for DMI, WDC, and industrial clients.

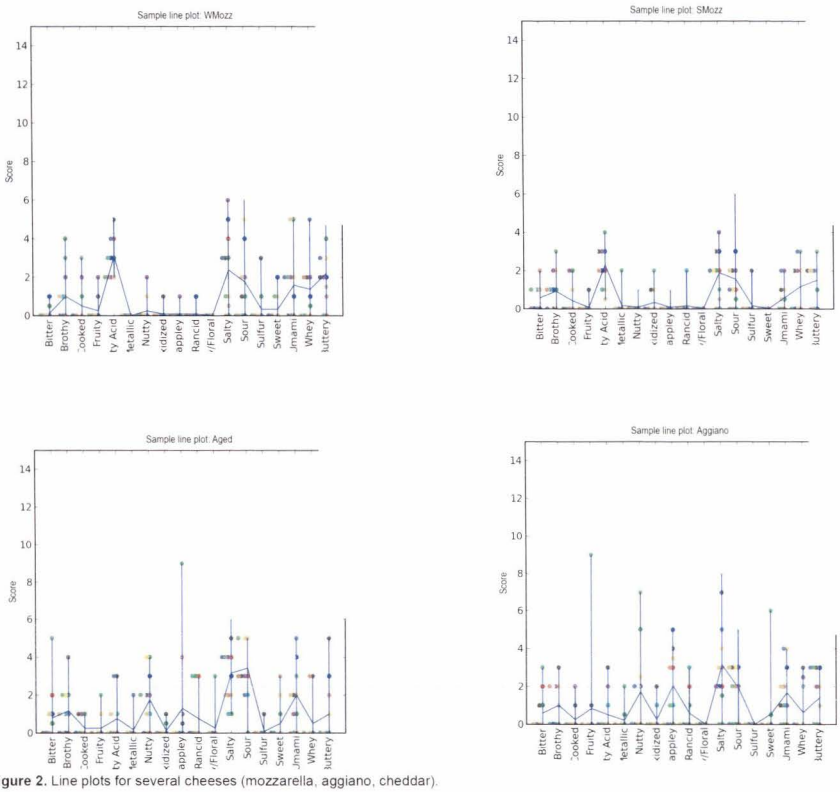

Figure 2. Line plots for several cheeses (mozzarella, aggiano, cheddar).

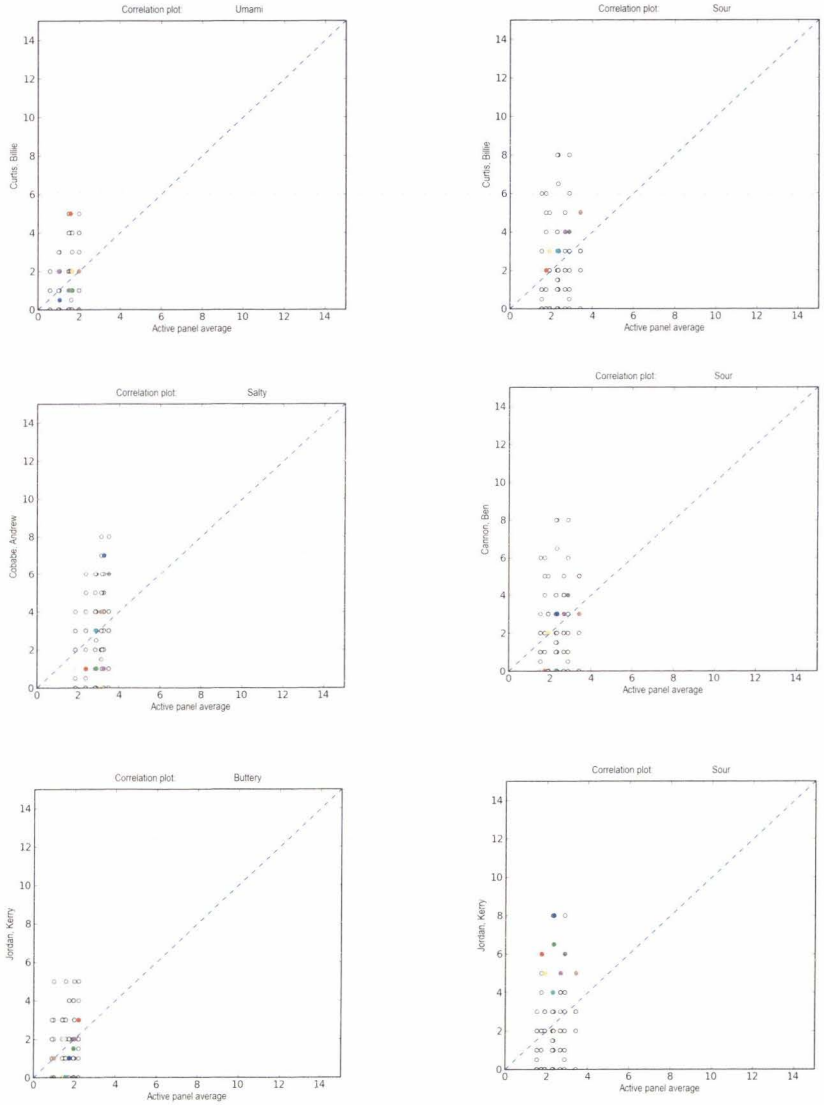

Figure 3. Panelist correlation plots.

Questionnaire 1

PRESCREENING QUESTIONNAIRE

HISTORY:

Name:_____ Gender:_____ Age:_____.
Phone: (Primary) (_____)_____ (Secondary) (_____)_____.
Where did you hear about this project?_____.

TIME:

1. Are there any weekdays (M-F) that you will not be available on a regular basis? If yes, please explain._____.
2. Are there any times of day that you are not available? If yes, please explain. _____.
3. How often do you travel or go on vacation? On average, how long are your trips or vacations? _____.
4. How much longer do you plan to live/work in Cache Valley? (Circle one.)

   <1 year *1 year* *2 years* *3-5 more years* *5-10 more years* *>10 year*

HEALTH:

1. Do you have any of the following?
   - Dentures (partial or full) _____
   - Diabetes _____
   - Oral or gum disease _____
   - Hypoglycemia _____
   - Food allergies _____ What allergies?_____.
   - Hypertension _____
2. Do you take any medications which affect your senses, especially taste and smell? _____.
3. What are your smoking habits? (Circle one.)

   Never *Only in the past* *Smoke occasionally* *Smoke regularly*

FOOD HABITS:

1. Are you currently on a restricted diet? If yes, please explain._____.
2. How often do you eat cheese in a week? _____.
3. What is (are) your favorite cheese(s)? _____.
4. What is (are) your least favorite cheese(s)? _____.
5. What foods can you not eat? _____.
6. What foods do you not like to eat? _____.
7. Is your ability to distinguish smell and tastes... (Circle one for smell. Circle one for taste.)

   SMELL: *Better than average* *Average* *Worse than average*
   TASTE: *Better than average* *Average* *Worse than average*

Does anyone in your immediate family work for a food company?_____.
Does anyone in your immediate family work for an advertising company or a marketing research agency? _____.

QUESTIONS:

1. What do you consider the most prominent characteristic of a ripe piece of fruit? _____.
2. If a recipe calls for thyme and there is none available, what would you substitute? _____.
3. What are some other foods that taste like yogurt? _____.
4. How would you describe the difference between flavor and aroma? _____.
5. How would you describe the difference between flavor and texture? _____.
6. What is the best one or two word description of grated Italian cheese (Parmesan or Romano)?_____.
7. Describe some of the noticeable flavors in mayonnaise. _____.
8. Describe some of the noticeable flavors in cola. _____.
9. Describe some of the noticeable flavors in sausage. _____.
10. Describe some of the noticeable flavors in Ritz crackers. _____.
11. What are some products that have an herbal smell? _____.
12. What are some products that have a sweet smell? _____.
13. How would you describe the difference between fruity and lemony? _____.
14. Describe the smell associated with Feta cheese. _____.
15. Describe some of the noticeable smells in a bakery. _____.

Questionnaire 2

Sample Cheese Descriptive Panel Screening Questionnaire Components

I. Taste Identification: You have been given [five] reference samples. Please taste each one, in the order presented, and identify the flavor attribute present in the sample. Rinse you mouth with water between samples. Expectorate the samples into the cup provided. Do not swallow.

Sample _____ Attribute_____
Sample _____ Attribute_____
Sample_____ Attribute_____
Sample_____ Attribute_____
Sample_____ Attribute_____

II. Odor Recognition: You have been presented with [five] containers. Please open only one of the containers at a time, proceeding in order. Open a container and carefully take three quick sniffs. *Immediately close the container.* Then try to identify and/or describe the contents based on the odor. If you do not recognize the odor, try to describe it. Re-open the containers and re-sniff the odor as often as you wish, but do not fatigue your olfactory system by sniffing too long or too often.

Fruity, nutty, fishy, rancid, buttery, rosy (floral)

Reference_____ Odor description_____
Reference_____ Odor description_____
Reference_____ Odor description_____
Reference_____ Odor description_____
Reference _____ Odor description_____
Reference_____ Odor description_____
Reference _____ Odor description_____

III. Intensity Ranking: Please identify and rank the intensity of the flavor attribute for each of the 12 samples.

Sample_____ Attribute_____

Attribute Intensity Ranking

☐ No Flavor ☐ Slight Flavor ☐ Moderate Flavor ☐ Strong Flavor ☐ Extremely Strong Flavor

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.
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(112 x's)

Projects Continuing in 2010

Innovative approaches for improving low fat mozzarella cheese

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Funded by: Dairy Management Inc., January 2008 - June 2010

ABSTACT

Low fat cheeses dehydrate too quickly when baked in a forced air convection oven that prevents proper melting on a pizza and limits consumer acceptance. To overcome this problem, attempts were taken to avail fat in free form in low fat cheese during baking which could release readily on surface during baking and prevent dehydration. Low fat Mozzarella cheese was made using direct acidification with vinegar to pH 5.9, containing 1.5%, 3.0%, 4.5%, or 6.0% fat in final curd. The cheese curd was salted and placed in a bowl chopper and then melted butter was added at the level 4.5%, 3.0%, 1.5%, or 0.0% respectively, along with glucono-δ-lactone so that the total fat in final cheese becomes 6%. Cheese manufactured was analyzed for melting characteristics, texture profile, free oil content, and dehydration performance when baked on a pizza at 250°C for 6 minutes in an impinger oven after 15, 30, 60, and 120 days of storage at 5 °C. Sensory evaluation of low fat cheese was also compared against full fat cheese for consumer acceptance. All cheeses contained 5.5% to 6.0% fat and 52% to 53% moisture at day 1 of storage. Cheese samples with butter had higher stretchability when tested with a fork, compared to the control cheese in which all 6% fat was from the curd. Melting characteristics also improved in contrast to the control cheese which remained in the form of shreds and did not melt. The treated cheeses had higher free oil content and less hardness, gumminess, and chewiness as compared to the control cheese. Average rating for overall consumer liking for low fat cheese samples were 6.08 which was slightly liked on hedonic scale.

BACKGROUND

Objective 1.

For good melting properties, a low fat cheese needs to have sufficient moisture and fat to prevent the cheese from dehydrating too much during baking, especially when baked on a pizza in an Impinger-type oven using hot air. The chemistry of the protein matrix can be adjusted so that it flows and has stretch properties, but in a low fat cheese there is insufficient free oil to prevent excessive surface dehydration which then results in charring. In a cheese containing only 6% fat, the fat globules are well entrapped within the protein matrix while in a full fat cheese (or even part skim mozzarella) some of the fat is no longer in globular form and is released from the cheese as it is heated.

In recent research at Utah State University we have observed that reduced fat cheese curd can be ground (or chopped) into small pieces and then filled into hoops and pressed. Such cheese knits together well and can be easily sliced. By using this technique, a cheese curd with fat content below the final target can be made and a small amount of fat added during grinding. The amount of fat cannot be too high or it will prevent fusion of the curd particles during pressing. The final cheese will then have tiny veins and droplets of fat distributed throughout that is not intrinsically entrapped by the protein matrix. This cheese can then be diced and when spread on a pizza (or other baking applications) there would be sufficient free oil that can be released to provide a thin coating to the cheese surface.

The maximum fat level allowable for a low fat cheese is 6% fat (max. 3 g fat per 50-g reference amount). One of the requirements to meet the AHA Heart Healthy guidelines, is that such a low fat food can contain no more than 1 g of saturated fat per 1 oz serving. And since butterfat contains 63.5% saturated fat, then such a cheese can only contain 1.57 g of fat per 28 g serving, or no more than 5.6% fat. This will be the target used in this research, and this can be fat present in the original milk or added during the grinding/blending process.

Objective 2.

The fibrous characteristic of mozzarella cheese comes about because during the *pasta filata* hot water cooking/stretching of the cheese curd, the fat globules undergo a type of phase separation from the protein matrix. During this process the fat globules gather and as a result of the stretching process are formed into long column of fat globules in serum with the protein consequently forming into strands that are separated by these fat-serum channels. Then when the hot cheese is extruded the protein strands are oriented so as to parallel with each other. In the absence of fat, there is nothing to keep the protein strands separated and the cheese loses stringiness unless some other substance is added that can also interfere with protein fusion to form the two phase system of protein strands and particle channels during the mixing-extrusion process. Polysaccharide materials that do not bind to the proteins have the potential to mimic the action of fat globules in physical blocking protein fusion and allowing protein strands to form.

The polysaccharide can be added in powdered form or as a slurry. Included in this would be starches and gums. Another source of polysaccharide is that produced by EPS$^+$ cultures such as the capsular forming *Streptococcus thermophilus* MR1C started culture that has been used for increasing moisture content of low fat mozzarella cheese and was observed in cheese to have a large polysaccharide capsule around the bacterial cells which form into chains (Perry et al., 1997,1998). This can be grown in a milk media and would have the advantage of being included in the ingredient list as part of the cultures.

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Perry, D., D.J. McMahon and C.J. Oberg. 1997. Effect of exopolysaccharide-producing cultures on moisture retention in low fat mozzarella cheese. J. Dairy Sci. 80:799-805.

RESEARCH PLAN

Objective 1. Improve the melting properties of low fat mozzarella cheese by having butterfat in the cheese in a more available form for release during baking.

Hypothesis: Mixing a small quantity of oil with comminuted cheese curd before pressing will prooduce a low fat cheese with increased oiling off characteristics during heating, thus preventing surface dehydration and charring of the cheese.

Objective 2. Develop a low fat mozzarella cheeese that has the same characteristics as string cheeese made from cheese containing 20% fat.

Hypothesis: Mixing a polysaccharide material into hot stretched low fat mozzarella cheese will create a channnell of polysaccharide that mimics the fat-serum channels present in higher fat cheeses and cause the protein to form into strands, thus giving the cheese a string cheese appearance.

MATERIALS AND METHODS

Objective 1.

Cheesemaking.

Four batches of 136.4 Kg (300 lbs) pasteurized milk each, obtained from Western Dairy Center, were utilized to manufacture low fat cheese curd with fat levels of 6.0, 4.5, 3.0, and 1.5% using direct acidification method with citric acid (DSM Nutritional Products, Parsippany, NJ) to pH 5.9. Direct acidification helped in quick ripening. The vats were cooked at 42°C with stirring for 30 minutes, and then whey was drained. The cheese curd was then milled using 16-mm curd knives, salted in three applications with 5 minutes apart at1.8%, and placed in a bowl chopper. Melted butter in 0.0, 1.5, 3.0, or 4.5% concentration, respectively, was added along with glucono-δ-lactone (GDL) at 11.00% level (JM Swank, ConAgra Foods Inc., North liberty, IA). The purpose of adding glucono-δ-lactone was to enhance the flavor of low fat cheese and to stabilize constant pH. The curd mixture was salted with comminuted using a Hobart bowl chopper (Hobart, Troy, OH), hooped inn 9kg (20 lbs) cheese blocks, and pressed at 100 kPa pressure on horizontal press. The blocks were then stored at 3°C until used for analysis. Cheesemaking was performed in two replicates. Cheese samples were analyzed in triplicate for proximate composition and water activity at day, pizza fork test, melt profiling, free oil determination, and texture profile analysis at days 15, 30, 60, and 120. A Confocal Laser Scanning Microscopic (CLSM) examination was also performed on all samples to observe the distribution

of fat particles and to confirm if melted butter added post processed to cheese samples was available in free form.

Proximate composition and water activity.

The moisture, pH, salt, and fat contents were determined in triplicate for all cheese samples at day one of storage at 3°C. The pH of cheese sample was determined by combining 20 g of ground cheese with 10 g of distilled water in a plastic bag and stomached (Stomacher Model 400, Seward, Riverview, FL) at 260 rpm for 1 minute. The pH of the cheese slurry so formed, was measured by using a Xerolyt combination electrode (Model HA405, Mettler Toledo, Columbus, OH) and an Accumet pH meter (model AR 25, Fisher Scientific, Pittsburgh, PA). Moisture analysis was done on CEM microwave oven (CEM Corp., Indian Trail, NC) by placing 2-3 g of finely grated cheese sample in between two pads, after taring the two pads on CEM oven inbuilt balance. The moisture content was recorded at the final beep. Fat content was determined using Babcock Method (Method 15.8.A: American Public Health Association, 1992). Salt content was measured by combining 5g of finely garted cheese with 98.2 g of distilled water and mixing for 4 minutes at 260 rpm in a Stomacher 400 (Steward). The mixture was filtered through Whatman no. 1 filter paper, and the filtrate was analyzed for sodium chloride analyzer (model 926, Corning Medfield, MA). Water activity was evaluated using AquaLab Lite water activity meter (Decagon, Pullman, Wash., U.S.A.), which recorded water activity in 5 minutes per sample.

Pizza baking and fork test.

The stretchability of mozzarella cheese was empirically measured by using a fork test (Gunasekaran and Ak, 2002, 2003) on melted mozzarella cheese on a pizza crust. Pizza baking was performed on days 15, 30, 60, and 120 of cheese storage. 150 g of shredded cheese was placed on half of the pizza crust (30 cm diameter) with 16 g of tomato sauce that was spread on the crust. One pizza crust was used for two types of samples. An Impinger oven (Lincoln Foodservice Products Inc., Fort Wayne, IN) with forced air convection was used to bake the pizza at 250°C for 6 minutes. The fork test, as it is called, is performed by stretching the lump of melted cheese on a pizza crust vertically by a fork until the bulk of the cheese strands break. A stainless steel fork was used to test the stretchability of low fat mozzarella cheeses, after baking them on a pizza crust and standing the baked pizza on a benchtop for 1 minute to perform the fork test. The vertical stretch was measured using a scale in triplicate from three different spots and recorded as an average.

Melting profile.

Cheese blocks were cut into thin slices (7 mm) with a hand-operated slicer (Model No. 1042, Rival Co., Kansas City, MO). The cylindrical specimens of 30 mm diameter were cut out with a cork borer. The cheese samples, 7 mm in thickness and 30 mm in diameter, were put in a ziplock bag until tested on UW-meltmeter at 65°C.

UW meltmeter, developed for measuring melting behavior of cheese, consists of an aluminum body with a doughnut shape heater inside (Kuo et al. 2000) and a temperature controller unit (CN 4400, Omega Engineering Inc., Stanford, CT) to control the heater. The heater is in contact with the stationary piston. The outer ring can be moved up and down around the stationary piston using the lever arm. A circular plate is attached to an LVDT (linear variable differential transformer, Schaevitz Engineering, Pennsauken, NJ) to monitor the flow of cheese upon melting. A personal computer with a data acquisition board (DAS 16G High Speed Analog I/O Board, Metrabyte Corp., Taunton, MA) and software (Easyest LX software, Asyst Technologies Inc., Rochester, NY) were used for data collection and analysis. The temperature of the meltmeter was set before the specimen was placed. Both meltmeter top surface and circular plate were lubricated with mineral oil to ensure lubricated squeezing flow. The cheese sample was heated while covered with a circular plate to prevent moisture loss. The temperature of the cheese that was in the sample well was monitored by a digital thermocouple thermometer inserted into the sample. The samples took about 5-7 minutes to reach the test temperature of 65°C. Once the sample reached the test temperature, the data acquisition system was started. The lever arm was raised to lower the outer ring and allow the sample to flow under the constant force or 0.67 N (the weight of the circular plate and the LVDT rod). The height of specimen was recorded as a function of time. Meltability was calculated as the difference between the initial height and the height of melted cheese at one second (Wang et al.1998).

Free oil determination.

A rapid method of free oil determination was conducted using the modified Babcock Method as described by Kindstedt and Rippe (1990). 36 g of ground cheese samples were weighed in Babcock bottles. The bottle with cheese was immersed in boiling water for 8.0 minutes to melt the cheese. Distilled water (20 ml at 57.5°C) was then immediately added, and the bottle was centrifuged hot (ca. 57.5°C) for 10 minutes. A portion of 1:1 distilled water: methanol (21°C) was then added to attain a final level in the upper region of the calibrated neck. The bottle was then centrifuged hot (ca. 57.5°C) for 2 minutes. At this point, the bottle was removed from the centrifuge and gently rocked by hand for 10 seconds. The bottle was tilted at a 45° angle with the rubber stopper oriented downward during rocking. The bottle was next centrifuged for 2 minutes (ca. 57.5°C), and then the 10 seconds, rocking 2 minutes centrifugation

sequence was repeated. Finally, the bottle was tempered in 57.5°C water for 5 minutes, and the fat column was measured using glymol to eliminate the meniscus. This procedure gave a well-defined straw colored fat column. Free oil was expressed as both percentage in cheese and percentage in cheese fat (FOFB), using the following equations:

Measured fat column FO (% in cheese) = Measured fat column/4

FOFB = (Measured fat column/4) / (% total fat in cheese)

Texture profile analysis.

Texture profile analysis (TPA) of low fat cheeses were performed at 15, 30, 60, and 120 days of storage, using a Texture Analyzer TA.XT plus (Stable Micro Systems, Godalming, Surrey, UK) equipped with 2 kg load cell. The cheese textural parameters evaluated were hardness, adhesiveness, cohesiveness, chewiness, and springiness. The cheese samples were cut into cylindrical specimens (20 mm X 10 mm) using a cork borer. The samples were tempered for 2 hours before analysis. A two bite compression test was conducted with 60% compression. Hardness, adhesiveness, cohesiveness, and springiness were evaluated and calculated as described by Bourne (1968). Measurements were performed in triplicate.

Laser Scanning Confocal Microscopy (LSCM).

Samples of cheese were cut into 8 cm^3 pieces, placed under a 2 kg weight with intent to hold the cheese, and pulled across a granite tile at a rate of 20 cm/s. Confocal Laser Scanning Microscopic (CLSM) imaging was conducted at 4°C. The LSCM staining for the fat used 0.1% Nile Red, and for the protein used 0.01% Fluorescein Isothiocyanate (FITC). The fat was imaged at 565 nm and the protein at 488 nm.

Sensory evaluation.

A consumer test, consisting of 90 panelists, was administered in the USU Sensory Facilities. A total of four samples were presented to the consumers. Pizza was made (as described earlier) with cheese samples, 6% fat, all from cheese curd (Control), 3% fat from cheese curd plus 3% fat from melted butter added post processed, commercial whole milk mozzarella cheese or regular, commercial part-skimmed low moisture mozzarella. Pizzas were then cut into equal sized slices and served while hot to the panelists. Samples were presented in random order with a three digit random number coding on a paper plate to minimize any bias. 90 panelists total participated in the test, and each tasted and rated the samples based on degree of liking on a typical 9-point hedonic scale in the following categories: overall, flavor, color, and texture. Sensory data was collected using SIMS 2000 (Morristown, NJ). Before sensory panel for tasting cheeses, approval from Utah State University Institutional Review Board was obtained to use human subjects.

Data analysis.

A randomized complete block design, which incorporated the four treatments (control, 1.5%, 3.0%, and 4.5% melted butter) and 3 blocks, was used to analyze the response variables. The proximate composition (moisture, fat, and salt %), fork test data, and free oil% values were evaluated as average values with standard deviation. The textural parameters (hardness, adhesiveness, cohesiveness, springiness, and chewiness) were evaluated by using PROC GLM and PROC CORR functions of SAS 9.1.3 (SAS Institute, Inc., Cary, NC). Statistical significance was identified at the 95% confidence level, and posthoc means comparisons were made based on p-values obtained using the Tukey-Kramer adjustment.

Objective 2.

Hot low fat mozzarella cheese was made from milk containing 0.7% fat and then manually mixed with hot polysaccharide slurries. The hot cheese was then placed in a conical pot and then extruded into string cheese, and cut into pieces, then cooled.

Cold milk was standardized to 0.5 % fat. 600 lbs of adjusted milk was used at 60.2°F. 48 g of TiO_2 and 1 ml of annatto were diluted in water and added to the milk. Following thorough mixing, 2100 ml of vinegar (5% acetic acid) was added, pre-acidifying the milk to a pH of 6.30. After 30 minutes of incubation, the milk was heated to 90°F, at which time 48 g of the starter culture St. thermophilus was added. The milk was allowed to ripen for 30 minutes. After ripening, 24 ml of chymosin was added, stirred for 2 minutes, and then left undisturbed until a firm set was obtained after approximately 25 minutes. The coagulum was cut vertically with large knives and allowed to heal for 5 minutes. The curd was then slowly stirred for 55 minutes. At this point 2/3 of the whey was drained, and the curd was stirred continuously until a pH of 5.4 was reached. The temperature was raised to 90°F. When the curd reached pH 5.4, the rest of the whey was drained and the curd was washed with 50°F water until the curd temperature dropped to 70°F. The wash water was drained, and the curd was dry stirred until a pH of 5.30 was reached, followed by the addition of salt at 1% (w./w.). The curd was divided into 6 lbs aliquots for hand stretching and addition of starches. A different starch was added to each aliquot: waxy corn, waxy rice, and instant tapioca.

The starches were allowed to hydrate to a concentration of 9% w/v. The starches were added to the appropriate cheese during the stretching and cooking step at 7% (v./w.). A control was made without starch added. The pasta filata cheese of 1.5 inches in diameter was extruded, cut into 6 inch lengths, and submersed in a cold 5% brine solution. Cheese was vacuum packaged and stored at 42°F. The cheese was analyzed for fat, moisture, salt, and pH.

RESULTS AND DISCUSSION

Objective 1.

Proximate composition and water activity.

The proximate composition of control was no different than the experimental cheeses ($p<0.05$). The fat content was found to be 6% or less in most of the cases. As summarized in Table 1, pH of all cheeses was maintained in the range 5.1–5.2 and the moisture content ranged between 52-56% of the cheese samples. All cheese samples were also analyzed for NaCl content, and the salt-in-moisture content of the cheeses were between 1.6-1.8%. As expected for low fat cheeses, moisture, fat, salt, and pH values were approximately 53%, 6.0%, 1.8%, and 5.2, respectively which were in accordance with standard of identity for low fat cheeses.

Stretchability test and free oil %.

The stretchability measurements were done using the fork test, and free oil determination was evaluated by using the modified Babcock Method as described earlier. The data was summarized in Table 2. The stretchability was measured in centimeters of length of cheese strands height until broken. After baking pizza in the oven, control cheese did not melt and had a dried surface with charred cheese and very less fusion. However, adding melted butter to cheese post processing became very fluid and flowed nicely on the pizza crust, and the stretch of cheese was greater than the control. The length of cheese strands were observed to be increasing with increasing level of melted butter in cheese samples. The fusion of cheese shreds added with melted butter helped in delaying dehydration of cheese during baking and allowed proper melting by applying hydrophobic coating on cheese surface. The results were in agreement with the results obtained by Rudan and Barbano (1998) when they tested vegetable oil spray on nonfat cheese shreds on pizza crust during baking. The stretchability data was highly correlated to free oil content as shown in Table 2. As the free oil content increased in cheese samples, stretchability also increased. The effect of aging on free oil content and stretchability was also investigated, and it was observed that both free oil and stretchability decreased with aging from 15 days to 120 days.

The free oil content was the evidence of availability of fat in free form which increased with the increasing level of melted butter (Table 2). The correlation coefficient (r) was very high when stretchability was compared with free oil content and ranged between 0.84 to 0.99 for four time points (15, 30, 60, and 120 days) in aging period.

Melting profile.

Meltability of mozzarella cheese is very important because this cheese is widely used for pizza. Lucey and others (2003) reported that melting properties of cheese are based on the interactions between casein molecules. However, a nonfat or low fat cheese lacks casein-casein interactions, resulting in poor meltability. When fat was provided in free form, as in this study, improved meltability to a greater extent. The graphs in Figure 1 (A-D) showed that cheese height increased with increasing levels of added butter, and time of melting decreased with increasing levels of added butter. Though the melting profile of all cheeses tended to be close to each other (Figure 1C), the overall meltability showed no differences with aging of cheese.

Textural analysis.

Texture of cheese is a very important feature for consumer acceptance. The changes in textural parameters (hardness, adhesiveness, springiness, cohesiveness, gumminess, and chewiness) were reported in Table 3. Fat reduction in mozzarella cheese was accompanied with increase in hardness and chewiness in control cheese samples, which was also reported by Awad and others (2005). However, adding butter post processed to cheesemaking significantly decreased the hardness and chewiness ($p<0.05$), as shown in Table 3. The cohesiveness was observed to decrease at 15 days of storage, with added butter, and this was due to comminuting the cheese curd for mixing melted butter. The cohesiveness was then increased at 30, 60, and 120 days of storage, which was due to knitting back of curd particles in the vacuum packaging. As summarized in Table 3, hardness, adhesiveness, cohesiveness, gumminess, and chewiness values were very low for cheeses added with melted butter, especially at 3.0 and 4.5% level and these values increased at 30 and 60 days of storage. This was again due to the comminuting of cheese curd and knitting back together after 30 days of storage and then continued to increase on further storage. The increase in hardness during ripening might result from the reduction in the level of free water, which increases cheese resistance to deformation as previously reported (McMahon et al., 1999; Beal and Mittal, 2000). As observed in Table 3, springiness of low fat cheeses remained unchanged with regard to control as well as the storage. This study helped in improving the texture of low fat mozzarella cheese without increasing the

Table 4. Summary of mean-scores, P-values, and significance. Test result code - PIZZ-4-09.

Attribute	Control 6% Fat	3% Cheese Curd / 3% Butter Fat	Commercial Whole Mozz	Commercial Part Skim	P-Value	Sig
	b	b	a	a		
Overall Liking	5.88	6.08	7.26	7.17	0.0001	***
	b	b	a	a		
Flavor Liking	5.85	6.09	7.14	7.17	0.0001	***
	c	b	a	a		
Color Liking	5.31	5.97	7.17	7.11	0.0001	***
	b	b	a	a		
Texture Liking	5.05	5.34	7.05	7.03	0.0001	***
	b	b	a	a		
Fat Content By Sample	20.8	23.09	30.77	29.15	0.0001	***

overall fat content.

Confocal Laser Scanning Microscopy (CLSM).

The results from CLSM were very consistent, and the representative images for control and treated cheese samples were included in Figure 2 (a-d). Cheese samples were majorly occupied with protein (casein) matrix with fat entrapped within the matrix. For low fat mozzarella control cheese, fat was observed to be tightly embedded in casein matrix and was not available as free form (Figure 2a). However, cheese samples with added butter showed more free fat droplets and not entrapped mostly in casein matrix. The amount of free fat droplets increased with increasing levels of added butter (Figure 2b-d). The fat droplets were more spherical in shape and smaller in size in the low fat cheese matrix (Figure 2a). However, low fat cheese samples with added butter tended more towards full fat cheese micrograph, not for increasing volume of fat but with the availability of fat in free form and disrupted fat globule membrane.

Consumer preference test.

The consumer preference test was performed on the low fat mozzarella cheese sample with 3% added butter level. It was chosen based on its better performance on pizza baking and better functionality than other treated cheese samples. The 4.5% added butter level showed better performance on pizza, except that it had browning (Figure 3) and it was soupy when flowed on pizza crust after baking. The 3% added butter cheese sample was served to the consumer panel along with control (low fat mozzarella cheese with no added butter), commercial whole milk, and part skimmed mozzarella cheeses (Leprino Foods, Denver, CO).

Each pizza was evaluated for liking, including overall liking and liking in flavor, color, and texture, as shown in Table 4. They were also evaluated for their perceived fat content, knowing that pizza typically has a fat content range of 20-30%. Panelists were able to choose a percentage of fat for each sample, based on how high or low they felt the fat content of that sample was. The number of times each sample was given a rating is also shown in Figures 4A-D. These charts are separated based on liking category and allow for a more in-depth picture of how each sample was rated. Out of the panelists that took part in this test, 50% were male and 50% were female. As summarized in Table 4, the average overall liking of experimental cheese (3% added butter) was 6.08, which were for "like slightly" on a categorical scale. It was a lower rating than the commercial cheese samples but slightly higher than the control cheese. The color rating was significantly higher for experimental cheese than the control cheese sample ($p<0.05$). The ratings for flavor and texture for experimental cheese were slightly higher than the control cheese but no different than the control ($p>0.05$).

Low fat mozzarella cheese manufactured in September for consumer studies was found to have too high a level of coliforms (>1000 cfu/g), and it was discarded and not used for sensory testing. Presumably this contamination occurred during the grinding process. Afterwards, the grinder was stripped down, thoroughly cleaned, and a new cleaning procedure was implemented.

Objective 2.

Cheese manufacture November 24, 2009.

Changes in the cheese make procedure included using fat percent of the milk used was slightly higher, at 0.77%. The cooking temperature was held at 94°F, instead of 90°)°F. Different additives were used in the cheese, namely: Guar gum, xanthan gum, guar plus xanthan, polydextrose, and

instant tapioca starch. The gums made were hydrated to 7% w/v and added to the cheese in the cooking stretching step at 8% w/w. The polydextrose was hydrated to 20% w/v and added to the cheese at 8% w/w. The starch was added to the cheese at 10% w/w and added to the cheese at 8% w/w.

CONCLUSIONS

The concept of a low fat mozzarella cheese with enhanced melting was shown to be feasible by adding melted butter post to cheese manufacturing and prior to pressing and storage of cheese, provides the free fat during baking cheese on pizza crust without increasing the fat percent. This helps in significant improvement of melting, as well as stretching properties of low fat mozzarella cheese. Though the higher the added butter, the higher the meltability, but due to the browning of cheese with 4.5% butter fat, the best combination was 3% butter added to 3% curd fat. Statistical analysis of the taste panel results show that there was an improvement in overall liking, flavor liking, and texture liking between the control cheese low fat mozzarella and cheese made from 3% cheesecurd and 3% butter fat. Commercial whole mozzarella and commercial part skim mozzarella were both significantly different in all attributes of comparison with the control cheese.

Objective 2.

The manufacture of low fat string cheese has required numerous modifications of the procedure for small scale manufacture of string cheese. Teflon coating of the interior of the extruder reduced the friction between the interior surface and the low fat cheese, thus allowing the cheese to pass through the extruder properly. The addition of starch to the cheese did visually improve the texture and stringiness. The starch that showed the best results, when stringiness was compared, was instant tapioca and was carried over into the second trial. The addition of xanthan and guar gum improved the stringiness of the cheese but were more difficult to incorporate into the cheese during cooking and stretching. Xanthan gum showed to produce the cheese with the best string formation. The addition of polydextrose showed no improvement but may need to be further examined. The temperature of the brine must be above 170°F to allow the incorporation of the starch or gums while stretching.

NEXT STEPS

Objective 1.

Continue writing the initial manuscript of the low fat mozzarella for baking on a pizza for submission to the Journal of Dairy Science.

Objective 2.

Plan and execute a new trial for addition of starch and gums using the procedures developed. Analysis of the cheese for texture at both refrigeration temperature and mouth temperature, as compared to commercial produced cheese (control group). Observe starch and gums structure within the cheeses using confocal microscopy. Conduct taste and descriptive panels.

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Inventions or Patents

A provisional patent application was filed but allowed to lapse.

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Effect of fat removal on cheese microenvironment and starter culture metabolism in cheddar cheese

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ABSTACT

Flavor development in bacterial-ripened cheese (e.g., Cheddar) is primary due to the action of lactic acid bacteria (LAB) and enzymes in the ripening curd. Knowledge of the mechanisms by which LAB affect cheese flavor has facilitated industry efforts to accelerate or intensify flavor development in many traditional cheese varieties. Unfortunately, empirical efforts to extend this information into low-fat cheese systems have not proved successful, and low-fat products continue to suffer from low intensity of desirable flavors and/or from pronounced off-flavor defects.

It is the hypothesis of this project that flavor problems in low-fat products are most likely explained by a scenario wherein starter physiology (and thus overall metabolism) is altered by differences in the physico-chemical environment in ways that affect the production of flavor- and aroma-active metabolites. To this hypothesis, we are working to determine how *Lactococcus lactis* metabolism is affected by the changes in cheese microenvironment, and especially S/M ratio, that occur as a consequence of fat reduction. Specifically, we are evaluating gene expression and volatile metabolite profiles from *L. lactis* strains cultured in Cheddar cheese extract (CCE) medium adjusted to reflect the differing microenvironments of low- and full-fat cheese.

Efforts to develop effective culture systems for low-fat cheese will require more specific knowledge of how the cheese physico-chemical environment affects starter cell physiology. Knowledge generated from this study will facilitate industry efforts to develop starter strains, through mutagenesis or other methods, that can enhance flavor development in low-fat and nonfat Cheddar cheese.

BACKGROUND

Transformation of bland-flavored curd into delicious mature cheese is a complex and dynamic process whose intricacies are scripted by the milk type and composition, the cultures and enzymes present or added to the cheese milk, and the manufacturing and ripening conditions used. Many cheeses need to be stored at low temperature for months or even years before they attain characteristic flavor and body attributes. During this time, termed the curing or ripening period, microorganisms and enzymes in the cheese matrix act on milk constituents in a manner that is partly dictated by the curd microenvironment (e.g., cheese pH, a_w, salt content, E_h, temperature, etc.) and which ultimately gives the desired product (Fox et al., 1993).

Types of LAB that occur in internally bacterial-ripened cheeses such as Cheddar, Dutch, Swiss, and Italian varieties, include deliberately added strains (e.g., starters and adjunct cultures) and adventitious species (primarily nonstarter LAB or NSLAB) that enter cheese through milk or processing equipment. Modern sanitation and Good Manufacturing Practices help minimize initial numbers of NSLAB in cheese, yet these organisms invariably appear and grow to high numbers during ripening (Peterson and Marshall, 1990). In Cheddar cheese, numbers of *Lactococcus lactis* starter bacteria frequently exceed 10^9 colony-forming units (cfu) per gram when ripening begins. As maturation proceeds, the harsh cheese ripening environment (little or no residual lactose, pH 5.0 to 5.3, 4-6% salt in moisture, 5-13°C) gradually takes its toll and starter viability declines. A fraction of the dying starter cells undergo autolysis, which releases intracellular enzymes and cellular components (e.g., sugars and nucleic acids) into the cheese matrix (Fryer, 1969). At the same time, NSLAB populations (whose initial numbers are typically less than 10^2 cfu/g in cheese made under good sanitary

conditions with high quality pasteurized milk) begin to grow and eventually plateau at cell densities of 10^7-10^8 cfu/gram after 3-9 mo of aging (Peterson and Marshall, 1990). Microbiological studies have shown NSLAB populations in bacterial-ripened cheeses may be quite diverse, but are usually dominated by facultatively heterofermentative species of lactobacilli or, far less frequently, by pediococci (Fryer, 1969; Beresford et al., 2001; Broadbent et al., 2003; Broome et al., 1990a; Crow et al., 2001; Sherwood, 1939). Depending on the species that is used (and whether or not the particular strain can grow in ripening cheese), populations of adjunct bacteria may mirror the trend for starter or NSLAB fractions.

Though a link between LAB activity and cheese flavor attributes was postulated more than 100 years ago (see Fryer, 1969), the variation and complexity that exists in cheese microbiota and enzyme content confounded early efforts to establish a causal role for these bacteria in flavor development. This limitation was overcome in the late-1950's, when sensory studies of aseptically manufactured Cheddar cheese showed that starter-free, gluconolactone-acidified cheese failed to develop Cheddar flavor, while cheese made with *Lc. lactis* starter bacteria developed characteristic, balanced flavor (Law et al., 1976; Reiter et. al., 1967). The same investigations also showed NSLAB could modify basic flavor notes and accelerate flavor development. More recently, use of *Lactobacillus* spp. NSLAB isolates as adjunct cultures for Cheddar cheese manufacture has indicated these bacteria may influence flavor in at least 3 ways: they may intensify (i.e., accelerate) typical flavor development, impart atypical (but desirable) flavor notes, or promote off-flavor development (Fryer, 1969; Crow et al., 2001; Sherwood, 1939; Broome et al., 1990b; Lynch et al., 1999; McSweeney et al., 1994; Swearingen et al., 2001). In addition, NSLAB have also been associated with cheese quality defects such as open body (via gas production) and formation of calcium lactate crystals (Fryer, 1969; Johnson et al., 1990; Khalid et al., 1990).

Given the causal role of LAB in flavor development, efforts to define the biochemical basis for flavor changes in cheese have logically focused on the microbiology and physiology of species found in cheese (for recent reviews see Beresford et al., 2001; El Soda et al., 2000; Fox and Wallace, 1997; Marilley and Casey, 2004; Rattray and Fox, 1999). Those efforts have identified many of the most important biochemical and chemical processes in maturation, and have shown starter, adjunct, and NSLAB have an intimate role in most of those processes including lactose fermentation, conversion of milk proteins (primarily caseins) into peptides and free amino acids, catabolism of amino acids into volatile aroma compounds, lipase/esterase activity, and citrate catabolism.

Knowledge of the mechanisms by which LAB affect cheese flavor has facilitated industry efforts to promote flavor development in many traditional cheese varieties, but empirical efforts to extend this information into low-fat cheese systems have not proved successful. As a result, low-fat or nonfat bacterial-ripened cheeses continue to suffer from low intensity of desirable flavor or pronounced off-flavors. From a purely technological perspective, many of the flavor limitations in low-fat cheese might be overcome through the addition of dairy flavors or enzymes. The costs associated with this technology are estimated to be 1-5 cents per pound (T. Bhowmik, pers. Comm.), however, and would be incurred on top of the already premium cost to make low-fat cheese. As a result, exclusive use of flavors or enzyme technologies to soilve the flavor problems in low-fat products is not an attractive option at this time. Industry concerns about product cost is supported by a recent consumer study that found there is little support for low-fat cheese product, if their cost is substantially higher than that of full fat cheese (M.A. Drake, pers. comm.).

A more cost-effective solution to the flavor problems in low-fat cheese can likely be found through combining flavors or enzymes with culture systems that deliver bettter flavor in these products. Compared to dairy flavors or enzyme addition, culture technology is an inexpensive means to secure flavor development, and one that should be further explored and optimized for industry to offset the price concerns associated with low-fat cheese products.

These deficiencies in low-fat cheese flavor are most likely explained by one of two scenarios: 1) starter physiology (and thus metabolic end-product profile)) is the same in all cheeses, but sensory perception of those metabolites is altered by differences in the physiico-chemical environment (e.g., fat, moisture, or salt in moisture contents); or 2) starter physiology itself (and thus overall metabolism) is altered by differences in the physiico-chemical environment in ways that affect the production of flavor- and aroma-active metabolites. A third possibillity, of course, is that each of these scenarios has a role in the atypical flavor profile of low- and nonfat cheese systems.

Though little information is available on the differences in flavor- or aroma-active metabolites in full versus reduced fat cheese, work by Milo and Reineccius (1997) noted important differences in volatile components from full fat versus 40% fat reduced Cheddar cheese, and suggested that these differences might be due to the higher water content in the reduced fat cheese. More recemtly, Carunchia Whetstine et al. (2006) investigated sensory properties and volatile chemical profiles in full- and 50 % reduced fat Cheddar manufactured by a novel fat removal process after aging is complete. Those authors discovered that the great majority of volatile aroma-active compounds remained in the aqueous-containing cheese matrix versus the removed fat fraction and, more interestingly, found that cheeses with either fat level had nearly identical flavor profiles. Given these findings, and the central role of lactic

acid bacteria in flavor development, it is our hypothesis that the second scenario provided above (i.e., starter physiology and metabolism is altered by perturbations in the cheese microenvironment) has the greatest effect on flavor development in low- or nonfat cheese. If this hypothesis is correct, efforts to develop effective culture systems for low-fat cheese will require more specific knowledge of how the cheese physico-chemical environment affects starter cell physiology. Thus, the goal of this study is to determine how *Lactococcus lactis* metabolism is affected by fat reduction and its concomitant changes in cheese make procedure impart on the cheese microenvironment (e.g., S/M ratio, lactate content, pH, etc.). This knowledge is expected to generate basic information industry needs to develop starter strains, through mutagenesis or other methods, which enhance flavor development in low-fat and nonfat Cheddar cheese. To attain the goals of this project, we will complete the following experiments:

RESEARCH PLAN

Objective 1.

Develop and utilize a model system to investigate the impact of different cheese microenvironments on the physiology of commercial *L. lactis* starter bacteria.

MATERIALS AND METHODS

Objective 1. Develop and utilize a model system to investigate the impact of different cheese microenvironments on physiology of commercial *L. lactis* subsp. *lactis* and *L. lactis* subsp. *cremoris* starter bacteria.

To ensure their relevance to cheese, experiments performed under this objective will utilize cells incubated in a Cheddar Cheese Extract (CCE) medium using laboratory-scale (1 to 2 L) bioreactors. The CCE medium for these experiments was prepared from a large batch (>500 lbs) of full and low-fat Cheddar cheeses so that we would have sufficient uniform CCE powder to meet all the needs of the project and the partner NSLAB project submitted by Dr. Steele entitled "Selecting bacterial cultures to enhance low-fat cheese flavor". Full and low-fat Cheddar cheeses were manufactured at the USU dairy plant and aged 6 weeks (the time at which HPLC analysis confirmed residual lactose and galactose levels had fallen below quantifiable levels), then shredded and frozen in preparation for subsequent processing into CCE powder. Because the lyophilization step for CCE preparation imposed a significant time delay, we eventually worked with the Western Dairy Center staff to develop a novel, high-throughput system for generating the final CCE aqueous extract using our pilot plant UF capabilities. Once complete, samples of the CCE were collected for analysis of residual lactose and galactose, pH, NaCl, D/L lactic acid, and small peptides and amino acids using standard methods (Marshall, 1993), then aliquots were frozen at -20°C. Some samples were shipped to Dr. Steele's lab for use in the partner NSLAB project.

To determine the impact of cheese microenvironment on starter volatiles production and transcriptional profile, we selected six different *L. lactis* starter bacteria that include strains recommended by industry suppliers for aged full fat or low-fat cheese production (Table 1). To avoid potential overlap in strains from different culture companies, the uniqueness of each strain was analyzed by plasmid DNA profiles. Working cultures were prepared from frozen stock cultures through two successive transfers (0.1% inoculum) in sterile skim milk at 30°C for 16-18 h without pH control.

Microbes in cheese are found in the aqueous fraction, so experiments to replicate the microenvironment of low-fat and full-fat cheese will be performed by adjusting CCE composition so reflect the aqueous fraction of either low-fat or full-fat cheese (Table 2). To accomplish this, CCE collected from low-fat cheese will be aseptically transferred into 1 L bioreactors, adjusted to obtain specific environmental conditions listed in Table 2, then incubated under temperature (10° or 30 ° C) and pH (5.1) control. *Important note: Test conditions listed in Table 2 were established by consultation between Dr. Broadbent and Drs. Donald J. McMahon, Mark E. Johnson, and James L. Steele.*

The first series of experiments will evaluate differences in the transcriptomes and in volatile compounds production by *L. lactis* strains during incubation in CCE designed to mimic low-fat or full-fat cheese microenvironments (Table 2) at 10°C, except that redox will not be adjusted. Those experiments will be followed by a series of independent experiments to investigate the effects of salt-in-moisture (3.7 vs 4.75%), temperature (10 vs 30°C), and redox (+340 vs -200).

For each of these experiments, CCE composition will be adjusted as desired (Table 2), then the bioreactor will be inoculated at approximately 1 x 10^6 CFU/ml with a single strain of fresh, milk-grown working culture and the pH will be maintained at 5.1 throughout incubation by addition of 15% (v/v) NH_4OH with an agitation rate of 100 rpm. Cells will be incubated for various times (see relevant sections below) before samples are collected for transcriptome or volatiles analysis.

Production of volatile flavor compounds.

The influence of cheese environment on volatiles production by the starter will be performed under the supervision of Dr. Robert Ward using a solid phase microextraction GC-MS approach essentially as described by Lee et al. (2007). Cells will be incubated in the CCE as

Table 1. Lactococcal cultures selected for the study.

Lactococcus lactis	Comment	Source
M70	Starter for 2006 Low-fat platform project	Danisco
D11	Commercial starter culture for LF cheese	Danisco
S2	Autolytic starter culture	J.L. Steele
SCO213	Commercial starter culture for aged cheese	Chr. Hansen
LL011	Commercial starter culture for LF cheese	DSM
LL071	Commercial starter culture for LF cheese	DSM

Table 2. Cheese environmental conditions to be used in the study.

Component[a]	Low-fat model	Full-fat model
Salt-in-moisture (%)	3.7%	4.75
Lactate	5500 ppm L-lactate 600 ppm D-lactate	5500 ppm L-lactate 600 ppm D-lactate
Temperature	10, 30°C	10, 30°C
Redox[b]	+340, -200, none	+340, -200, none
pH	5.1	5.1

[a]Reflects typical S/M (at press) and lactate contents (at 3 mo) of washed curd low-fat and full fat cheese made and analyzed under the 2006 DMI collaborative low-fat platform project.
[b]Stable oxidative or reducing conditions (Eh = 340 ± 30 mV or -200 ± 30 mV, respectively) will be generated as described by Kieronczyk et al. (2006) with addition of 24 mmol potassium ferricyanide [$K_3Fe(CN)_6$] or 30 mmol dithiothreitol (DTT) to the medium.

described above then samples will be collected for volatiles analysis at time 0 and after 72 h. Once volatiles analyses are complete, Drs. Broadbent, Ward and Steele will individually review the volatiles data obtained from these experiments and prioritize the lactococcal RNA samples that should be included in the transcriptome component of this work. Priority for these selections will be based on pair-wise comparisons that each professor feels will provide the greatest insight to effect of microenvironment on cell physiology. It is our expectation that prioritization will allow us to reduce the number of strains that will be included in transcriptional profiling from 6 down to 3 or fewer.

Transcriptional studies.

The influence of cheese environment on the transcriptional profile of different starter bacteria will be performed under the supervision of Dr. Jeff Broadbent using a lactococcal full-genome Affymetrix microarray that is available through the USU Center for Integrated Biosystems. RNA samples for microarray studies will be extracted from 20-100 ml of cell culture (depending on growth phase and cell density) collected at time 0 and after 72 h as described above. The cells will be harvested by centrifugation at 4000 rpm for 4 min in a rotor prewarmed to culture growth-temperature because preliminary microarray hybridizations indicated cell exposure to a chilled rotor resulted in the induction of several cold-shock genes (Wechter and Steele, unpublished). The cell pellet will immediately be suspended in 10 ml of RNAprotect (Qiagen, Valencia, CA) and incubated at room temperature for 10 min. After RNAprotect treatment, the cells are pelleted by centrifugation, suspended in 1 ml RNAse-free sterile H_2O containing 20 mg/ml lysozyme, 8 U mutanolysin, and 75 uL rifampicin (25 mg/mL in methanol). The cells are incubated at 37°C for 15 minutes in a shaker incubator (240 rpm), then total RNA is isolated using the Aurum Total RNA mini Kit (Bio-Rad Laboratories, Catalog #732-6820) scaled to 10X as directed by the kit supplier. The resulting total RNA sample is frozen at -80°C until needed.

Synthesis and biotin labeling of cDNA from selected total RNA samples will be performed using a series of protocols recommended by Affymetrics (see GeneChip® Expression Analysis Technical Section 3: Prokaryotic Sample and Array Processing; www.affymetrix.com/support/technical/manual/expression_manual.affx). Hybridization of cDNA samples to microarrays and array scanning will be performed in the Affymetrix core facility at the USU Center for Integrated Biosystems. Statistical analysis and interpretation of microarray data will be performed by personnel in Drs. Broadbent's lab as described previously (Smeianov et al. 2007; Broadbent et al. 2010).

Measurement of microarray spots intensity levels is, hypothetically, directly correlated with the abundance of the corresponding mRNA. However, the intensity unit is arbitrary, and ratios are relative between samples

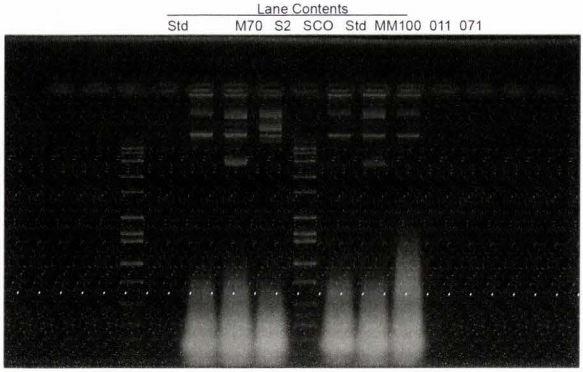

Figure 1. Plasmid DNA content of lactococci selected for the study.

being compared. As a result, there is a need for absolute quantification of mRNA (or indirectly via cDNA) levels between samples, in order to confirm the findings suggested by microarray data. Real-time RT-PCR is a method that allows confirmation and quantification of microarray results with a higher throughput and accuracy than those of Northern blots or RNA slot blots. Thus, key genes and operons identified in the microarray analysis will be confirmed by real-time RT-PCR. Genes will be chosen for analysis by real-time RT-PCR based upon the level and reproducibility of changes in expression observed in the microarray experiments. Equipment and methods for real-time RT-PCR are established in Dr. Broadbent's laboratory (Smeianov et al. 2007; Broadbent et al. 2010).

In summary, it is our expectation that the combined results from volatiles profiling and transcriptional studies will generate critical knowledge needed to understand how starter physiology is altered by perturbations in the cheese microenvironment. The molecular information derived from this work should allow us to build a "metabolic fingerprint" for flavor production in low-fat and full-fat cheese that may reveal new strategies to develop starter strains, through mutagenesis or other methods, that produce more intense and desirable flavor notes in low-fat Cheddar cheese.

RESULTS AND DISCUSSION

Strain characterization.

As part of our initial preparation for this work, we collected and characterized six strains of *Lactococus lactis* for use in this study: *L. lactis* M70, *L. lactis* MM100, *L. lactis* S2, *L. lactis* SCO213, *L. lactis* LL 071, and *L. lactis* LL 011. The cultures were grown in M-17 lactose broth at 30°C, streaked for single colony isolation on M-17 lactose agar, and their identity as *Lactococcus lactis* was confirmed by PCR and sequencing of the 16S rRNA gene. The plasmid DNA profile of each strain was also determined, and results suggest some of these strains may be related (Figure 1). To address this possibility, we customized a software program called "r" to suit the lactococcal microarray that will be used to analyze gene expression profiles in CCE-grown lactococci, then used that program to perform comparative genome hybridizations with genomic DNA from each of the six strains against the *L. lactis* Affymetrix custom microarray. Results from those experiments confirmed the suitability of our microarray methodology, and also provided us with details of the genetic similarities and differences between these strains.

CCE preparation.

As was noted above, we worked with the Western Dairy Center staff to develop a novel, high-throughput system for CCE production using our UF equipment. Importantly, this process is also amenable to using frozen shredded cheese as the input, and gives an aqueous concentrate that we believe will allow us to completely eliminate the need for lyophilization in future CCE preparations. Experiments confirmed that NSLAB growth was comparable in CCE concentrate versus powder prepared from the same cheese, although compositional analysis of CCE concentrate and powder revealed minor differences in sugar and organic acids content (Table 3). Because of this finding, we have opted to rely exclusively on concentrate for this project and use the powder for other experiments.

Table 3. Composition of powder and liquid concentrate forms of CCE prepared from low-fat cheese.

	Powder % (g/100mL)		Liquid % (g/100mL)	
	mean	***st.dev***	***mean***	***st.dev***
Acetate	0.00	0.00	0.00	0.00
Formate	0.00	0.00	0.00	0.00
D-Lactate	0.03	0.00	0.02	0.00
L-Lactate	1.68	0.01	0.90	0.07
Galactose	0.00	0.00	0.05	0.00
Lactose	0.01	0.00	0.06	0.00
Citrate	0.03	0.00	0.05	0.00
Salt	1.07	0.05	1.19	0.06

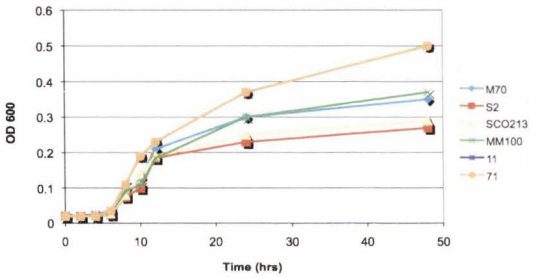

Figure 2. Growth of *L. lactis* starter cultures in CCE with 0.2% lactose at 30°C.

Incubations in CCE.

Because the liquid CCE contained over 0.1% residual sugar (Table 3), we initially believed these cultures would display modest growth upon inoculation into this medium (as is normally the case when starters transition from milk to fresh curd). However, our initial experiments in 10 ml CCE samples indicated growth was negligible. Because opportunity for modest growth is desirable to our goals to mimic the cheese environment, we performed follow-up studies to establish such conditions. As is shown in Figure 2, we have found that adjustment of the lactose concentration to 0.2% does allow such growth and provides for a 1.5-2 log increase in cell numbers after incubation for two days at 30°C. As a result of these studies, we have decided to supplement our liquid CCE with lactose to 0.2% final concentration for our subsequent transcriptomics and volatiles studies.

Finally, we have also begun to analyze the volatiles content of CCE fermented with different starter cultures (Table 4 and Figure 3). To ensure results from this study can be directly compared with data collected from the DMI low-fat cheese platform study, we have coordinated our methodology for volatiles analysis with Dr. Mary Anne Drake at North Carolina State University, so that.

Finally, recent research has expanded our understanding of the CCE model for cheese in another very important way. As is shown in Fig. 4, we discovered that addition of milk fat globular membrane (MFGM) material promotes growth of some lactococci at 10°C. This finding indicates MFGM may contribute to cytoplasmic membrane fluidity (and thus membrane function, including solute uptake and efflux) and/or some components of MFGM may serve as substrattes (and thus contribute to the profile of volatile aroma compounds in cheese). Since either of these possibilities has obvious implications on flavor development in low-fat versus full fat cheese, we have recently prepared a large amount of MFGM so that it can be included, at an appropriate concentration, in CCE designed to mimic each type of cheese.

CONCLUSIONS

Results to date have confirmed the suitability of our methodology for microarray and volatiles studies of lactococci incubated in CCE. Results also show that the

Table 4. Examples of aroma compounds detected in CCE after incubation with *L. lactis* starter cultures[1]

Compound	Flavor	Compound	Flavor
2-Butanone, 3-hydroxy	sour milk	Nonanal	green, fatty
2,3-Butanediol, [S-(R*,R*)	cheesey, caramel	Octanoic Acid	body odor
Butanoic acid	cheesey, rotten, sharp	Decanal	green
Butanoic acid, 3-methyl	tootsie roll	Nonanoic acid	goat
2-Heptanone	fruity, spicey, fatty	Pyrazine, trimethyl	nutty, musty, beans
Methional	roast potato	Heptanal	soapy
Pyrazine, 2,5-dimethyl	nutty, roast grain	Heptanoic acid	rancid
Benzaldehyde	almond	Acetic acid	vinegar
Hexanoic acid	popcorn goaty	Pyrazine, tetramethyl	raw potato, beans
Benzeneacetaldehyde	rossy	Acetophenone	sweet

[1]A total of 150 compounds were detected.

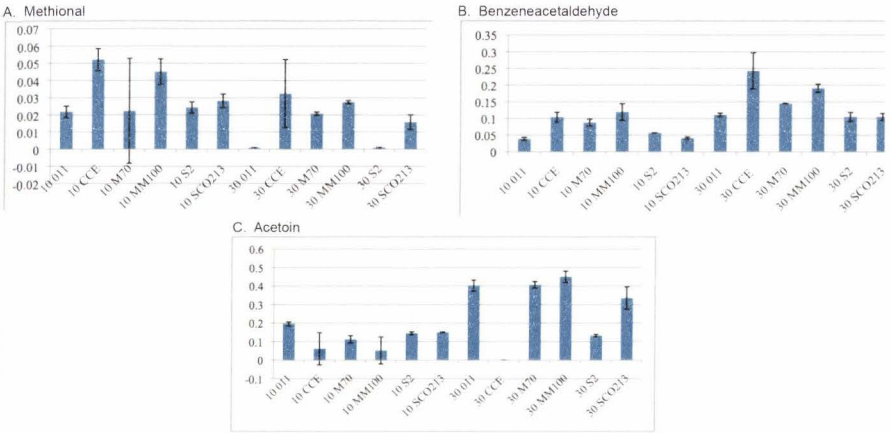

Figure 3. Production or degradation of the aroma compounds methional (A),benzeneacetaldehyde (B), and acetoin (C) in CCE by different *L. lactis* during incubation at 10° or 30°C. CCE = cell-free medium.

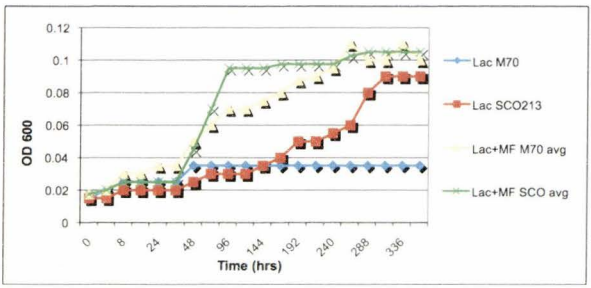

Figure 4. Growth of *Lactococcus lactis* M70 or SCO213 in CCE with or without added MFGM.

lactoccal strain we have selected for the study produce different levels of aroma compounds in CCE, and suggest the presence of milk fat may be important to starter growth and metabolism.

NEXT STEPS

Based on the outcomes described above, we have decided to initiate our transcriptome and volatile studies using *L. lactis* M70. Our rationale for selecting this strain first include: 1) It was the culture used for the low-fat platform study, which will allow us to tie data from the CCE trials to chemical data collected in the platform study; and 2) Plasmid profiles, CGH, growth curves in CCE, and volatiles data indicate some strains are highly similar (e.g., M70 and MM100) while others appear to be quite different (e.g., S2). To avoid expensive and unnecessary redundancy in our transcriptome study, we will work with Dr. Jim Steele at the University of Wisconsin to perform a series of small volume fermentations in CCE to assess similarities and differences in production of volatiles when strains are incubated under low-fat and full fat like environments. Results from those experiments will be used to determine which of the other strains should move forward for more detailed transcriptome analysis.

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Improving the flavor of low fat cheese by adding innovative cultures and/or flavoring systems

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ABSTACT

Use of adjunct lactobacilli cultures in low fat cheese has not shown an improvement in flavor over use of lactococci culture alone. Further testing with non-lactobacilli adjunct cultures is in progress.

No improvement in flavor was obtained by addition of cheese flavor preparations to comminuted cheese with subsequent re-pressing. Such cheeses typically had an increase in lipolytic flavor but non increase in "cheddar cheese" flavor.

BACKGROUND

A two pronged approach will be used to investigate ways to get a better flavored low fat cheese that includes the use of adjunct starter cultures that have been shown to increase, or provide specific flavor attributes to, the flavor level of other cheeses, as well as direct addition of complete flavor systems to the cheese curd prior to pressing the curd into a block. For the culture portion of the study, a variety of cultures will be tested, and the resultant cheese will be screened for flavor development. Those cheeses that show an improvement in flavor will be subjected to sensory analysis, and those that do not will be discarded. Two sensory tests will be performed to obtain a description of the flavor profile, as well obtaining information on how well consumers like the low fat cheeses. The other part of the study involves working with flavor companies, and to use their expertise with flavors to obtain a flavoring system that provides the missing components of cheddar cheese flavor that are needed to produce a low fat cheese that is liked and acceptable to consumers.

RESEARCH PLAN

Objective 1.

Determine the extent to which more cheese flavor can be developed in a low fat cheese by adding adjunct cultures.

Objective 2.

Determine if cheddar cheese flavor can be obtained in a low fat cheese by adding flavorings to the low fat cheese during manufacture.

MATERIALS AND METHODS

Objective 1.

Contact and obtain from cultures companies, adjunct cultures that increase flavor production in cheese. These will include cultures proposed for use in full fat cheddar cheese and cultures used for non-cheddar varieties to see if they have a positive effect on cheese flavor in a low fat cheese system.

Use these cultures to manufacture low fat cheese using 300-lb milk per vat. Cut the 20-lb block of cheese into ten 2-lb blocks, vacuum package them, store them at 4°C for 14 days and then store half at 6°C and the other half at 4°C. Cheeses will be made in duplicate.

The cheeses will be screened for flavor improvement compared to the flavor of the cheese manufactured as part of the Low Fat Strategic Flavor Platform project, by cheese experts at Utah State University when they are 1mo old. If an improvement in flavor is evident then cheeses will be tested using consumer preference sensory evaluation when they are 2 mo old. Any cheeses with high consumer preference scores will be sent to North Carolina State University for flavor profile analysis at 3 mo of age, and a

consumer preference and acceptance sensory panel will be conducted at Utah State University.

Any cultures that demonstrate a positive influence on flavor development will be used in combination and the above cheesemaking and tests repeated. Some of the cultures that will be tested include:

- cultures, such as *Lactobacillus helveticus* CNRZ32, that has been shown to be effective in increasing the cheese flavor intensity and eliminating bitterness in cheddar cheeses (Broadbent and Steele, 2006; Sridhar et al. 2005).
- a *Lactococcus lactis* culture that was recently shown to increase the nutty flavor intensity of cheddar cheese (Carunchia Whetstine et al. 2006). This would be important in a low fat cheese because such cheeses in the past have tended to have a more brothy flavor than typically occurs in cheddar cheese.
- other cultures recommended by culture suppliers but not usually used for cheddar cheese such as *Brevibacterium linens*, *Propionibacteria*, yeasts.

Q3 2009.

Standardized make procedure for testing cultures in low fat cheese was developed that incorporate addition of sodium gluconate, which should allow better comparison of the influence of adjunct cultures on cheese flavor. (Table 1.)

Q4 2009.

Cultures and make procedure used for making low-ffat cheddar cheese. (Tables 2-3.)

Objective 2.

At least two different flavor companies will be contacted with the intent to obtain flavoring systems that can be added directly to the low fat cheese curd at the time of salt addition during cheese manufacture. There are two alternatives that will be pursued in developing this flavor system:

- have a neutral cheese base and add a flavoring system that can provide virtually the entire flavor profile of a 3 mo old cheddar cheese, or
- select a culture system that provides some cheese flavor development, and then supplement this with a flavoring system that provides the missing components as well as potentially masking the any non-preferred flavors.

Low fat cheese will be made by two different methods:

Table 1. Standardization make procedure for low fat cheese.

Process Step:	Time Line	Min. to Next Step	Temp. In (F)	pH	
Start Filling Vat	-2:00	45	73	6.2	During filling add Dilute L - lactic acid (88% dilute 1:166 in DI water) to reach a pH of 6.20. (600:9600)
Vat Filled, begin heating	-1:15	45	90		Adjust milk temp. to 92 F while agitating at 15 RPM
Add Starter, (start of ripening)	-0:30	20	90		DVS 850, **150 g** Single strength (34ml/1000 lbs of milk). Dilute 20:1 im chlorine free, cold water, **55 ml**
Add Color	-0:10	10	90		Double strength (34ml/1000 lbs of milk). Dilute 20:1 in chlorine free, cold water, **60 ml.** Stir for 2 min (16 RPM) before stopping agitators.
Add Coagulant, Set Vat	0:00	20	90		Cut 1 min @ 10 RPM
Vat Cut	0:20	5	90		Cut 1 min @ 14 RPM Cut 1 min @ 4 RPM Cut 2 min @ 5 RPM Watch cut to ensure curd isn't too small Cut 2 min @ 5 RPM

Table 1 Continued. Standardization make procedure for low fat cheese.

Process Step:	Time Line	Min. to Next Step	Temp. In (F)	pH	
Start Rest (5 minutes)	0:25	5	90		Cut 1 min @ 6 RPM Cut 1 min @ 7 RPM Cut 1 min @ 8 RPM Watch cut to ensure curd isn't too small Cut 10 min @ 9 RPM
Start Forework (10 minutes)	0:30	10	90		Cut 30 sec @ 14 RPM Cut 20 min @ 9 RPM Stir continuously to prevent clumping and assist syneresis (water expulsion) 12 RPM. Warm drain table to match temp of cooked curd. Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.
Start Cook	0:40	20	90		Continue stirring until pH 5.95 - 6.0 is reached Drain whey completely. Continue stirring until pH 5.5 is reached Weigh curd, then return curd to drain table. Wash the remaining curd with water at 47-50 F using 4lbs water/10 lbs curd to lower the curd temperature to 72 F.
Start stir-out (30 minutes)	1:00	30	90		Apply salt to the curd at a rate of 0.22lb of salt per 10 lbs of curd. 3 applications 5 minutes apart.
Start Pump over	1:30	15	90		Separate the curd into 2 piles of 60 lbs each. Add sodium gluconate to the 2 piles as shown below.
Wet Acid Development	1:45	30	90	5.95-6.0	0.8% Sodium Gluconate - 218 grams
Drain Whey	2:15	5	89	5.95	1.6% Sodium Gluconate - 436 grams
Dry Stir Remaining Curd	3:20	65	83	5.45	
Weigh Curd	3:30	10	82		Sanitize both the cheese hoops and the cheese cloth in chlorinated water at 150-200 ppm. Place 28 lb of salted cheese in each hoop.
Wash Curd	3:40	10	72		Place pressed cheese in barrier bags and seal in vacuum chamber. Label each bag and box with Date, Block, Weight and Type of cheese, then place in cold storage.
Salt the Curd	3:50	15	72		
Add Sodium Gluconate	4:05	10	72		Apply salt to the curd at a rate of 0.22lb of salt per 10 lbs of curd. 3 applications 5 minutes apart. Separate the curd into 2 piles of 60 lbs each. Add sodium gluconate to the 2 piles as shown below. 2 blocks at 0.8% Sodium Gluconate - 218 grams 2 blocks at 1.6% Sodium Gluconate - 436 grams
Cheese Hooped	4:15	18 hrs	72		
Cheese Wrapped	Next Day		72	5.05-5.2	Sanitize both the cheese hoops and the cheese cloth in chlorinated water at 150-200 ppm. Place 28 lb of salted cheese in each hoop.

Table 2. Cultures sets for low fat cheddar cheese with adjunct cultures, Rep 1 and 2.

Vat #	Culture Type	Culture Description	Chr. Hansen Code
Vat 1 & 12	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
	ADJ	*Lactococcus lactis* ssp.	CR540
	ADJ	*Lactobacillus* ssp.	
Vat 2 & 11	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
	ADJ	*Lactobacillus helveticus*	LH32
Vat 3 & 8	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
Vat 4 & 9	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
	ADJ	*Lactobacillus helveticus*	Emfour
	ADJ	*Lactobacillus acidophilus*	
Vat 5 & 10	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
	ADJ	*Lactobacillus helveticus*	LH32
	ADJ	*Lactococcus lactis* ssp. *cremoris/ lactis*	CR319
Vat 6 & 7	S	*Lactococcus lactis* ssp. *cremoris/ lactis*	DVS 850
	ADJ	*Lactobacillus paracasei*	CRL431

Table 3. Make procedure used during Q4 for low fat cheddar cheese with adjunct cultures and includes addition of titanium dioxide.

Process Step:	Time Line	Min. to Next Step	Temp. In (F)	pH	
Start Filling Vat	-2:10	45	68	6.2	During filling add Dilute L - lactic acid (88% dilute 1:166 in DI water) to reach a pH of 6.20. (700:11200)
Add Titanium Dioxide	-1:25	10	73		Add 340g of TiO2 diluted in 1000mL of Distilled water.
Vat Filled, begin heating	-1:15	45	90		Adjust milk temp. to 90 F while agitating at 15 RPM
Add Starter, (start of ripening)	-0:30	20	90		DVS 850, **150 g**

Table 3 Continued. Make procedure used during Q4 for low fat cheddar cheese with adjunct cultures and includes addition of titanium dioxide.

Process Step:	Time Line	Min. to Next Step	Temp. In (F)	pH	
Add Color	-0:10	10	90		Single strength (34ml/1000 lbs of milk). Dilute 20:1 in chlorine free, cold water, **55 ml**
Add Coagulant, Set Vat	0:00	20	90		Double strength (34ml/1000 lbs of milk). Dilute 20:1 in chlorine free, cold water, **51 ml**. Stir for 2 min (16 RPM) before stopping agitators.
Vat Cut	0:20	5	90		Cut 1 min @ 10 RPM
					Cut 1 min @ 14 RPM
					Cut 1 min @ 10 RPM
					Cut 2 min @ 10 RPM
					Watch cut to ensure curd isn't too small
Start Rest (5 minutes)	0:25	5	90		Cut 5 min @ 11 RPM
					Watch cut to ensure curd isn't too small
Start Fore work (10 minutes)	0:30	10	90		Cut 10 min @ 9 RPM
					Cut 30 sec @ 14 RPM
Start Cook	0:40	20	90		Cut 20 min @ 10 RPM
Start stir-out (30 minutes)	1:00	30	90		Stir continuously to prevent clumping and assist syneresis (water expulsion) 12 RPM. Warm drain table to match temp of cooked curd.
Start Pump over	1:30	15	90		Set pump at 60 Hz. Drain off some whey at start of pump over. Set RPM to 19.
Wet Acid Development	1:45	30	90	5.95-6.0	Continue stirring until pH 5.95 - 6.0 is reached
Drain Whey	2:15	5	89	5.95	Drain whey completely.
Dry Stir Remaining Curd	2:20	70	83	5.50	Continue stirring until pH 5.5 is reached
Wash Curd	3:30	10	72		Wash the remaining curd with water at 47-50 F using 4lbs water/10 lbs curd to lower the curd temperature to 72 F.
Weigh Curd	3:40	10	72		Weigh curd, then return curd to drain table.
Salt the Curd	3:50	15	72		Apply salt to the curd at a rate of 0.22lb of salt per 10 lbs of curd. 3 applications 5 minutes apart.
Add Sodium Gluconate	4:05	10	72		Separate the curd into 2 piles of 60 lbs each. Add sodium gluconate to the 2 piles as shown below.
					Separate the curd into 2 piles of 60 lbs each. Add sodium gluconate to the 2 piles as shown below.
					0% Sodium Gluconate – 0 grams (control)
					0.8% Sodium Gluconate - 218 grams
Cheese Hooped	4:15	18 hrs	72		Sanitize both the cheese hoops and the cheese cloth in chlorinated water at 150-200 ppm. Place 28 lb of salted cheese in each hoop.
Cheese Wrapped	Next Day		72	5.05-5.2	Place pressed cheese in barrier bags and seal in vacuum chamber. Label each bag and box with Date, Block, Weight and Type of cheese, then place in cold storage.

using a starter culture selected for use in low fat cheese, and by direct acidification without any cultures. These will then be sent to the flavor companies for selection of potential flavor systems that can be added.

- ☐ to the curd at the time of salting,
- ☐ to the curd after mechanical chopping to reduce curd pieces to about 2 mm in size.
- ☐ to 2-month old cheese that has been cut into pieces then mechanically reduced in size to about 2 mm pieces.

The flavored cheese will then be pressed overnight and vacuumed packaged and stored at 6°C and examined for flavor 2 wk, 1 mo, 2 mo, and 3 mo after flavor addition and pressing.

Any improvements in cheese flavor compared to the flavor of the cheese manufactured as part of the Low Fat Strategic Flavor Platform project will be noted, and those with acceptable flavor stored for 1 month and retested. If an improvement in flavor is evident then cheeses will be tested using consumer preference sensory evaluation when they are 2 mo old. Any cheeses with high consumer preference scores will be sent to North Carolina State University for flavor profile analysis at 3 mo of age.

Cheeses with promising flavor profiles will be made in duplicate.

The best cheeses will be resent to the flavor companies for possible modifications to the flavor system to provide cheddar cheese-like flavor. Additional vats of cheese will be made with adjustments in flavor systems as needed. Other compounds such as sodium gluconate that have influenced low fat cheese flavor will also be tested as part of this work.

Q1 2009.

Low fat cheddar cheese with 7% fat was manufactured at the WDC facility. The cheese was aged for two weeks at 5°C and then ground using a Urschel grinder with 1.5 mm grind size head. The cheese was added with flavor, mixed thoroughly, and repressed. An informal sensory panel was conducted in the Western Dairy Center with four panelists for flavor profile analysis of cheeses added with different levels of concentrated flavors (Cargill Flavors Inc.) using a 9-point hedonic scale of degree of liking. Based on the feedback or results obtained from first set of observations, cheeses were formulated with a different combination of flavors. Another sensory flavor profile test was done with four panelists, and the best four among eight cheeses were kept for further aging.

RESULTS AND DISCUSSION

Objective 1.

Q4 2008.

LF Mozz with S.thermophilus culture has been made, and no flavor improvement was observed.

Q1 2009.

No improvement in flavor by adding Lb. helviticus LH32. Similar effects were observed by Mark Johnson (WCDR), thus this objective has been discontinued until work on adding flavors is completed.

Q2 2009.

Further trials were conducted on being able to manufacture low fat cheese with 54% moisture, 6% fat, 1.8% salt, and pH 5.05 to 5.20, plus the addition of sodium gluconate to inhibit formation of burnt brothy flavor.

Q3 2009.

Two vats of low fat cheese were made with 0.8% and 1.6% sodium gluconate added, based on the make procedure developed at the Western Dairy Center. These are being aged, and at two months of age the cheeses with 1.6% sodium gluconate had a good mild cheese flavor and will be further evaluated by the DMI Expert Cheese Group.

Q4 2009.

Six vats of low fat cheese were made in replicate based on the above make procedure developed at the Western Dairy Center, five with adjunct cultures aimed at producing cheddar flavor. Sodium gluconate at 0.8% was added to half of each vat. Each treatment made two blocks, one held at normal ripening temperatures while the other underwent an accelerated ripening step. Cheeses are being aged.

Objective 2.

Q3 2008.

Flavor samples received from Cargill and tested.

Q4 2008.

LF cheddar was ground and flavors added and repressed. Initially twelve different formulations (including control) were used with ground cheddar cheese and mixed with different combination of Cargill flavors (140-30010 paste and 145-00153 powder). An informal taste panel was done (July 30, 2008), and reformulation (8 combinattions) was based on taste panel feedback. Proximate analysis was conducted on selected combinations. An informal taste panel was conducted (Aug 28, 2008) again on 8

Table 5. Proximate data: low fat cheese with adjunct cultures, Rep 1.

Vat #	Description of Vat	Block	pH	% Fat	% Moisture	% salt
Vat 1	DVS 850 + CR540, CONTROL	A2	5.05	5.5	52.82	1.87
	DVS 850 + CR540 + 8% Sodium Gluconate	B2	5.13	5.5	54.28	1.97
Vat 2	DVS 850 + LH 32, CONTROL	A2	4.99	5.5	53.79	1.87
	DVS 850 + LH 32 + 8% Sodium Gluconate	B2	5.02	5.5	55.59	1.88
Vat 3	DVS 850 only, CONTROL	A2	5.13	6.0	51.82	1.85
	DVS 850 only + 8% Sodium Gluconate	B2	5.14	5.5	53.43	1.96
Vat 4	DVS 850 + Emfour, CONTROL	A2	5.14	5.8	52.23	1.83
	DVS 850 + Emfour + 8% Sodium Gluconate	B2	5.14	5.5	54.31	1.95
Vat 5	DVS 850 + LH 32 + CR 319, CONTROL	A2	5.12	5.5	53.12	1.8
	DVS 850 + LH 32 + CR 319 + 8% Sodium Gluconate	B2	5.14	5.5	54.58	1.96
Vat 6	DVS 850 + CRL431, CONTROL	A2	5.12	5.5	53.12	1.8
	DVS 850 + CRL431 + 8% Sodium Gluconate	B2	5.14	5.5	54.58	1.96
	Mean		5.11	5.6	53.64	1.89
	Std. Dev.		0.05107	0.14878	1.04117	0.06256

Table 6. Proximate data: low fat cheese with adjunct cultures, Rep 2.

Vat #	Description of Vat	Block	pH	% Fat	% Moisture	% salt
Vat 7	DVS 850 + CRL431, CONTROL	A1	5.18	5.5	51.80	2.12
	DVS 850 + CRL431, CONTROL	A2	5.19	5.0	52.09	2.27
	DVS 850 + CRL431 + 8% Sodium Gluconate	B2	5.19	5.0	53.55	2.28
Vat 8	DVS 850 only, CONTROL	A1	5.23	5.0	54.58	1.95
	DVS 850 only, CONTROL	A2	5.22	5.5	54.61	1.92
	DVS 850 only + 8% Sodium Gluconate	B2	5.32	5.0	55.30	2.03
Vat 9	DVS 850 + Emfour, CONTROL	A2	5.07	3.5	52.44	1.81
	DVS 850 + Emfour + 8% Sodium Gluconate	B2	5.09	4.0	54.27	1.93
Vat 10	DVS 850 + LH 32 + CR 319, CONTROL	A2	5.18	4.0	54.92	1.99
	DVS 850 + LH 32 + CR 319 + 8% Sodium Gluconate	B2	5.28	5.0	55.77	2.05
Vat 11	DVS 850 + LH 32, CONTROL	A2	5.24	4.0	52.25	1.84
	DVS 850 + LH 32 + 8% Sodium Gluconate	B2	5.23	4.0	53.64	2.16
Vat 12	DVS 850 + CR540, CONTROL	A2	5.35	4.0	53.78	1.92
	DVS 850 + CR540 + 8% Sodium Gluconate	B2	5.43	4.0	55.01	2.01
	Mean		5.23	4.5	53.86	2.02
	Std. Dev.		0.09203	0.63987	1.24076	0.13918

Table 7: Formulation of cheese with flavor and feedback from informal panel.

Date	Formulation	Comments
Cheese make – 07/02/08	Control	Dry
	0.5% 140-30010 paste	Fishy
	0.75% 140-30010 paste	Oily
	1.00% 140-30010 paste	Rancid
Ground – 07/17/08	1.00% 145-00153 powder	No flavor
	1.5% 145-00153	Sour
	2.0% 145-00153	Buttery
Evaluated for flavor – 07/30/08	1.00% 145-00153+0.25% 140-30010 paste	Sulfury
	0.5% 145-00153+0.5% 140-30010 paste	Cheesy
	0.5% 140-30010 paste + 0.5% butter	Rancid
	1.00% 145-00153 + 0.5% butter	Buttery
	0.5% 145-00153 + 0.5% 140-30010 paste + 0.5% butter	Buttery

Table 8. Proximate composition on day 1 of low fat, low salt cheddar cheese for Innovative Flavor project

Date	Cheese Type	Moisture %	pH	Fat %	Salt %
07/29/08	Stirred curd	50.00	5.3	8	1.6
	Control cheese	48.45	5.28	8	1.72
	Cheddar cheese with 0.5% 140-30010+0.5% 145-00153+0.5% 0f butter	50.84	5.18	8	1.8

combinations, and the four best combinations were kept for more aging at 38°F.

Q1 2009.

Informal taste panel and proximates results have been analyzed and are shown in Tables 7 and 8.

CONCLUSIONS

Objective 1.

A satisfactory cheese make procedure has now been developed for consistent manufacture of low fat cheddar cheese with moisture content of 53%, 2% salt, and pH~5.1 to 5.2. No improvement in cheese flavor was apparent after 2 month of aging.

Objective 2.

Addition of flavors to cold comminuted cheese was not successful in producing more cheese flavor.

NEXT STEPS

Objective 1.

Q4 2009.

Evaluate differences in flavor of cheeses through informal tasting, descriptive analysis, and consumer panels of cheeses manufactured in Q3. Determine if addition of any of the lactobacilli adjuncts produces more cheddar cheese flavor when they are used in low fat cheese. Conduct a second trial using non-cheddar cheese adjuncts (propionibacteria, brevibacteria, leuconostocs) to determine if cheese flavor can be increased even if it is not cheddar flavor.

Objective 2.

No further work is planned for this objective.

Production of high protein cheddar cheese with an improved extrusion-modified texture

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ABSTACT

This project will investigate the use of extrusion technology to improve the texture of high protein (low-fat) cheddar cheese. High protein cheddar cheese has a composition of approximately 54% moisture, 6% fat, and 34% protein. This composition results in a cheese that has flavor defects and a rubbery texture. Therefore, several blends of cheddar cheeses (full-fat aged and nonfat) will be grated and blended prior to extrusion in a twin-screw extruder. Extruder conditions of low temperatures (40° to 60°C), screw and paddle configurations of medium shear, and moderate pressures (~350 psi) will be explored to improve the cheese texture by disruption of the casein matrix and evenly distributing fat, moisture, and air cells. Additionally, the use of fat replacers (Temp Pro WPC, Novagel, Simplesse, Vitacel MCG, and Vitacel Plus) to improve the texture of the extruded cheese will be investigated. All cheeses will be analyzed for protein, moisture, and fat content, and the texture will be determined and compared to full-fat aged and nonfat cheddar cheeses. The goals of this project are to optimize the extruder configurations (screw and paddle sequences) and the physiochemical parameters (formulation, temperature, and pressure) to allow the extrusion modification of cheddar cheese texture and to investigate the influence of fat replacers on the texture of the extruded cheese. The use of full-fat, aged cheeses blended with nonfat cheese in conjunction with extrusion may yield a product with enhanced flavor and texture compared to currently available high protein cheddar cheeses.

BACKGROUND

Literature review.

Low fat cheese (Table 1) approaches the definition of a high protein product with approximately 9.5 g protein in a 28 oz serving, while nonfat cheese has over 10 g protein per serving. Ten grams of protein per serving is classified as high (20% or more of RDI of a nutrient) based on DRV (daily reference value) of 50 g considering 2,000 calories/day. The higher protein content of low and nonfat Cheddar cheese is a result of the fat reduction. Unfortunately, the lower fat with higher moisture results in a product with textural and flavor deficiencies compared to the full or reduced fat Cheese products.

Low fat cheeses properties.

Reducing the fat content in Cheddar cheese results in a product that is high in protein. Fat plays a critical role in the flavor, texture and appearance of Cheddar cheese and low and nonfat cheeses are usually identified as bland, firm, rubbery, and defective in color. The higher moisture in low-fat cheeses results in a lower salt content in the moisture phase. This change in the microenvironment is related to the changes in the sensory characteristics as well as in the microbiology and chemistry of the cheeses (Mistry, 2001).

Flavor defects arise from the lack of butterfat and the development of bitterness. Recent research (McMahon, unpublished data) shows that reduced fat Cheddar cheese has less milkfat flavor and higher sulfur, brothy and bitter flavors.

Texture profile and sensory analysis of low fat cheese show that lowfat cheese exhibits more springiness, firmness, and cohesiveness with a higher fracturability (McMahon unpublished data). This profile results in a rubbery texture to consumers. The structure of low fat cheese as determined by confocal microscopy is different from full or reduced fat. The fat in the cheese forms cavities and

channels that help give the cheese an open structure. Typically, in full and reduced fat cheeses, the protein matrix is open, with spaces occupied by fat globules. In low fat cheese, the matrix is compact (Rahimi et al, 2007; Aryana and Haque, 2001; McMahon, 1996). The low fat cheese, without fat cavities and channels, is a dense aggregation of casein, hence the rubbery texture. In addition, there is limited breakdown of α_{s1}-casein during ripening which contributes to the firm texture (Mistry, 2001)

To overcome these deficiencies, three different categories of modifications have been investigated. The categories include cheese-make process modifications, starter and adjunct culture use, and the use of fat replacers. Increasing the moisture content has been suggested to improve the properties of low-fat cheeses (Rodriguez, 1998), yet others have suggested that it is necessary to maintain the same moisture in nonfat as found in full fat (Mistry, 2001). One popular approach is the use of fat replacers to maintain the same moisture:solids ratio.

Use of fat replacers in low fat cheeses.

The definition of fat-free is less than 0.5 g fat per serving size (approx 1.8 % fat in cheese). Low fat is 3 g fat per serving size (approx 10.7% fat in cheese) (21 CFR 101.62). Therefore, the high protein cheeses that will be produced in this project fits into the low fat claim category. According to the CRF (21 CFR 130.10) "deviations from noningredient provisions of the standard identity are permitted in order that the substitute food possesses performance characteristics similar to those of the standardized food". Other ingredients are also allowed "to improve texture, add flavor, prevent syneresis, extend shelf life, improve appearance, or add sweetness so that the product is not inferior in performance characteristics to the standardized food ...". The fat replacers proposed in this project do have defined functionalities which may improve the texture, prevent syneresis, and improve appearance of low fat/high protein cheese.

The use of fat replacers has been extensively explored to improve the texture and appearance of low and nonfat cheeses (Rahimi et al., 2007). Fat replacers based on microparticulated protein or carbohydrates have been recommended for use in cheese products (Romeih et al., 2002). They can mechanically entrap water giving the sense of lubricity and creaminess, but cannot positively impact the flavor defects. Table 2 lists some of the recent publications that have investigated the use of fat replacers in cheese. The fat replacers were added to the milk (or blended with cream) prior to cheese making. Only one reference estimated the amount of fat replacer retained in the curd (McMahon, 1996). This reference assumed 70% retention. Therefore, the use levels given in Table 2 can be reduced by 30% as the starting concentration since no loss is expected if the fat replacers are added to the cheese blends prior to extrusion.

The microstructure of low-fat cheese made with different fat replacers (Simplesse, Dairy Lo, Stellar, and Novagel) was compared to a full fat and a low-fat Cheddar cheese (Aryana and Haque, 2001). The electron micrographs of cheeses in this study containing full fat showed numerous holes and had a smooth surface. The holes were from the delipidation of the samples. The low-fat cheese showed less holes and a rippled surface. The addition of protein based fat replacers (Simplesse and Dairy-Lo) showed fewer ripples, and the authors correlated the amount of ripples to the hardness of the cheeses, in this case, the low-fat cheeses made with protein based fat replacers were less hard than the low-fat cheese without fat replacers.. The Simplesse particles (0.75 micron) were visible in the matrix, interrupting the casein network. The surfaces of low-fat cheeses made with carbohydrate fat replacers were undulated and rough. The cheese made with Novagel showed large particles (50 micron) that were fewer in quantity than the Simplesse, but also interrupted the network. Their conclusions were that Simplesse and Novagel softened low-fat Cheddar cheese by imparting discontinuity to the casein matrix. McMahon et al. (1996) investigated the microstructure of low-fat Mozzarella cheese and low-fat Mozzarella made with two protein based (Simplesse and Dairy-Lo) and two carbohydrate based (Stellar and Novagel) fat replacers. The only fat replacer that increased the openness of the cheese structure was Novagel because it was too large to be embedded in the protein matrix; instead it created large serum channels in the cheese. The Novagel particles were 30-300 micron compared to 0.5 microns for Stellar while the protein based fat replacers contained particles of 5-10 and 0.5 to 1.0 micron for Dairy-Lo and Simplesse respectively.

Applications of extruders in food processing.

Extruders can be configured for low, medium, or high shear by the sequence of the screws and paddles in the barrel. The screws promote conveyance and the paddles interrupt the flow and create shear and back pressure. Twin-screw extruders are more functional than single screw extruders and they can be used at higher moisture levels (>40 %) as compared to single screw extruders (<35) (Walsh and Carpenter, 2008). Extruders are used in the food industry for the production of direct expanded (high shear), low density snacks, flat breads, and breakfast cereals; medium shear products such as animal feeds and textured vegetable protein; and low-shear products including pasta.

A recent patent (Mueller 2005, USP 6942888) describes the use of a twin-screw extruder for producing pieces of cheese from blocks of compressed aged or unaged cheese curds. This process is to produce pieces of cheese that have an exact weight from bocks of compressed curds with an overall goal to eliminate storage and aging of the

Table 1. Approximate composition of Cheddar cheeses produced at the Western Dairy Center.

Cheese	Moisture (%)	Fat (%)	Protein (%)	g fat/28 g	g protein/28g
Full fat, aged	37	32	25	9	7
Aggiano	32	35.7	28.6	10	8
Reduced fat, aged	48.5	16	30	4.5	8.4
Low fat	54	6	34	1.68	9.52
Non fat	60	0.3	38	0.1	10.65

Table 2. Some studies that have investigated the use of fat replacers in low fat cheeses.

Cheese type	Fat replacers used individually	Functional changes compared to low or reduced fat	Reference
Low-fat white-brined cheese	0.7 or 1.4% Oat beta-glucan	Improved texture but lower flavor and color	Volikakis 2004
Low-fat fresh Kashar	1% Simplesse 100 or 1% Dairy-Lo or 5% Raftiline HP	Simplesse and Raftiline improved the texture and sensory properties up to 60 days	Koka 2004
Imitation Mozzarella cheese	8-43 % dry basis Novelose 240 (fiber)	Decreased hardness	Noronha 2007
Low-fat Iranian White Cheese	0.75 % Gum Tragacanth	Improved texture, water binding, decreased hardness	Rahiml 2007
Low-fat Cheddar	Beta-glucan Nutrim	Decreased hardness and sensory scores	Konuklar 2004
Low-fat white pickled cheese	0.5% Simplesse 100 or 0.5% Dairy-Lo or 0.5% Perfectamyl or 0.4% Satiagel	Dairy-Lo and Satiagel were similar in texture to low-fat sample	Kavas 2004
Low-fat white brined cheese	1% Simplesse 100 or 0.125 % Novagel NC200	Improved texture, Simplesse also showed improved appearance	Romeih 2002
Low-fat Cheddar cheese	1% Dairy Lo or 1.5% Simplesse or 1.2% Stellar, or 0.2% Novagel	Simplesse and Novagel imparted discontinuity to the casein matrix	Aryana 2001
Low-fat Mozzarella	0.6% Simplesse or 0.6% Stellar or 2.5% Dairy-Low or 2.5% Novagel	Cheeses with Stellar and Simplesse showed greater initial meltability but all cheeses showed the same meltability after 21 days.	McMahon 1996

large cheese blocks for later cutting into consumer-seized pieces of cheese. This process uses temperatures of 30 C in the extruder and a cooling section at 7.2 C and the extruder is configured with intermeshing feed screws, which increase in thickness from the input to the cheese output end. This arrangement is to increase pressure in the system to compress the cheese pieces into a uniform, homogenous cheese flow. The cheese then enters a cooling device at 7.2 C where is cheese solidifies so a properly shaped end product results. In the process described in this proposal, a cooling devise was not included but may be added to the extruder if needed. We have previously used a cooling device for the production of textured whey protein for use as a meat extender. Additionally, we may need to press the extruded product to remove excessive air.

The majority of extruded snacks on the market fall into the direct expanded category and the most popular are the corn based such as corn curls, balls and rings. Other corn-based snacks such as Doritos are also extruded products along with potato snacks including Pringles, Baked Lays and Munchos. Seafood based snacks are produced from shrimp and fish mixed with a starch to form a dough that is expanded via extrusion. Co-extruded snacks have an extrusion produced outer shell with a filling such as peanut or cheese filled pretzels or fruit filled cereal based products. Examples of breakfast cereals that are derived from extrusion include crisp rice, sugarcoated fruit flavored rings, cornflakes, cinnamon and sugar graham shapes, coco balls, bran/wheat flakes, frosted stars and flakes.

Textured vegetable protein is an example of medium shear and it can be formed into meat-free (vegetarian) hot dogs, hamburgers, chicken patties/shapes, hams, and sausage, pepperoni, and bacon. Lose meat products are incorporated into tacos, canned chili and spaghetti sauces. Textured vegetable protein is currently produced from soy flour, sesame flour, wheat gluten and canola and rapeseed

concentrates.

In conjunction with the screw and paddle sequence, the speed of the co-rotating screws and the temperature during extrusion significantly influence the texture of the final product. High temperatures will melt (denature the proteins) to allow for new protein:protein interactions. The rate of speed will influence the amount of input shear, residence time in the barrel, and also denature the proteins. For this project, we are looking to produce a product using medium shear conditions at moderate temperatures with the goal to change the protein network and evenly distribute the fat, moisture, protein and air to positively impact the Cheddar texture.

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RESEARCH PLAN

Objective 1.

Various USU cheddar cheeses (USU Aged Old Jfumiper, Aged Aggiano, and nonfat Cheddar) will be groumd to yield particle sizes of 0.5, 1, and 2 mm to determine the appropriate size that would be suitable to use as a starting material for twin-screw extrusion.

Objective 2.

The screw and paddle configuration of the twin-screw extruder will be adjusted to allow low temperature (40° to 60°C), medium-shear, and moderate pressure extrusion (~350 psi). A minimum of four combinations of screw and paddle configurations will be used to modify the texture of cheddar cheeses.

Objective 3.

Ground cheddar cheeses will be blended to yield a final protein content of >10 g per 28 oz serving size and will be extruded based on the extruder configurations defined in objective 2 (a minimum of four different cheddar cheese blends).

Objective 4.

Texture enhancing ingredients (fat replacers Temp Pro WPC 80, Novagel, Simplesse, Vitacel MCG, and Vitacel Plus HF) will be mixed into the ground cheese blends at two different concentrations and extruded as in objective 3.

Objective 5.

All extruded cheeses (objectives 3 and 4) will be analyzed for texture using the TPA assay initially and over a six month shelf life. The protein, moisture, and fat contents, as well as the color of the cheeses, will also be determined.

MATERIALS AND METHODS

All cheeses used in this study will be manufactured in the USU Dairy Products Laboratory under the direction of Don McMahon.

The protein concentration will be determined by the Kjeldahl Method and converting the nitrogen measurement to protein content by multiplying by 6.38. The texture will be analyzed using a Texture Analyzer (TA-XT) equipped with a flat plunger as described by Volikakis et al., 2004 and McMahon (unpublished data). The color of cheese at days will be determined with a Hunter Lab system to generate l, a, and b values. The moisture will be determined by drying in an oven, and the fat content will be determined by

Table 3. Blends of cheeses to achieve 10g protein per 28g serving size.

Number	Full fat, aged Old Juniper (%)	Full fat, aged Aggiano (%)	Amt Low fat (%)	Amt nonfat (%)	g fat/28 g	g protein/28 g
1	15			85	1.4	10.1
2	10			90	1	10.27
3		15		85	1.6	10.25
4		10		90	1.1	10.37

Table 4. Texture modifying ingredients.

Product name and manufacturer use level for cheese	Composition	Functionality
[1]Temp ProTM Use level is 0.4 to 4%	80% WPC	Heat stable whey protein, stays fluid at retort temperatures, will not interact with casein, matrix interruption
[2]Novagel NC 4230 cellulose gel Use level is 0.125-2%	Microcrystalline cellulose	Fat replacer. Gel particles interrupt casein structure by reacting with kappa casein to form a curd that can entrap moisture
[3]Simplesse 100 Use level 0.5-1%	54 % whey protein	Microparticulated whey provides matrix interruption, creaminess, UHT and retort stable, provides for uniform moisture distribution
[4]Vitacel MCG 611F Use level 0.4-2 %	Insoluble microcrystalline cellulose	Matrix interruption, fat imitation
[4]Vitacel Plus HF Use level 0.4-2 %	Insoluble and soluble fibers	Matrix interruption, fat imitation

[1]Leprino Foods
[2]FMC BioPolymer, Can be blended in 0.4% Temp Pro™ and dispersed into ground cheese
[3]CP Kelco Co
[4]JR Rettenmaier

the Babcock or Leco methods.

All analysis will be done in triplicate for each cheese sample that shows improved texture compared to nonfat and reduced-fat cheddar cheese over a shelf life of four to six months. All data will be analyzed by ANOVA to determine the significant differences among treatments.

Project Design.

Full fat aged and nonfat cheeses will be ground to determine the suitable size (0.5 to 2 mm) for extrusion using a Hobart mixer/grinder. Cheeses will be added to the Hobart mixer/grinder based on the composition of each cheese as stated in Table 1 and below in Table 3.

Values will vary slightly (increase in protein and fat) due to the moisture content of the blends. There is room for an additional 1% of non-protein ingredient before protein concentrations fall below 10 g/serving. This can also be offset by the use of Temp Pro WPC 80 in combination with the non-protein ingredients.

The full fat, aged Cheddar cheeses can get pasty if they are ground in the Hobart alone, therefore, the full fat aged and nonfat cheeses will be added together into the Hobart mixer/grinder to reduce the pastiness of the cheese. We believe the smallest particle size (0.5 mm) will be optimum as a starting material for the extruder if the texture is not pasty. Otherwise, we may need to increase the cheese particle size to reduce the pastiness.

The extruder we will be using is an APV Baker MPF19 twin-screw extruder with a length/diameter barrel of 25. The temperature zones (5) along the barrel are controlled with a CAL3200 Autotune temperature controller. The sample is introduced into the barrel via a KTron volumetric dispenser. A new twin auger will need to be purchased for the dispenser based on the particle size of the cheese as the starting material. Based on the size of the cheese particles, a double or single screw auger will be purchases to replace the current dry feed auger. The extruder also has a liquid feed inlet that dispenses in the first barrel zone.

The blended cheeses will be extruded at various extruder screw and paddle configurations using a twin screw extruder at temperatures between 40 and 60 C to determine the optimum extruder configurations that yield a cohesive product with evenly incorporated air pockets. The screw and paddle configurations will be sequenced to provide a moderate amount of shear. Generally, one screw and paddle sequence can be tried per day per cheese blend.

Initially, the cheeses will be extruded and screw speed and temperature varied to visually inspect the changes in the cheese matrix. The cheeses will also be analyzed for tex

ture as stated below in the methods to determine the influence of screw speed, screw configuration and temperature on the final product texture. We will start with a medium shear configuration and temperature and screw speed settings to allow product flow through the extruder. We want to limit the temperature to approximately 60 C to eliminate any volatile flavor loss and extensive protein:protein crosslinking. We may need to press the cheese after extrusion if there is excess air in the product.

Fat replacers that we will investigate as texture modifiers are given below in Table 4. The fat replacers will be added to the cheeses while they are in the Hobart mixer/grinder to ensure even distribution. Initially we will use 30% of the lowest use level of each fat replacer. We will also use double that amount to investigate the influence of the fat replacer on the cheese texture.

Additionally, we can use both the Temp ProTM heat stable whey in conjunction with the other fat replacers to increase the amount of protein if necessary in the sample. We also may need to add liquid to the sample during extrusion if hydration of the fat replacer is necessary. The liquid addition can be set to the microgram volume and increased to hydrate the fat replacer to ensure the cheese flows through the extruder.

All extruded Cheddar cheese samples will be analyzed for texture using the Texture Profile Analysis assay. Additionally, the moisture, protein and fat concentrations and color of each cheese will be determined.

All analysis will be done in triplicate for each cheesse sample that shows improved texture compared to nomfaat and reduced fat Cheddar cheese over a shelf life of 4-66 months. AOVA will be used to determine the significannt differences in texture based on the treatments (fat repllacc-ers, amount, extrusion conditions etc).

RESULTS AND DISCUSSION

Objective 1.

Q4 2008.

Various USU cheddar cheeses (USU Aged Old Juniperr, Aged Aggiano, and nonfat Cheddar) will be ground tto yield particle sizes of 0.5, 1, and 2 mm to determine thae appropriate size that would be suitable to use as a startingg material for twin-screw extrusion.

We have initiated this objective, and we have grounad various cheeses (Aggiano, nonfat Cheddar, and Ageed Old Juniper cheese) to a particle size of about 2 mm. Thae nonfat cheese is facile to grind, while the full-fat cheesees are sticky. We plan to blend the cheeses prior to grinding tto minimize the stickyness of the grounded cheeses. We havve also changed the configuration of our extruder to be able tto

Table 5. Extruder conditions for the extrusion of cheddar cheese.

Extruder Conditions	Start Up Conditions	Collection Conditions
Dry Feed (rpm)	400	800
Screw Speed	150	200
Set Temp °C /Recorded Temp.		
Zone 1	35/ 28	41
Zone 2	15/ 38	46
Zone 3	35/ 37	37
Zone 4	35/ 39	36
Zone 5	20/ 24	24
Melt Temp	30.4	45.1
Pressure psi	-110	-30
Torque %	35%	25%

Table 6. TPA hardness data on extruded cheeses.

Treatment	Mean Hardness	Grouping	Post Extrusion Treatment
Control cheddar unextruded	3271.55	A	
Cheddar extruded with Vitacell	1660.44	B	30 psi / 4 hrs
Cheddar extruded with Novagel	1507.55	BC	5 psi / 0.5 hrs
Cheddar extruded with Novagel	1316.70	C	30 psi / 15 min
Cheddar extruded with Temp Pro	1032.01	D	20 psi / 0.5 hrs
Cheddar extruded	927.02	D	30 psi / 20 hrs

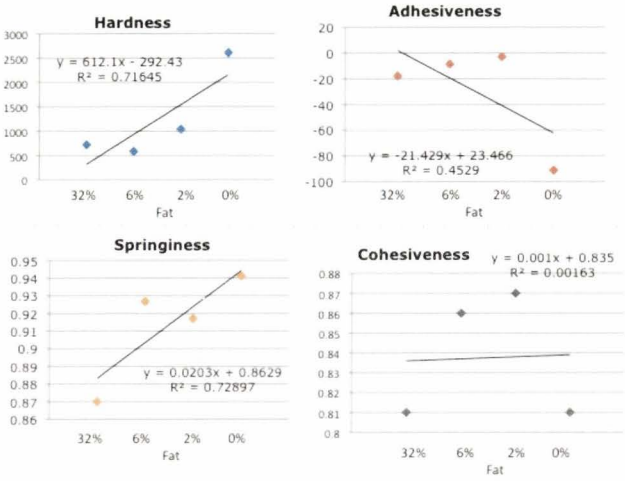

Figure 1. TPA resuits from control Cheddar cheeses.

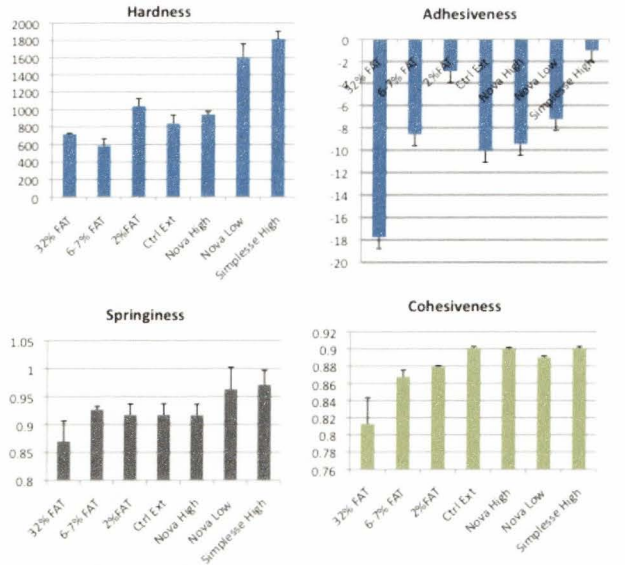

Figure 2. TPA parameters of control and extruded cheeses.
- Ctrl Ext = 2% fat cheese extruded with no fat replacer
- Nova High = Novagel RCN15 with highest concentration (2%)
- Nova AvgM = Novagel RCN15 with 1.06% fat replacer
- Nova Low = Novagel RCN15 with 0.125%
- Simplesse High = simplesse with 1% fat replacer

feed the cheeses through the hopper into the extruder.

Objective 2.

Q1 2009.

We have determined the extruder conditions that allow for extrusion of ground cheese. We are currently using a screw and paddle configuration that promotes high shear without pressure. Table 5 lists the extruder conditions that are effective. Note, we strive to keep the temperature below 45°C; higher temperatures melt the cheese, and we feel the texture is negatively affected.

Objective 4.

Q1 2009.

Prior to starting objective 3, we have conducted some initial research on the effects of fat replacers on the texture of 6% fat cheese that has been extrusion modified. We have used the fat replacers Novagel RCN15 (2%) (cellulose based), Temp Pro (4%) (whey protein based), and Vitacel MCG (2%) (microcrystalline cellulose) at the concentrations listed, which are the highest concentrations recommended by the manufacturers. We have conducted TPA analysis of the samples to obtain preliminary data on the use level of fat replacers. Since the Aged Aggiano cheese is costly, we would like to confirm the use levels of the fat replacers prior to starting objective 3.

The extruded cheeses are collected in small (1 lb) presses, and various pressing conditions (pressure and temperature) were used to determine the influence of pressing on the knitting of the cheese. TPA analysis of 6% fat extruded cheese with fat replacers, compared to the original 6% fat cheese, shows that the hardness of the extruded cheeses was significantly less than the un-extruded cheese. The lowest hardness values were obtained with the addition of 4% Temp Pro and cheese with no addition of fat replacers. This is preliminary data and will be replicated to confirm results. (Table 6.)

Q3 2009.

We have been investigating the extrusion modification of 2% fat cheese by the addition of fat replacers, as described in the methods section, and we are also correlating the fat content of cheese to the TPA properties of hardness, adhesiveness, springiness, and cohesiveness, which are the most important characteristics.

Figure 1 shows the standard curves of percent fat vs the TPA measured parameters. The parameters of hardness and springiness show a fair correlation coefficient (0.7), while those of adhesiveness and cohesiveness are not linear. We are adding more data points to these graphs this month to improve the correlation coefficients.

We ultimately would like to use the TPA measurements to be able to correlate the effects of extrusion and the addition of fat replacers to the perceived fat level in the extruded cheeses. TPA measurements of hardness, adhesiveness, springiness, and cohesiveness of control and extruded cheeses is shown (Figure 2). This is preliminary data, since this represents one of three replicates for each extrusion treatment. We will be able to make more confirming conclusions once the whole data set is extruded and analyzed.

CONCLUSIONS

We were successful in grinding the cheeses to the suitable size to feed into the extruder.

Extrusion alone and with the addition of fat replacers can reduce the hardness of 6% cheddar cheese.

We will be able to correlate the TPA parameters to the % fat in control cheeses to predict how extrusion and extrusion with fat replacers changes the characteristics of

Table 7. Predicted texture attribute of cheeses extruded with fat replacers.

Texture Parameter	Fat Replacer	Concentration of Fat Replacers		
		High	*Middle*	*Low*
Hardness	***Novagel***	48.79+/- 6%	36.54 +/- 2%	41.79 +/- 6%
	Simplesse	27.47 +/- 1%	34.25+/- 2%	36.2 +/- 2%
	Temp Pro	22.12 +/- 1%	23.65+/- 1%	26.75 +/- 1%
Cohesiveness	***Novagel***	0.0	0.0	0.0
	Simplesse	33.12 +/- 0%	34.2+/- 0%	35.29 +/- 0%
	Temp Pro	34.2 +/- 0%	33.1 +/- 0%	34.2 +/-0%

the cheeses with respect to perceived fat level.

Simpless and TempPro show promise to modify the texture of extruded 2% fat cheese so the hardness and cohesiveness values are more similar to full fat cheese.

Objectives 4 and 5.

Q4 2009.

Texture analysis of cheddar cheese at three fat concentrations (2%, 13%, and 32%) resulted in a linear relationship between fat content, hardness, and cohesiveness, allowing the determination of effectiveness of the fat replacer. The R^2 Values for the different texture parameters are:

Hardness : $y = -21.43x + 1466.425$, $R^2 = 0.9326$

Cohesiveness : $y = 0.0092064x + 0.5951$, $R^2 = 0.9251$

Low-fat cheddar cheese (2% fat) was extruded with three different fat replacers at three concentrations based on manufacture recommendations: Temp Pro (4%, 0.40%, 2.20%), Novagel RCN15 (2%, 0.125%, 1.06%), and Simplesse 500 (1%, 0.50%, 0.75%) in triplicate. Extruded cheeses were analyzed for the texture parameters of hardness and cohesiveness over storage time. We are able to give predictive fat concentrations based on the control cheeses. (Table 7.)

Using the standard curve for hardness, the use of the fat replacers gave varying results, although all the cheeses gave predicted fat levels higher than the 2% fat cheese used. Based on the data in Table 7, the use of Novagel and Simplesse at the middle concentrations resulted in cheeses with a texture similar to full-fat cheddar, based on hardness scores. Although, there is not a dose response with the use level of fat replacers. In fact, the use of Simplesse at the highest concentration gave a lower predicted fat level that at the lowest use level. The use of TempPro resulted in an increase in predicted fat level at all concentrations, yet the values did not approach the levels seen with Novagel or Simplesse.

The use of the cohesiveness standard curve also showed interesting results. In this case, the use of Novagel at all three concentrations did not yield predicted fat levels higher than the original 2% fat cheese used. The use of simples and TempPro at all levels resulted in predicted fat levels approaching or greater than full-fat cheese.

The results in Table 7 are the results of TPA analysis of extruded cheeses after one week of storage. We are currently investigating the TPA data for one month of cheese storage.

Once we narrow the fat replacer based on all the TPA data, we will be blending the 2% fat cheese with the fat replacer, the full-fat aged cheese, and extruding samples.

NEXT STEPS

We have no changes in the original proposal at this time. We will continue to extrude 2% fat cheese with three levels of fat replacers with three replicates.

We will continue with the analysis of cheeses extruded with three different fat replacers at three concentrations to conclude at one month of storage. We will then pick the most appropriate fat replacer and extrude 2% fat cheese with the fat replacer and aged full-fat cheese.

Effect of high intensity ultrasound (HIU) on functional properties of whey proteins

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ABSTACT

This project will test the hypothesis that high intensity ultrasound (HIU) can improve the functional properties of whey proteins. HIU will be applied to whey protein solutions, and the effect of acoustic waves on functional properties crucial to the manufacture of beverages will be evaluated. The functional properties that will be analyzed are: heat stability, clarity/turbidity, and flavor. Different HIU settings (power, duration of the signal) will be tested in combination with several environmental conditions such as protein concentration (to simulate the concentrations found in the whey protein production process) and temperature. HIU treated whey will be analyzed for flavor and exposed to different temperatures to reproduce a hot filling, pasteurized and UHT processing at three pH values, and analyzed for clarity/turbidity and solubility. The presence of off-flavors such as overall aroma intensity, cooked, diacetyl, sourness, cucumber, brothy, cardboardy, soapy, bitterness, and astringency will be quantified among the treatments and compared to a control whey protein solution (non-HIU treated). The acoustic setting that results in the greater improvement on heat stability and turbidity will be chosen to evaluate the effect of protein concentration on these properties.

BACKGROUND

Introduction.

During the last decade food scientists have been interested in ultrasonic applications due to its reliability, reproducibility, high efficiency and capability to analyze opaque and concentrated materials.

Results from several studies showed the effect of HIU on the functional properties of whey proteins. Kresic *et al.* (2008) showed an increase in water solubility of whey proteins treated with HIU. They suggested that HIU enhanced protein solubility by changing protein conformation and by decreasing its molecular weight. Jambrak *et al.* (2008) evaluated the effect of ultrasound on the solubility and foaming properties of whey protein suspensions. They found that both functional properties were improved when HIU was used. However, the results were dependant on the acoustic frequency used. Higher frequencies (40 kHz) were not as efficient as lower ones (20 kHz).

Stathopulos *et al.* (2004), and Villamiel and de Jong (2000) reported conformational changes in HIU treated proteins. The first group of researchers reported the formation of aggregates with high β-content in non-dairy proteins such as myoglobin and lysozyme. The second group of researchers found denaturation of whey proteins when ultrasound, in combination to heat was applied to milk.

Even though some of the research described above indicates that HIU improves the functional properties of proteins, there is no research showing its effect on the flavor of whey proteins. We propose to apply HIU to whey proteins and evaluate the effect of the acoustic waves on their functional properties such as heat stability (solubility) and clarity/turbidity, for application in beverages. We would like to couple these experiments with the analysis of the effect of HIU on the flavor of the whey protein solutions.

As described by Patist and Bates (6) ultrasonic technologies have the capability for a large commercial scale-up and a good payback on capital investment. Some of the reasons are:

- Availability of high amplitude/power units for large commercial operations

- Improved energy efficiency of the equipment
- Easy to install and/or retrofit systems
- Competitive energy costs
- Low maintenance costs
- Strong potential for intellectual property

The effect of acoustic waves in reducing pathogens in milk suggests that high intensity ultrasound can be used as an additional technique to extend the shelf life of pasteurized milk without affecting its flavor. In addition, due to the association between acoustic waves and molecular entities, the effect of HIU on the flavor of UHT milk will be evaluated. Although several papers related to ultrasonic pasteurization have been published, none of them address the effect of this technique on the flavor of milk.

Experimental procedure.

Previous research has shown that ultrasonic pasteurization is effective against a variety of common spoilage and pathogenic microorganisms, including *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Lactobacillus acidophilus, Streptococcus thermophilus*, and *Sacharomyces cerevisiae* (1-5). In this proposal, high intensity ultrasound (HIU) will be used to extend the shelf life of pasteurized milk and to improve the sensory quality of UHT milk. The experimental design will be divided in 3 parts.

Part I. Optimization of the ultrasonic conditions to extend the shelf life of pasteurized milk.

1. HIU will be applied to fleshly pasteurized milk using different settings:

   a. Acoustic power (n = 2): 50 and 100 W/cm^2
   b. Frequency (n = 2): 20 kHz and 500 kHz
   c. Signal duration (n = 2): 5 and 15 min
   d. With and without temperature control: (n = 2): T = 25 °C. One set of sample will be sonicated a room temperature (25 °C) without any temperature control. An increase (up to 50 °C) in the sample temperature is expected as a consequence of ultrasound application. A water bath will be used to control the temperature in the other set of samples. Temperature will be set at 25 °C.

2. The shelf life of the pasteurized milk after HIU treatment will be evaluated as a function of time. Total aerobic microbial count after sonication and during storage at 4 °C will be performed. Samples for microbial count will be taken weekly until microbial spoilage is detected. Non-sonicated pasteurized milk will be used as a control.

Part II. Evaluation of the flavor quality of thee pasteurized milk with extended shelf life.

Sonicated milk samples that show an extended shelf life will be evaluated in a discrimination sensory tesst for significant overall differences against non-treateed pasteurized milk.

Part III. Improve the sensory quality of UHT mililk using ultrasound.

The same ultrasonic settings used in Part I of thiis proposal will be used to evaluate the possibility of flavoor improvement in UHT milk. HIU will be applied to UHTT milk and the overall sensory quality of the treated milk willl be evaluated against a control (non-treated UHT milk). Iin case of significant differences in the overall flavor profilde of the milks, a descriptive panel will be used to identiffy and rate the different flavors present in the milks. Some oof the attributes evaluated will be cooked, stale and sulfurouus notes.

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RESEARCH PLAN

Objective 1.

To apply HIU to whey protein solutions.

Objective 2.

To evaluate the effect of HIU on the heat stability of whey proteins at different pH values and heat treatments (hot filling, UHT and pasteurization conditions).

Objective 3.

To evaluate the clarity/turbidity of HIU whey proteins treated at different temperatures and pH values.

Objective 4.

To evaluate the effect of HIU on the flavor profile of whey proteins, with emphasis on bitterness and astringency.

Objective 5.

Analyze the data obtained and submit it for publication in a scientific journal.

MATERIALS AND METHODS

Objective 1.

HIU was applied as described in the proposal. The only change is that these first preliminary tests were performed with 6 and 26% solid solution of WPC80. An additional treatment was included which consisted on the application of HIU using the same time/power combination but without controlling the sample temperature.

HIU was applied to fresh whey (6% solid, 0.93% protein) provided by Glanbia. The acoustic power levels and intensities for the HIU conditions were calculated.

HIU was applied to liquid whey samples provided by Glanbia with 22, 26, and 34% protein approximately.

Objective 3.

The turbidity of the samples was analyzed as described in the proposal. A calibration curve was performed with a WPC80 solution at 6% solids to evaluate the reproducibility of the method. After HIU application, WPC80 solutions were freeze dried and the turbidity of the samples were measured as the transmittance of a 1% solid suspension of the product after a 10X dilution.

This method was optimized for the fresh whey. The optimization consisted on measuring transmittance of a 1% solution at 600 nm (instead of 420 nm).

Objective 5.

Turbidity data is being analyzed and prepared for a manuscript submission and possible patent application. Manuscript will be submitted to the Journal of Food Engineering in January 2010.

RESULTS AND DISCUSSION

Objective 1.

WPC80 solutions were prepared at final concentrations of 6 and 26% solids. HIU was applied as described in the materials and methods section. Samples were immediately frozen and freeze dried. These samples were used to perform the experiments in Objective 1. No significant problems were encountered in this step. No visual changes were observed in these solutions during sonication. When sonication was applied without temperature control, the sample temperature increased from 20 – 40°C, approximately. This procedure was repeated for a fresh whey solution of approximately 6% solids (0.75% protein). The same ultrasound treatments were applied to liquid whey solutions of 22, 26, and 34% solids.

Objective 3.

A calibration curve was obtained for the turbidity measurement. Dilutions of the 6% WPC80 were performed to obtain the following concentrations: 0.0638, 0.306, 0.585, 3.19, and 6.38 mg/ml. The solutions were prepared in duplicate, and their transmittance (T%) was measured at 420 nm. A negative exponential relationship (R^2 = 0.9975) was found between the transmittance measured and the solution concentration (Figure 1). The higher the concentration in the solution, the lower the transmittance, since fewer solids are dissolved and less light is transmitted through the spectrophotometer cell, indicating a higher turbidity. This relationship shows that the transmittance of light measured at 420 nm can be used as a measurement of the turbidity of the solution. This is an inversely proportional relationship, where a high transmittance is associated with

a low turbidity in the solution or dispersion. It is important to note here that, in this report, concentrations are expressed in terms of solid % or solid content and NOT in terms of protein content.

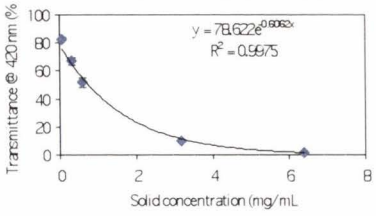

Figure 1. Calibration curve for WPC80 solutions.

After validating the use of transmittance (T%) as a tool to measure turbidity in a solution, the T% of the sonicated WPC80 solutions was measured. Controls (WPC80 solutions without sonication) were also used. Each sonication treatment was performed once and, for each treatment, T% was measured in triplicate. Results are shown in Tables 1-6.

From Tables 1-6, we can observe that HIU application time (5 or 15 minutes) does not significantly affect the T% measurements. However, in some of the WPC80 solutions when higher power settings were used, an increase ($p < 0.05$) in the T% of the sample was observed, indicating a lower turbidity. This is the case of 6% WPC80 sonicated at 20°C using power 3 and 15W for 15 minutes (Table 1), 26% WPC80 solution sonicated at 60°C for 15 minutes using 3W of power, and for the 6% WPC80 solution sonicated without any temperature control. In this case, the temperature in the sample increased from 20°C to approximately 40°C during sonication. Changes in these samples were the most evident ones, showing an increase in the T% from approximately 41% in the control to 55% in the sample sonicated for 15 minutes using 15W of power.

To evaluate the effect of pH values on the T% measurements, samples were prepared as described before and the pH adjusted to 3.5, 4.5, and 6.8. The T% of these samples was measured, and the results are presented in Tables 7-12, 13-18, and 19-24, respectively.

For the T% measured at pH 3.5 (Tables 7-13), a significant difference ($\alpha = 0.05$) between the application time and ultrasound power is observed, especially for higher powers (15 W and longer times 15 minutes) for the 6 and 26% WPC80 solution sonicated at 20°C. Interestingly, for these same solutions but sonicated at 60°C the T% trend was to decrease with acoustic power (the higher the power used, the lower the T% observed). A significant increase ($\alpha = 0.05$) in the T% with acoustic power was also observed in the WPC80 samples that were sonicated without temperature control.

Tables 13-18 show the T% of the sonicated samples when these were measured at pH = 4.5. As expected, the T% values were lower than the ones measured at neutral pH and at pH = 3.5. This is due to the lower solubility of the whey proteins and pH = 4.5 (pH close to the isoelectric point). Also, no differences were found between the different acoustic conditions. The only exception was the 26% WPC80 solution sonicated without temperature control, where a slight increase in T% was observed at high acoustic powers.

Tables 19-24 shows the same results but for solutions at pH = 6.8. It can be seen that these are very similar to the values observed in Tables 1-6, with no differences between application times and differences ($\alpha = 0.05$) with acoustic power.

The fresh whey solution containing 6% of solids (0.75% of protein) was sonicated as described before, freeze dried, and then re-suspended to form a 1% solid solution. The transmittance of these solutions was measured at 420 nm. Transmittance results were significantly lower than expected (in the order of 13%) due to the presence of a yellowish color that absorbs energy at 420 nm. To avoid the interference of the yellowish color, a new calibration curve at 600 nm (Figure 2), where this substance does not have an absorption spectrum, was performed and the turbidity measurements were done at this wavelength.

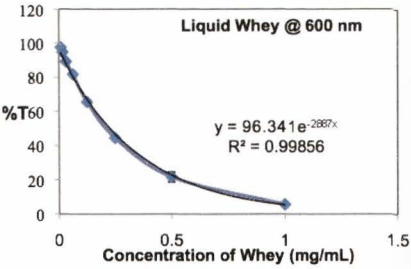

Figure 2. Transmittance (T%) calibration curve at 600 nm for liquid whey.

The T% of the 6% solids liquid whey was measured before and after sonication. The T% was measured using water as a solvent and using different buffer solutions (pH = 3.5, 4.5, and 6.8). Results obtained are shown in the tables below. Table 25-27 shows the T% values of the 6% solid fresh whey after being sonicated, freeze dried, and re-suspended in water. No significant differences in the T% were observed in the T% values of these samples.

Tables 28-30 show the T% of the same solutions but

when they were re-suspended in a pH3.5 buffer. The T% values reported in these tables were lower than the ones obbserved when the samples were re-suspended in water (TTables 25-27). This is an expected result since a lower ssolubility of the protein is expected at lower pHs. No sisignificant differences were observed in the T% values foor the samples sonicated at 20°C (Table 28). However, ssonication time was a significant factor for the samples ssonicated at 60°C (Table 29), resulting in a higher T% (and thherefore increased solubility) of samples that have been ssonicated for longer periods of time (15 minutes). When ssamples were sonicated without temperature control, power leevel was a significant factor (Table 30). Surprisingly, TT% values for the sonicated samples were lower that the ccontrol ones.

Tables 31-33 show the T% values of the sonicated wwhey (and controls) after they have been freeze dried and ree-suspended in a buffer solution of pH=4.5. No significant dilifferences in the T% values were observed when the ssamples were sonicated at 20 and 60°C under the HIU cconditions mentioned above (time and power) (Table 31 annd 32). Significant differences were observed, however, wwhen HIU is applied with no temperature control (Table 33). SSimilar to the results obtained for pH 3.5, lower values of TT% was observed for 3W of power for the samples sonicated dduring 5 minutes.

Tables 34-36 show the T% values of samples re-suspended inn a 6.8 buffer. T% values are higher than the ones observed at 3.5 and at 4.5 due to the higher solubility of whey proteins at thhese pHs. No significant differences in the T% values obtained foor the different HIU conditions for the samples sonicated at 200°C and the samples sonicated without temperature control wwere observed (Tables 34 and 36). Significant differences in thhe T% for the samples sonicated at 60°C at different power leevels were observed (Table 35).

Liquid whey was also obtained at different strategic pooints during whey processing. Samples taken out of the pprocessing line were: 22, 26, and 34% solids. These samples ccontain approximately 15, 34, and 80% protein on a dry bbasis, respectively. These samples were sonicated using the cconditions mentioned before. After sonication, they were frreeze dried, re-suspended in a 1% solid solution, and the ttransmission at 600 nm was then measured.

Tables 37-39 show the transmission of the 22% solution wwhen samples were sonicated at 20°C, with no temperature ccontrol and at 60°C, respectively. From these tables, we ccan observe that ultrasound application increased the ttransmittance of the samples, suggesting a less turbid system. NNo significant differences were found between application tiime or application power (3 vs. 15W) for samples sonicated att 20°C (Table 37), while significant differences were obbserved between these power levels for the samples ssonicated without temperature control (Table 38). In this ccase, higher power levels and longer times (especially for thhe higher power) result in an increase in the transmittance. TThe same behavior was observed for sample sonicated at 60°C.

Tables 40-42 show the transmittance (T%) values for the 26% solid liquid whey obtained from the processing line. It is important to notice here that the T% of the control samples are significantly lower than the ones obtained for the 6 and 22% samples. This is due to the higher protein content of these samples. An exponential decay on the T% values is expected as protein concentration increases (Figure 2). Although the T% of the controls were low, as shown in the following tables, the T% increases significantly as a function of ultrasound power and time. This increase is more evident in the solutions sonicated without temperature control where at 55 and 89% increase in T% is observed.

Tables 43-45 show the data for the 34% solid solution. T% values are similar to the ones observed for the 26% samples; however, ultrasound did not affect the T% of the samples. In fact, when the samples were sonicated at 60°C there was a significant decrease in the T%, suggesting that proteins are being denatured and aggregated.

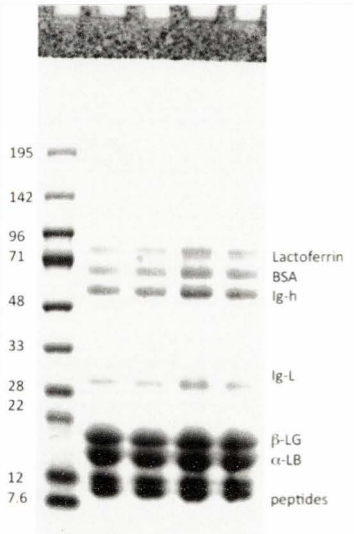

Figure 3. SDS-PAGE analysis of 26 and 34% whey solids. From left to right, lane 1 is molecular weight markers, lanes 2 and 3 are 26% solids: control, and sonicated for 15 min using 15W of power, respectively; and lanes 4 and 5 are 34% solids: control, and sonicated for 15 min using 15W of power, respectively.

SDS-PAGE analysis was conducted on the samples that showed drastic differences in turbidity (26 and 34% solids).

The 34% solids sample showed an increase in turbidity at 60°C and 3 and 15 W, while the 26% sample showed more clarity with these treatments. SDS-PAGE analysis was conducted to determine if the sonication treatments resulted in hydrolysis of the whey proteins, leading to more clarity in the 26% solids. The analysis shows no difference in protein banding patterns therefore we conclude that no hydrolysis was occurring. The changes may be due to the high protein concentration of the 34% sample heated to 60°C, leading to aggregation and an increase in turbidity.

Onset and peak temperatures were not significantly affected by sonication, however, the enthalpy of samples sonicated for 15 minutes at 3 W were significantly lower than the controls and the samples sonicated using higher power levels. In addition, enthalpies for the 26% solution were significantly lower than the ones for the 34% ones. The lower denaturation enthalpy in samples sonicated for 15 minutes using 3W of power suggests that the protein was denatured under this condition. However, comparing these results with the transmission ones, we can assure that a denatured protein does not necessarily result in a less turbid solution (compare transmission values of the 26% sample sonicated for 15 minutes using 3 and 15W of power).

CONCLUSIONS

These results show that the measurement of T% at 420 nm is an appropriate tool to evaluate the turbidity of whey protein solutions. We also proved that differences in protein solubility can be detected by this measurement. The technique was optimized and is ready to be used for the next period of this grant, where HIU will be applied to the samples provided by Glanbia.

We validated the use of transmittance measurements at 600 nm as a method to quantify solubility of whey protein in fresh whey.

T% at 600nm was measured for the control and sonicated liquid whey samples or 22, 26, and 34% solids.

A significant increase in the T% was observed in the 22 and 26% samples, suggesting that less turbid samples are obtained as a consequence of sonication. This increase in transmittance was more significant for the 26% sample.

The best sonication conditions to improve the turbidity of whey samples is when ultrasound is sonicated for 15 minutes using 15W of power and without using temperature control.

Sonication was shown not to produce hydrolyzed protein samples.

Some protein denautration was observed in samples sonicated for 15 minutes using 3W of power as evidenced by differential scanning calorimetry.

NEXT STEPS

During the next period, we will sonicate the samplees provided by Glanbia (6 and 26% solids), and the turbiditity of these solutions will be measured at different pH (3.5.5, 4.5, and 6.8). Sonication will be performed in triplicate. IIn addition, the sonicated solutions will be heated using thhe methodology described in the proposal, and the turbiditity will be measured after heat treatment.

Fresh whey from different sections of the whey prooduction line will be sonicated and evaluated for solubilitity using the 600 nm transmittance method. Once these meaasurements are performed, the best sonication condition wiill be chosen and the stability of the treated whey protein soolution will be evaluated at different pHs and after the heaat treatments proposed in the original plan of work.

The 26% solid sample will be sonicated and otheer functional properties will be measured. Such functionaal properties include: sensory evaluation, heat stability, annd protein solubility at different pHs.

In January 2010, we expect to sonicate liquid wheey samples with approximately 26% solids and perform thhe sensory and heat stability experiments.

The manuscript will be submitted and, if appropriate,, a patent application will be filled by the TCO office at USU.

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Manuscripts and Abstracts

Influence of Time, Temperature and Intensity of High Intesitity Ultrasound on the Turbidity of Whey Proteins, 101th AOCCS Annual Meeting and Expo (poster-accepted).

Martini, S, Potter, R., Walsh, M.K. Optimizing the use of higgh intensity ultrasound to decrease turbidity in whey proteein suspensions. Submitted to the *Journal of Food Engineering* (March 2010)

Table 1. T% values for the 6% WPC80 solutions sonicated at 20 °C

6% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	41.36a ± 3.0	44.83a ± 5.03	46.10a ± 3.72
15	41.36a ± 3.0	48.98b ± 1.91	49.75b ± 3.96

No significant differences between application time (rows)
For the same row, values with same letter are not significantly different

Table 2. T% values for the 6% WPC80 solutions sonicated at 60 °C

6% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	44.86 ± 3.14	41.70 ± 2.21	39.34 ± 4.04
15	37.67 ± 3.41	35.21 ± 4.55	39.44 ± 2.15

No significant differences between application time (rows) or HIU power (columns)

Table 3. T% values for the 26% WPC80 solutions sonicated at 20 °C

26% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	47.85 ± 2.06	42.89 ± 2.44	44.72 ± 1.50
15	47.85 ± 2.06	45.10 ± 2.93	48.57 ± 3.20

No significant differences between application time (rows) or power (columns)

Table 4. T% values for the 26% WPC80 solutions sonicated at 60 °C

26% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	42.58a ± 3.58	44.02a ± 0.99	46.73a ± 2.22
15	38.82a ± 2.44	46.98b ± 0.95	42.38ab ± 3.28

No significant differences between application time (rows)
For the same row, values with same letter are not significantly different

Table 5. T% values for the 6% WPC80 solutions sonicated with no temperature control

6% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	41.36a ± 3.00	49.52b ± 2.42	48.23b ± 0.96
15	41.36a ± 3.00	49.52b ± 1.99	55.07c ± 3.41

No significant differences between application time (rows)
For the same row, values with same letter are not significantly different

Table 6. T% values for the 26% WPC80 solutions sonicated with no temperature control

26% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	47.85 ± 2.06	49.37 ± 2.33	51.31 ± 3.35
15	47.85 ± 2.06	50.49 ± 5.31	53.58 ± 1.87

No significant differences between application time (rows) or power (columns)

Table 7. T% values for the 6% WPC80 solutions sonicated at 20 °C (pH = 3.5)

6% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	24.03 ± 2.79	27.09 ± 3.63	28.07A ± 1.48
15	24.03a ± 2.79	29.85b ± 2.74	36.44cB ± 0.69

Capital letters: differences between rows

Table 8. T% values for the 6% WPC80 solutions sonicated at 60 °C (pH = 3.5)

6% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	29.90aA ± 0.35	24.45b ± 1.62	26.03abA ± 1.30
15	35.35aB ± 3.49	28.58b ± 2.96	32.44abB ± 1.85

Capital letters: differences between rows

Table 9. T% values for the 26% WPC80 solutions sonicated at 20 °C (pH = 3.5)

26% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	25.96a ± 2.25	28.31ab ± 0.79	35.07bA ± 2.42
15	25.96a ± 2.25	30.65a ± 4.60	43.69bB ± 5.32

Capital letters: differences between rows

Table 10. T% values for the 26% WPC80 solutions sonicated at 60 °C (pH = 3.5)

26% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	32.29 ± 1.56	26.07 ± 2.32	27.85 ± 1.11
15	30.72 ± 7.42	28.57 ± 3.72	30.13 ± 1.36

No significant differences between application time (rows) or columns (power)

Table 11. T% values for the 6% WPC80 solutions sonicated with no temperature control (pH = 3.5)

6% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	24.03a ± 2.79	31.29b ± 2.39	34.12b ± 2.14
15	24.03a ± 2.79	36.44b ± 1.38	39.75b ± 3.37

No significant differences between application time (rows)

Table 12. T% values for the 26% WPC80 solutions sonicated with no temperature control (pH = 3.5)

26% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	30.85ab ± 5.45	28.49a ± 0.86	35.70ab ± 1.31
15	30.85a ± 5.45	34.47a ± 1.42	41.89b ± 2.48

No significant differences between application time (rows)

Table 13. T% values for the 6% WPC80 solutions sonicated at 20 °C (pH = 4.5)

6% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	31.21 ± 2.70	26.44 ± 5.38	20.93 ± 2.25
15	31.21 ± 2.70	27.95 ± 8.94	29.92 ± 5.67

No significant differences

Table 14. T% values for the 6% WPC80 solutions sonicated at 60 °C (pH = 4.5)

6% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	30.48 ± 5.52	23.39 ± 6.58	31.12 ± 5.26
15	23.94 ± 2.87	27.71 ± 5.35	29.29 ± 2.30

No significant differences

Table 15. T% values for the 26% WPC80 solutions sonicated at 20 °C (pH = 4.5)

26% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	24.28 ± 5.19	26.69 ± 6.87	23.67 ± 7.11
15	24.28 ± 5.19	24.95 ± 5.87	26.00 ± 9.81

No significant differences

Table 16. T% values for the 26% WPC80 solutions sonicated at 60 °C (pH = 4.5)

26% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	24.30 ± 8.23	21.13 ± 1.72	25.11 ± 4.22
15	23.80 ± 0.38	15.02 ± 2.65	19.23 ± 7.24

No significant differences

Table 17. T% values for the 6% WPC80 solutions sonicated with no temperature control (pH = 4.5)

6% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	31.21 ± 2.70	30.82 ± 4.94	30.45 ± 4.70
15	31.21 ± 2.70	29.04 ± 5.23	29.43 ± 2.97

No significant differences

Table 18. T% values for the 26% WPC80 solutions sonicated with no temperature control (pH = 4.5)

26% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	21.13a ± 6.01	25.31ab ± 2.53	31.58b ± 4.63
15	21.13a ± 6.01	30.55ab ± 3.53	31.01b ± 4.44

Table 19. T% values for the 6% WPC80 solutions sonicated at 20 °C (pH = 6.8)

6% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.75a ± 3.06	55.89bc ± 3.11	54.91 ac ± 4.62
15	48.75a ± 3.06	55.88 ± 1.65	53.18ac ± 2.94

No significant differences between time (rows)

Table 20. T% values for the 6% WPC80 solutions sonicated at 60 °C (pH = 6.8)

6% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	54.00Aa ± 1.64	43.76Abc ± 5.14	46.05Aac ± 2.39
15	40.12Ba ± 4.61	49.02Ab ± 4.75	50.11Ab ± 4.88

Table 21. T% values for the 26% WPC80 solutions sonicated at 20 °C (pH = 6.8)

26% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.51 a ± 2.10	52.22a ± 2.44	50.30a ± 0.68
15	48.51 a ± 2.10	53.80ab ± 6.12	57.27b ± 3.52

No significant differences between time (rows)

Table 22. T% values for the 26% WPC80 solutions sonicated at 60 °C (pH = 6.8)

26% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	45.92a ± 3.50	48.60a ± 3.47	44.50a ± 2.09
15	43.72a ± 2.82	54.14b ± 6.43	43.05a ± 5.52

No significant differences between time (rows)

Table 23. T% values for the 6% WPC80 solutions sonicated with no temperature control (pH = 6.8)

6% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.75a ± 3.06	52.89a ± 3.67	56.52 ± 5.87
15	48.75a ± 3.06	55.37ab ± 3.33	56.70b ± 2.88

No significant differences

Table 24. T% values for the 26% WPC80 solutions sonicated with no temperature control (pH = 6.8)

26% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.51a ± 2.10	53.93b ± 1.11	59.29c ± 4.05
15	48.51a ± 2.10	56.03b ± 1.06	58.05b ± 1.42

Table 25. T% values for the 6% solid fresh whey solutions sonicated at 20 °C

6% solids-20 °C	HIU power		
Application time (min)	*0*	*3*	*15*
5	52.35 ± 4.52	50.21 ± 1.93	53.60 ± 2.77
15	52.35 ± 4.52	55.06 ± 2.05	58.56 ± 4.35

No significant differences between application time and power (α = 0.05)

Table 26. T% values for the 6% solid fresh whey solutions sonicated at 60 °C

6% solids-60 °C	HIU power		
Application time (min)	*0*	*3*	*15*
5	52.35 ± 4.52	49.75 ± 1.45	56.00 ± 1.21
15	52.35 ± 4.52	56.23 ± 2.49	55.02 ± 0.88

No significant differences between application time and power (α = 0.05)

Table 27. T% values for the 6% solid fresh whey solutions sonicated without temperature control

6% solids-no temp control	HIU power		
Application time (min)	*0*	*3*	*15*
5	51.99 ± 2.44	54.24 ± 1.77	58.64 ± 2.44
15	52.70 ± 1.51	55.20 ± 2.82	55.69 ± 13.87

No significant differences between application time and power (α = 0.05)

Table 28: T% values for the 6% solid fresh whey solutions sonicated at 20 °C (pH=3.5)

6% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.26 ± 5.16	35.98 ± 1.70	33.64 ± 1.63
15	48.26 ± 5.16	32.46 ± 2.38	40.42 ± 3.75

No significant differences between application time and power (α = 0.05)

Table 29. T% values for the 6% solid fresh whey solutions sonicated at 60 °C (pH=3.5)

6% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	45.18a ± 0.46	41.16a ± 0.53	43.83a ± 0.65
15	45.96a ± 1.94	46.16b ± 2.70	47.89b ± 1.45

Significant differences between application time (α = 0.05).
For each column, same letter indicates no significant differences

Table 30. T% values for the 6% solid fresh whey solutions sonicated without temperature control(pH=3.5)

6% solids-no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	48.26a ± 5.16	36.38b ± 0.61	35.26b ± 0.41
15	48.26a ± 5.16	33.00b ± 1.13	34.64b ± 3.25

Significant differences between power levels (α = 0.05).
For each row, same letter indicates no significant differences

Table 31. T% values for the 6% solid fresh whey solutions sonicated at 20 °C (pH=4.5)

6% solids-20 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	40.29 ± 2.54	36.84 ± 1.71	34.20 ± 2.66
15	40.29 ± 2.54	37.37 ± 1.35	38.12 ± 1.93

No significant differences between application time and power (α = 0.05)

Table 32. T% values for the 6% solid fresh whey solutions sonicated at 60 °C (pH=4.5)

6% solids-60 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	39.27a ± 0.89	37.43b ± 1.91	39.46a ± 1.79
15	38.94a 1± 1.56	39.02a ± 2.17	39.28a ± 1.83

No significant differences between application time and power (α = 0.05)

Table 33. T% values for the 6% solid fresh whey solutions sonicated without temperature control (pH=4.5)

6% solids-no temp control	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	40.29a ± 2.54	36.05b ± 1.52	37.67a ± 1.79
15	40.29a ± 2.54	38.96a ± 1.16	36.84a ± 1.05

Significant differences between power levels (α = 0.05). For each row, same letter indicates no significant differences

Table 34. T% values for the 6% solid fresh whey solutions sonicated at 20 °C (pH=6.8)

6% solids-20 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	54.70 ± 3.45	56.61 ± 1.20	54.63 ± 1.13
15	54.70 ± 3.45	53.49 ± 3.69	57.98 ± 1.87

No significant differences between application time and power (α = 0.05)

Table 35. T% values for the 6% solid fresh whey solutions sonicated at 60 °C (pH=6.8)

6% solids-60 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	50.33a ± 4.60	55.72ac ± 0.84	61.05bc ± 1.47
15	50.07a ± 1.29	57.30b ± 0.29	57.19b± 4.05

Significant differences between power levels (α = 0.05). For each row, same letter indicates no significant differences

Table 36. T% values for the 6% solid fresh whey solutions sonicated with no temperature control (pH=6.8)

6% solids-no temp control	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	54.70 ± 3.45	56.31 ± 1.65	50.48 ± 1.03
15	54.70 ± 3.45	53.39 ± 0.31	53.56 ± 1.75

No significant differences between application time and power (α = 0.05)

Table 37. T% for the 22% solid fresh whey solutions sonicated at 20 °C

22% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	37.24a ± 0.91	46.09b ± 0.60	46.08b ± 0.65
15	37.24a ± 0.91	46.27b ± 0.31	47.49b ± 0.41

No significant differences in time. Significant differences for power ($p<0.05$)

Table 38. T% for the 22% solid fresh whey solutions sonicated with no temperature control

22% solids- no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	37.24a ± 0.91	45.34b ± 1.16	47.79c ± 1.32
15	37.24a ± 0.91	45.30b ± 0.54	49.82c ± 0.41

No significant differences in time. Significant differences for power ($p<0.05$)

Table 39. T% for the 22% solid fresh whey solutions sonicated at 60 °C

22% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	39.71a ± 1.80	44.83b ± 0.66	45.32b ± 1.03
15	38.98a ± 0.65	43.25b ± 1.17	50.07c ± 0.72

No significant differences in time. Significant differences for power ($p<0.05$)

Table 40. T% for the 26% solid fresh whey solutions sonicated at 20 °C

26% solids-20 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	8.28a ± 0.04	11.20b ± 0.43	12.22c ± 0.25
15	8.28a ± 0.04	11.45b ± 0.31	13.54d ± 0.19

Significant differences between time and power ($p<0.05$)

Table 41. T% for the 26% solid fresh whey solutions sonicated with no temperature control

26% solids- no temp control	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	8.28a ±0.04	10.80b ± 0.17	12.86c ± 0.47
15	8.28a ± 0.04	10.94b ± 0.42	15.63d ± 0.16

Significant differences between time and power ($p<0.05$)

Table 42. T% for the 26% solid fresh whey solutions sonicated at 60 °C

26% solids-60 °C	HIU power (W)		
Application time (min)	*0*	*3*	*15*
5	8.92a ± 1.32	7.77b ± 1.77	14.09d ± 0.71
15	9.23a ± 0.65	10.51c ± 0.30	16.17d ± 0.45

Significant differences between time and power ($p<0.05$)

Table 43. T% for the 34% solid fresh whey solutions sonicated at 20 °C

34% solids-20 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	10.35a ± 0.20	9.83b ± 0.25	9.98b ± 0.15
15	10.35a ± 0.20	10.28a ± 0.06	10.16a ± 0.26

Significant differences between time and power ($p<0.05$)

Table 44. T% for the 34% solid fresh whey solutions sonicated with no temperature control

34% solids- no temp control	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	10.35a±0.20	10.11ac ± 0.07	10.04bc ±0.14
15	10.35a±0.20	10.18a ± 0.16	9.60b ± 0.19

Significant differences between power ($p<0.05$)

Table 45. T% for the 34% solid fresh whey solutions sonicated at 60 °C

34% solids-60 °C	HIU power (W)		
Application time (min)	***0***	***3***	***15***
5	6.93a ± 0.25	6.61a ± 0.39	3.58d ± 0.49
15	4.34b ± 0.39	3.60c ± 0.37	0.07e ± 0.01

Significant differences between time and power ($p<0.05$)

Table 46. Differential Scanning Calorimetry (DSC) denaturation parameters. T_{on}: Onset temperature (°C); T_p: Peak temperature (°C), and ΔH: denaturation enthalpy (J/g). Liquid whey samples C, and D with solid content of 28.2 and 30.2% and protein content of 25.6, and 88.0, respectively.

Samples	T_{on} (°C)	T_p (°C)	ΔH (J/g of protein)
26% control	82.1 ± 0.44 a	85.3 ± 0.23 a	3.64 ± 0.61 a
26% 60 °C 15 min	80.5 ± 1.48 a	84.9 ± 0.17 a	4.56 ± 0.15 a
26% 60 °C 15 min 3W	81.8 ± 0.64 a	84.8 ± 0.34 a	2.16 ± 0.18 b
26% 60 °C 15 min 15W	80.9 ± 0.80 a	85.1 ± 0.19 a	3.34 ± 0.13 a
34% control	69.8 ± 0.09 b	75.0 ± 0.39 b	8.59 ± 0.76 c
34% 60 °C 15 min	69.1 ± 2.29 b	74.9 ± 0.13 b	7.44 ± 0.88 c
34% 60 °C 15 min 3W	71.8 ± 0.11 b	75.4 ± 0.69 b	3.81 ± 0.09 d
34% 60 °C 15 min 15W	72.0 ± 0.52 b	75.0 ± 0.03 b	7.29 ± 0.03 c

For the same column, DSC values with the same letter are not significantly different ($p<0.05$)

Flavor comparison between UHT milk heated by conventional methods and electrical resistive heating

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ABSTACT

Ultra-high temperature processing extends the shelf life of milk up to a year, but also causes several chemical changes to milk components that are reflected in the products' flavor and acceptance. Different heat treatment methods are used to increase milk temperature and their effect on milk quality is unclear. Electrical resistive heating may produce fewer heat-induced changes than indirect heating due to its rapidity and may result in shelf-stable milk that is more preferable than current UHT milks. We compared the flavors of UHT milk from electrical resistive heating to the standard methods of making (i.e., steam injection and indirect heating) for differences in flavor and consumer preference during 9-mo storage at room temperature. Flavor preference, descriptive flavor analysis, and analysis of flavor volatiles were used to determine the attributes responsible for the improved flavor of electrical resistive heated milk. All UHT treatments were performed on the same batch of milk simultaneously. UHT milks were compared to pasteurized milk for flavor preference. We found that pasteurized milk was preferred over UHT milk. Initially electrical resistive heated milk was preferred at 4 wks age but at 16 wks of age the different UHT milks were all equally preferred. Brothy flavor perception increased at 16 wks age. By 36 wks pasteurized or electrically resistive UHT milks were less bitter than steam and plate-heated milks. Headspace analysis showed that dimethyl sulfide is the most abundant volatile sulfur compound in UHT milks. Concentration of volatile sulfurs generally decreased over UHT milk storage time, with the exception of dimethylsulfide increasing in steam UHT-processed milk. All UHT milks had similar carbon disulfide concentrations at 16 and 36 wks of storage. Methanethiol, diimethyldisulfide, and dimethyltrisulfide concentrations were highly variable in UHT milks. Among carbonyls, apart from hexanal, most followed similar trends by milk type. In electrically resistive UHT milk an initial increase in carbonyls from 4 to 16 wks was followed by a decrease to initial or lower levels by 36 wks. Conversely a marginal increase of carbonyls in direct steam and plate UHT milks between 4 and 16 wks was followed by a higher increase (2-4-fold) by 36 wks. Differences in volatile compounds among UHT milks correspond to a better acceptance of electrically resistive UHT milk, suggesting that these differences may be responsible for its acceptance.

BACKGROUND

Liiterature review.

Ultra-high temperature milk.

Pasteurized milk typically has a 14-day shelf life at refrigeration temperatures while ultra-high temperature (UHT) processing allows milk to be stored 1 to 2 years at room temperature (Burton, 1988). However, increased temperature treatments used in UHT processing may cause cooked flavors that are objectionable to many U.S. consumers (Blake et al., 1996). Commonly disliked flavors observed in UHT milk include cooked, heated and stale flavors (associated with heating and room temperature storage) as well as bitter and rancid flavors (caused by residual proteases and lipases). Flavor of UHT milk and changes that occur during storage have recently been reviewed by Al-Attabi et al.(personal communication, 2008). Stale and oxidized flavor in UHT is of concern and it is thought that the predominant flavor chemicals responsible for these off flavors are methyl ketones and saturated aldehydes that develop during storage of UHT milk (Perkins et al., 2005).

Electrical resistive heating.

The operation of passing electric current through a food product causing the food to heat is known as resistive, ohmic or electroheating. Such heating provides very fast heating rates and because the heating occurs throughout the entire through which the electrical current passes, heating occurs volumetrically and the product does not undergo large temperature variations or come in contact with surfaces hotter than the fluid itself (Sastry, 1992). Also because the voltage being applied can be easily and rapidly changed, the product temperature can be very accurately controlled and no residual heating occurs when the current is shut off. Thus, fouling of the system or scorching of the product is minimized and resistive heating systems have the potential for operating for longer time than indirect heat exchangers in which fouling and build up of a biofilm requires frequent cleaning to be performed.

Resistive heating has been applied for some time to thermal processing of a variety of foods (e.g., Skudder, 1989; Qihua et al., 1993). The temperature of liquid at the outlet of a resistive heating system is a controlled by the flow rate, electrical conductivity of the fluid, applied voltage gradient and dimensions of the heating unit (Qihua et al.,1994).

References.

Blake, M. R.; Weimer, B. C.; McMahon, D. J.; Savello, P. A. 1996. Sensory and microbial quality of milk processed for extended shelf life by direct steam injection. J. Food Protection 58: 1007-1013

Burton, H. 1988. Ultra-High Temperature Processing of Milk and Milk Products. Elsevier Applied Science, London.

Perkins, M. L.; Elliott, A. J.; D'Arcy, B. R.; Deeth, H. C. 2005. Stale flavour volatiles in Australian commercial UHT milk during storage. Austl J. Dairy Technol. 60:231–237.

Qihua, T.; Jindal, V. K.; Winden, J. van. 1994. Design and performance evaluation of an ohmic heating unit for liquid foods. Computers and Electronics in Agriculture 9:243-253.

Skudder, P. J. 1989. Ohmic heating in food processing. ASEAN Food Journal 4:162-163

Preliminary findings.

In an earlier study (Irudayaraj et al.), reduced fat milk was UHT processed using electrical resistive heating to 135, 145 and 155°C and compared to a commercial UHT reduced fat milk for and sensory attributes. The milks processed by resistive heating had higher sweet scores and tended to have lower metallic, cardboard, bitter and butter scores than the commercial UHT milk. Overall liking was greatest for the resistive heated UHT milks with a score equivalent to "like slightly" compared to "dislike slightly" for the commercial milk sample. When asked to compare the UHT milks to the milk they usually drink, about 75% rated the commercial UHT milk as "worse than" and only 15% rated it as "equal to" the milk they usually drink. In comparison, the resistive heated UHT milks only received scores for "worse than" of from 35 to 47% depending on the heat treatment, and 39 to 51% rated them as "equal to" the milk they usually drink. The shortcoming of this study was that there was no flavor compound analysis performed that could be correlated with the sensory findings or provide directions on what chemical reactions are causing the off flavors that arise during storage of UHT milk. Furthermore, the control milk was a commercial milk available in the retail market and no information was available regarding how it was processed.

RESEARCH PLAN

Objective 1.

Using the same batch of 2% fat milk (in duplicate), manufacture UHT milk using steam injection, indirect heating, and electrical resistive heating all with a holding time of four seconds at 140°C.

Objective 2.

Store milk at room temperature for 36 weeks, and test samples at 4, 16, and 36 weeks for sensory flavor, flavor chemistry, and enzymatic and physical changes.

Objective 3.

Perform sensory descriptive analysis of UHT milks, and determine changes during shelf life and differences caused by heating method.

Objective 4.

Perform flavor chemical analysis of UHT milks, and determine changes during shelf life and differences caused by heating method.

Objective 5.

Perform sensory preference analysis of UHT milks, and determine consumer preferences based on heating method and storage time after manufacture.

Objective 6.

Statistical analysis and manuscript writing.

Objective 7.

Investigate different heating and time combinations

using electrical resistive heating that may provide further improvements to milk flavor.

Objective 8.

The differences in heating conditions between electrical resistive heating, indirect heating, and steam injection have the potential to produce a shelf stable UHT milk that retains more of the flavor characteristics of pasteurized milk.

MATERIALS AND METHODS

Milk processing.

Milk will be obtained from the Utah State University Caine Dairy Research & Teaching Center and delivered by tanker to the Gary H. Richardson Dairy Products Laboratory. It will be standardized to 2% fat, pasteurized, homogenized, cooled, and packed into 5-gal bags. Two-thirds of the milk will be stored refrigerated at Utah State University and the other portion shipped overnight (cold) to the Raztek Corp., pilot plant facility in Patterson, California.

At Utah State University, Logan, the milk will be UHT processed to 140°C for 4 s in an Alfa-Laval Sterilab UHT system, using both steam injection and indirect plate heat exchangers, and packaged under a HEPA-filtered positive air pressure hood into sterile plastic 4-oz and 8-oz containers. At Raztek Corp., Patterson, the milk will be UHT processed to 140°C for 4 s in an Electroheating system, and packaged using a chlorine-sanitized glove box into sterile plastic 4-oz and 8-oz containers. The milk processed in Patterson, will then be shipped back to Logan. Duplicate trials will be performed.

Storage and sampling.

All samples will be stored at room temperature at Utah State University. Any samples that had lost sterility during packaging and coagulated during storage will be deleted from the study and sent for disposal. At 3, 15 and 35 wk of storage, samples of milk will be sent to Oregon State University for flavor chemistry analysis, tested for enzyme activity at Utah State University, and a portion (approx. 4 L)) of each milk treatment will pooled, tested for microbial quality, and then stored (about 4 d) refrigerated while microbial testing is completed, and then used for sensory analysis.

Aroma identification.

Off-aroma compounds in UHT milk will be identified by gas chromatography-olfactometry-mass spectrometry method. In addition to solvent extraction-SAFE method, Solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) methods will be used to extract highly volatile and less volatile compounds to give a complete spectrum of volatile off-aroma compounds in milk. For SPME method, we will evaluate carboxen/PDMS, Carboxen/DVB/PDMS, and Wax fibers to have extractions of both nonpolar and polar off-flavor compounds in UHT milk. After the identification, we will confirm the off-aroma compounds in UHT milk by aroma recombination in fresh milk.

Flavor chemical analysis.

Volatile aldehyde and ketone analysis.

Hexanal, heptanal, octanal, nonanal, decanal, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and dimethyl sulfide will be analyzed using headspace solid-phase microextraction and gas chromatography with flame ionization detection (HS-GC/FID) as described previously (Vazquez-Landaverde et al. 2005). Twenty g of sample will be extracted with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber at 35°C for 1 h. Volatiles will be analyzed on a gas chromatograph with a HP-5 capillary column. Calibration curves will be constructed in raw milk using the standard addition technique by spiking the standards in the range 0.1 to 150 mg/kg. Five internal standards, trans-2-hexenal, 3-heptanone, 3-octanone, trans-2-nonenal, and 4-decanone, will be used to quantify the volatile compounds.

Volatile sulfur analysis.

Hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide will be analyzed using headspace SPME and gas chromatography with pulsed-flame photometric detection (HS-SPME/GC-PFPD) technique previously developed (Vazquez-Landaverde et al. 2006). Ten g of sample will be extracted with a 1-cm 85 µm carboxen-polydimethylsiloxane (CAR/PDMS) fiber at 30°C for 15 min and analyzed with a Varian CP-3800 gas chromatograph equipped with a DB-FFAP capillary column and a pulsed-flame photometric detector (PFPD). Calibration for the selected sulfur-containing compounds will be constructed in milk. Quantification will be achieved using isopropyl disulfide and ethyl methyl sulfide as the internal standards.

Lactones and other off-flavor compounds.

SBSE method will be used to extract lactones and other off-aroma compounds in milk samples. GC-MS will be used for quantification (Fang and Qian 2006).

Sensory flavor analysis.

Sensory descriptive analysis will be performed at Utah State University by a trained panel at 4, 16 and 36 wk of storage. Consumer preference panels will be conducted at the same time points using an untrained consumer panel. Pasteurized milk (1 wk old) will be used as a reference.

Enzyme analysis.

Milk will be tested for lipase and protease activity and any evidence of age gelation or sedimentation at Utah State University.

References.

Vazquez-Landaverde, P. A.; Velazquez, G.; Torres, J. A.; Qian, M. C. 2005. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. J. Dairy Science 88:764–3772

Vazquez-Landaverde, P. A.; Torres, J. A.; Qian, M. C. 2006. Quantification of trace volatile sulfur compounds in milk by solid-phase microextraction and gas chromatography-pulsed flame photometric detection. J. Dairy Science 89:2919–2927

Vazquez-Landaverde, P. A.; Torres, J. A.; Qian, M. C. 2006. Effect of high-pressure-moderate-temperature processing on the volatile profile of milk. J. Ag. Food Chem. 54:9184–9192

Yu Fang; Qian, M. C. 2006. Quantification of selected aroma-active compounds in Pinot Noir wines from different grape maturities. J. Ag. Food Chem. 54:8567–8573

RESULTS AND DISCUSSION

Objective 1.

Q1 2009.

Preliminary trials for all three heating systems have been conducted to evaluate manufacturing methods and resolve logistical problems.

Q2 2009.

Three experimental reps for all three heating systems (steam injection, indirect heating, and ohmic heating) have been manufactured using the same batch of 2% fat milk.

Q3 2009.

Sensory panels for the three experimental reps for all three heating systems (steam injection, indirect heating, and ohmic heating), that were manufactured using the same batch of 2% fat milk, continue. This was accompanied by chemical analysis to identify the volatile sulfur compounds present in the different milk treatments.

Q4 2009.

Because of problems with the sterility of the direct steam and ohmic treatments, Rep 3 was successfully repeated.

Objective 5.

Q1 2009.

Consumer sensory preference panels were conducted on samples obtained in the preliminary trials. Consumer preference ranking of one week old samples for each heat treatment is shown in Figure 5.1. Panelists were asked to rank the samples based on overall liking. Fifty percent of the panelists ranked the pasteurized milk as the best. The percent of panelists ranking the plate heat exchange, direct steam, and electric resistive heated produces were 17%, 17%, and 14% respectively.

Consumer preference rating for four week old samples processed by electrical restive (ohmic) heating, four week old samples processed by plate heating, and one week old pasteurized control samples is shown in Figure 5.2. The pasteurized product was preferred over the ohmic and plate heated products.

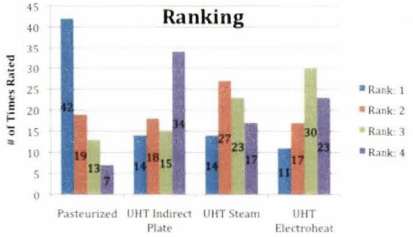

Figure 5.1. Ranking of one-week-old samples for each heat treatment by consumer sensory preference panels.

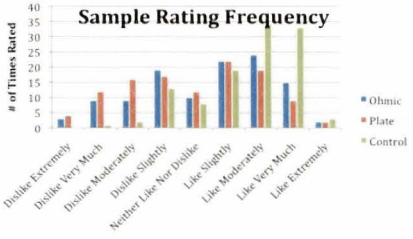

Figure 5.2. Consumer preference rating frequency of milk processed by electric resistive (ohmic) heating at 4 weeks of age, plate heat exchange at 4 weeks of age, and pasterized control at 1 week of age.

Q2 2009.

Consumer sensory preference panels were conducted at one week and four weeks on samples obtained in Rep 1 and at four weeks age for samples from Rep 2. Consumer preference rating of samples from Rep 1 (one week), Rep 1 (four weeks), and Rep 2 (four weeks) for each heat treatment are shown in Figures 5.3, 5.4, and 5.5, respectively.

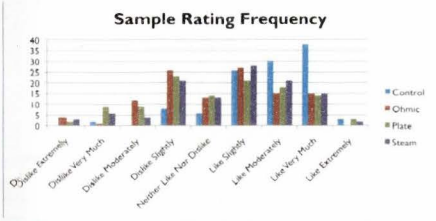

Figure 5.3. Consumer preference rating frequency of Rep 1 milk processed by electric resistive (ohmic) heating, plate heat exchange, indirect steam heating, and pasterized control at 1 week of age.

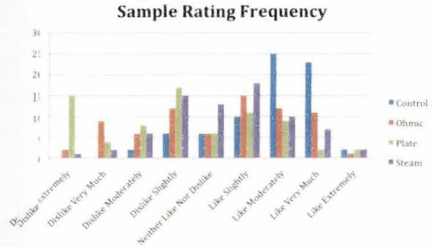

Figure 5.4. Consumer preference rating frequency of Rep 2 milk processed by electric resistive (ohmic) heating, plate heat exchange, indirect steam heating, and pasterized control at 1 week of age.

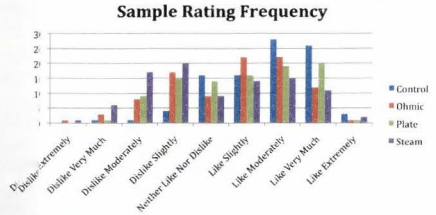

Figure 5.5. Consumer preference rating frequency of Rep 2 milk processed by electric resistive (ohmic) heating, plate heat exchange, indirect steam heating, and pasterized control at 4 weeks of age.

Q3 2009.

At sixteen weeks of storage of UHT milks from Rep 1, pasteurized milk (control) was still preferred over the UHT milks. However, there is no difference in preferred UHT milk by treatment. (Figure 5.6)

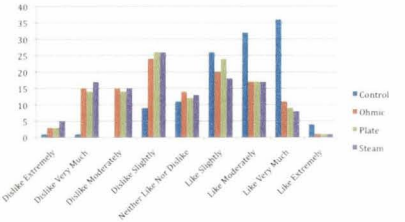

Figure 5.6. Consumer preference rating frequency of Rep 1 milk processed by electric resistive (ohmic) heating, plate heat exchange, indirect steam heating, and pasterized control at 16 weeks of age.

Volatile sulfur compounds present in UHT milks.

The major volatile sulfur compound found in UHT milk was dimethyl sulfide (DMS) (Figure 5.7). Several other volatiles such as methanethiol (MeSH), carbon disulfide (CS_2), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) were also present at lower concentrations than DMS.

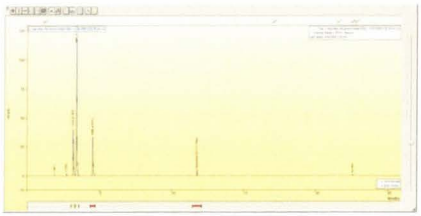

Figure 5.7. Representative GC-PFPD chromatogram of UHT milk.

Milk samples with different UHT treatments had different DMS concentration at the very beginning of the storage (one week; Figure 5.8), with the plate-heated sample having the highest, followed by ohmic heating, and the steam injection heated sample. The study of those compounds is still under progress. A pasteurized milk sample was used as the control, and DMS concentration in pasteurized milk was lower than in the ohmic heated samples.

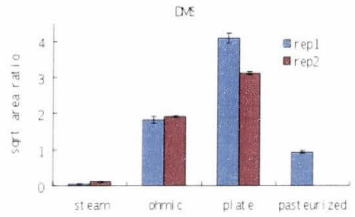

Figure 5.8. Square root area ratio of DMS in 1week milk samples with different treatments.

The concentrations of other sulfur compounds (CS_2, MeSH, DMDS, and DMTS) were also monitored. In general, there were greater variations for the replicates.

The ohmic heating sample had the highest concentration of CS_2, while steam heating and plate heating are similar to the pasteurized control samples (Figure 5.9).

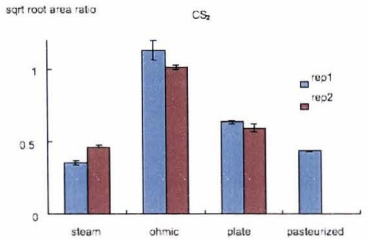

Figure 5.9. Square root area ratio of CS_2 in 1 week milk samples with different treatment.

MeSH was below the detection limit in the control sample, but was present in all three UHT milks at the same levels (Figure 5.10).

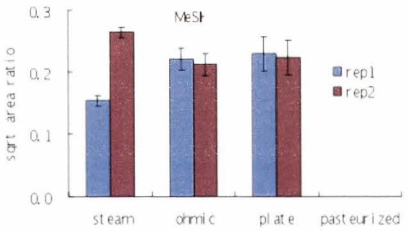

Figure 5.10. Square root ratio of MeSH in 1 week milk samples with different treatments.

DMDS and DMTS showed the same trend in two reps; both of them had a much higher concentration in Rep 2 than in Rep 1 (data not shown). Due to the variation in replicates, the effects of processing treatments are not comparable for these two compounds.

Volatile sulfur change during storage was compared at 1, 4, and 16 weeks and suggested that the two replicates were inconsistent (Figure 5.11). More replicates and/or storage data points are needed to understand the variability in volatile sulfur compounds for UHT milks.

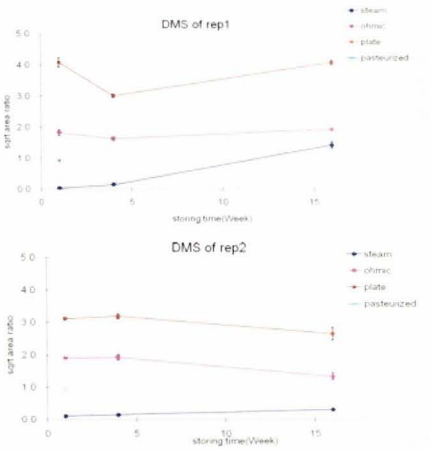

Figure 5.11. Square root ratio of DMS in two different replicates of UHT milk samples.

Q4 2009.

Sensory and chemical analysis of samples for the three reps is being completed on schedule.

CONCLUSIONS

The preliminary consumer preference panels indicate that the pasteurized projects are preferred. At four weeks of age, the ohmic heated milk was preferred over milk treated by plate heat exchange.

Consumer preference panels for both Rep 1 and Rep 2 show that the pasteurized milk samples are preferred at all time points. In Rep 1, the Ohmic is preferred over the plate heated samples at four weeks age, while Rep 2 shows the Ohmic is preferred over the steam heated samples at four weeks of age.

At sixteen weeks of storage, while pasteurized milk was preferred over UHT milk, there was no preference within the different UHT milks due to heat treatment.

Chemical analysis of milks shows that dimethyl sulfide is the most abundant volatile sulfur compound in UHT milks, and plate heated milk had the highest level of this compound at one week of storage. Carbon disulfide, however, was found to be the highest in the ohmic heated milk; while there was no difference in methanethiol levels of UHT milks.

NEXT STEPS

Taste panels and chemical analysis will continue as scheduled.

Influence of starter culture growth on the development of rosey and burnt-brothy flavors during aging of low fat cheese

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Funded by: Dairy Management Inc., January 2009 - October 2010

ABSTACT

Adding sodium gluconate to low fat cheese during salting slows the production of homofuraneol that has been associated with development of a burnt taste. There was no difference in starter culture or nonstarter lactic acid bacteria populations during storage between low fat cheeses made with or without added sodium gluconate. All the low fat cheeses also developed a rosey off-flavor.

BACKGROUND

Liiterature review.

The Low Fat Systematic Project reported that low fat cheeses develop a burnt brothy flavor and not the typical flavor of full fat cheddar cheese. Previous studies, and commercial experience have confirmed a lack of typical flavor development in fat reduced Cheddar cheeses (Drake and Swanson, 1995; Banks, 2004). Milo and Reineccius (1997) compared volatile profiles (no sensory analysis) of a full fat and 60% reduced fat cheese purchased commercially. They noted distinct differences in volatile profiles of the 2 cheeses. More recently, Carunchia Whetstine et al. (2006) evaluated aged full fat cheeses with and without fat removal (after aging) by a novel centrifugation process. They documented that flavor profiles of aged full fat cheese remained similar after fat removal and (as a direct result) texture alteration. Volatile profiles were also similar. These results quantitatively confirmed that lack of typical flavor in fat reduced cheeses was not due to texture and flavor release differences but that the fundamental biochemistry and flavor formation of fat reduced cheeses was altered.

High salt in moisture can inhibit starter growth while lower salt in moisture may allow their proliferation and survival for extended periods. However, it is unknown whether the starter culture or non-starter bacteria play a role in the development of the burnt, brothy flavor in cheese. It could be one or the other, or both. Preliminary work suggests a relationship between high acidity levels (low pH), fast acid producing mesophilic cultures and the burnt brothy note. Use of thermophilic strains such as *Streptococcus* tend not to produce the defect. These observations tend to link the flavor to the starter culture. However, preliminary investigations also link the wash or curd rinse (higher pH), ripening time, and temperature to the development of the flavor defect. This suggests the involvement of the metabolism of non-starter lactobacilli. A common link, regardless of the fat content of the cheese is a lower salt in moisture. A lower salt in moisture alters the microflora and chemistry of the cheese undoubtedly increases the likelihood of a cheese developing the burnt brothy flavor. Typical salt in moisture of low and reduced fat cheeses are below 3.8 % while a full fat cheddar is typically > 4.5 %.

References.

Banks, J.M. 2004. The technology of low fat cheese manufacture. Int J. Dairy Technol. 57:199-207.

Carunchia Whetstine, M.E., Drake, M.A., Nelson, B.K. Barbano, D. 2006. Flavor Profiles of Full Fat, Reduced Fat and Cheese Fat made from Aged Cheddar with the Fat Removed using a Novel Process. J. Dairy Sci. 89:505-517.

Drake, M.A. and B.G. Swanson. 1995. Reduced and Low Fat Cheese Technology: A Review. Trends in Food Science & Technology. 6(11):366 - 370.

Milo, C. and Reineccius, G.A. 1997. Identification and quantification of potent odorants in regular fat and low-fat mild cheddar cheese. J. Agricultural and Food Chemistry 45(9):3590-3594.

Preliminary findings.

In the Low Fat Systematic Project it was observed that there is less die-off of the starter culture and more rapid development of high numbers of nonstarter lactic acid bacteria in low fat cheese than in full fat cheese. This is thought to reflect the differences in salt-in-water content of these cheeses. Volatile analysis confirmed increased relative abundance and higher flavor dilution (FD) factors for furanones in low fat cheeses compared to full fat cheeses in the systematic project (Tables 1, 2) which is consistent with a previous publication on commercial cheeses (Milo and Reineccius, 1997). The aromas of these compounds are burnt/brothy in character and their thresholds are quite low (low ppb range). Further, preliminary studies with model cheeses indicated that addition of these compounds to a bland cheese matrix results in a burnt brothy flavor. Taken as a whole, these compounds (individually or as a group) are a source of imbalance and off flavor in low fat cheeses. . A rosy/floral off-flavor is also evident in the aftertaste of low fat cheese. The source of this off-flavor which can also be a problem in aged full fat cheese has been pinpointed to phenylacetic acid and phenylacetaldehyde (Carunchia Whetstine et al., 2005). Currently, techniques are not optimized for recovery and measurement of these compounds (so results provided below for furanones only are a rough estimate) and the technique applied for extraction (SAFE) is time-consuming and cumbersome (3-4 weeks per sample). An optimal instrumental method (in addition to sensory analysis) for recovery and quantitation of these compound(s) is needed to effectively evaluate efficacy and develop methods to prevent or minimize their formation.

References.

Milo, C. and Reineccius, G.A. 1997. Identification and quantification of potent odorants in regular fat and low-fat mild cheddar cheese. J. Agricultural and Food Chemistry 45(9):3590-3594.

Carunchia-Whetstine, M.E., Cadwallader, K.R. and Drake, M.A. 2005. Characterization of Rosey/Floral Flavors in Cheddar Cheese. J. Ag. Food Chem. 53:3126-3132.

RESEARCH PLAN

Objective 1. (Wisconsin Center for Dairy Research)

Manufacture low fat cheddar cheeses using salt sensitive and salt tolerant starter cultures, and determine bacterial populations, furanone content, and flavor development during 9 months of storage.

Objective 2. (Southeast Dairy Foods Research Center)

Optimize and streamline technique for furanone extraction, identification, and quantitation from cheese, and identify which furanones are the primary contributor(s) to burnt brothy flavor. Optimize extraction and quantitation of phenylacetaldehyde and phenylacetic acid (source of the rosy off-flavor in low fat cheeses).

Objective 3. (California Dairy Products Technology Center)

Manufacture low fat cheddar cheese using rennet with normal and reduced proteolytic activity, and store at normal and low temperatures and then determine extent of proteolysis, bacterial populations, furanone content, and flavor development during 9 months of storage.

Objective 4. (Western Dairy Center)

Manufacture low fat cheddar cheese with and without a bacterial static agent, and determine bacterial populations, furanone content, and flavor development during 9 months of storage.

Objective 5.

Collect cheese and lactic acid bacteria samplings from different low fat cheeses during 9 months of storage, and store at -80°C.

Objective 6.

Manufacture low fat cheese using standardized procedures in California, Utah, and Wisconsin, and compare flavor and textural properties at 4 months of age.

Objective 7.

Write and submit results of research for scientific publication regarding development of burnt brothy flavor in low fat cheese and its correlation with furanone production in low fat cheese, as influenced by activity of the starter and nonstarter bacteria during storage.

MATERIALS AND METHODS

Objective 1.

Manufacturing procedures.

Cheese will be made at the Wisconsin Center for Dairy Research using standard cheese making procedures and equipment.

Cheese composition.

Cheese will be measured for moisture using vacuum oven fat by Mojonnier salt by chloride electrode, protein by Kjeldhl and pH by quinhydrone electrode.

Bacterial population.

Bactrial populations will be determined using selective media and incubation conditions as follows, after making a cheese homogenate by stomaching the cheese sample in a sterile 2% sodium citrate warmed to 45°C (standard method). Make appropriate dilutions and use the spread plate methhod on the following media:

- Lactococci using M17 agar incubated aerobically at 30°C for 18 h.
- Total LAB using MRS with dextrose+sorbitol as the sugars, incubated anaerobically at 37C for 48 h..
- Nonstarter lactobacilli using MRS+vancomycin incubated anaerobically at 37°C for 48 h.
- Streptococci using MRS incubated anaerobically at 45°C for 48 h (When *St. thermophilus* starter used)

Preserving cheese bacteria for DNA testing:

- Place 1000 g of cheese in a whirl-pak bag and seal.
- Label the bag with:
  - Cheese type
  - Cultures used
  - Name of study for which cheese was made
  - Location of manufacture
  - Date of manufacture
  - Date of sampling date
  - Storage temperature
- Place the labeled sample inside a second whirl-pak bag and seal.
- Freeze at -80°C.

Preserving cells recovered during microbiological sampling of the cheese:

- Using the 10^1 dilution of the total LAB media above, and other selected media if desired.
- Add 1 ml of 0.85% saline onto the media surface.
- Scrape the surface with a "hockey stick".
- Transfer 0.2 ml into duplicate cryotubes that contain sterile MRS plus 11% glycerol.
- Label as above plus the media/incubation conditions used
- Store at -80°C.

Cheese flavor.

Cheese will be sent to North Carolina State University for sensory descriptive flavor analysis as performed during the Low Fat Systematic Project, and for volatile compound analysis to determine the levels of furanone compounds that have been identified as being correlated with development of the burnt brothy flavor typical of low fat cheese.

Objective 2.

Solid phase micro extraction (SPME) is a solvent-less headspace extraction technique that has been applied previously applied by our lab and others for extraction and highly sensitive quantitation of selected cheese and dried ingredient volatiles (Frank et al., 2004; Wright et al., 2006; 2008; Drake et al., 2007; 2008; Carunchia Whetstine et al., 2007). This technique, once optimized, lends itself directly to high throughput since multiple extractions and analyses can be completed in 1 day (unlike solvent (SAFE) extractions). Previous studies with other foods (Pinho and Bertrand, 1995; Adahchour et al., 1999; Ferreira et al., 2003) as well as preliminary SPME GC-MS scans in our lab suggest that we can apply this method to extract and quantify furanones and phenylacetic acid and phenylacetaldehyde from cheeses. A central composite design with response surface methodology will be used to optimize extraction of furaneol, sotolone, homofuraneol, phenylacetic acid and phenylacetaldehyde from cheeses. Full fat and low fat cheeses will be evaluated with and without spiking with the three compounds. Limit of detection (LOD) and limit of quantitation (LOQ) will be determined. This technique will then be applied in conjunction with sensory analysis to track efficacy of control of these compounds and burnt brothy flavor development. PI Drake will manage this objective and will also manage sensory and volatile compound analysis of cheeses in the other objectives.

A central composite design with response surface methodology (CCD RSM) will be used to optimize extraction of furaneol, sotolone, and homofuraneol from cheeses by SPME GC MS. Factors that will be evaluated include: fiber type, exposure time, exposure temperature and salt concentration. Full fat and low fat cheeses (young < 1mo and aged 9-12 mo) will be evaluated with and without spiking with the three compounds. GC-MS conditions of maximum sensitivity will be determined. Both an Agilent quadrupole mass selective detector and a Varian ion trap mass spectrometer will be evaluated. A separate CCD RSM will be conducted to optimize extraction of phenylacetic acid and phenylacetaldehde. A separate experiment is required since these two compounds are much less volatile than furanones and longer SPME fiber exposure times will likely be required for optimum recovery. Preliminary testing with SPME in our lab suggests that this objective (high throughput with sensitivity for these 3 compounds) is

achievable. In the unlikely event that it is not, conditions for solvent extraction will be optimized. Chiefly, amount of product, extraction time, and solvent type will be evaluated for maximum extraction of the three compounds from low fat cheeses.

Objective 3.

Low fat cheese will be made at California Polytechnic State University using the starter culture (DVS850). Cheese will be made to the target composition of 54% moisture, 2.0% salt, 6% fat, pH 5.15.

Manufacturing procedures.

Cheese manufacture will follow a make procedure similar to that used in the Systematic Low Fat Cheese Project for low fat cheese. Milk will be pre-acidified to pH 6.30 prior to ripening, and the curd will be cold water washed and salted so as to produce low fat cheddar cheese with moisture content and pH at the above targets. Cheese will be pressed overnight at 8 psi (as applied to cheese hoop) and vacuum packaged.

Ten 1-lb blocks will be stored at each temperature for pH and bacterial population testing. Six 2-lb blocks will be stored and used for furanone and flavor testing.

Cheese composition.

Cheese will be measured for moisture using vacuum oven, fat by Babcock, salt by chloride analysis, protein by N, and pH by glass electrode.

Bacterial population.

Bacterial populations will be determined using selective media and incubation conditions as follows:

- lactococci using M17 agar incubated aerobically at 30°C for 18 h.
- total lactic acid bacteria using MRS with dextrose+sorbitol as the sugars, incubated anaerobically at 37C for 48 h.
- nonstarter lactobacilli using MRS+vancomycin incubated anaerobically at 37°C for 48 h.

Preserve cheese samples and cells as described in Objective 1.

Cheese flavor.

Two 2-lb blocks of cheese will be sent to North Carolina State University for sensory descriptive flavor analysis as performed during the Low Fat Systematic Project, and for volatile compound analysis to determine the levels of furanone compounds that have been identified as being correlated with development of the burnt brothy flavor typical of low fat cheese.

Objective 4.

Duplicate vats of low fat cheese will be made at Utah State University using the same starter culture (DVS850) as in Objective 3 but with a modified make procedure to allow addition of sodium gluconate to part of the cheese curd.

Manufacturing procedures.

Cheese manufacture will follow a make procedure similar to that used in Objective 3 except that the cheese curd will be held for longer after draining to allow the pH to drop to pH 5.35. The curd will then be divided into portions (28 lb) and salted or salt mixed with sodium gluconate so that the amount of sodium gluconate added is 0.8, 1.6, or 2.4%. The lower salting pH is necessary as the gluconate will inhibit further fermentation by the starter culture. The cheeses will also have a slightly lower moisture content than in Objective 3 because of the longer time the curd is stirred before salting. Target cheese composition is 52% moisture, 2.0% salt, and pH 5.25.

Cheese composition.

As in Objective 3.

Bacterial population.

Bacterial populations will be determined using selective media and incubation conditions as follows:

- lactococci using M17 agar incubated aerobically at 30°C for 18 h.
- total lactic acid bacteria using MRS with dextrose+sorbitol as the sugars, incubated anaerobically at 37C for 48 h.
- nonstarter lactobacilli using MRS+vancomycin incubated anaerobically at 37°C for 48 h.

Further information about the microbial populations will be obtained by picking 10 colonies from some of the agar plates, isolating them as pure cultures, and using API typing to characterize them.

Also, preserve cheese samples and cells as described in Objective 1.

Cheese flavor.

As in Objective 3.

RESULTS AND DISCUSSION

Objectives 1.

See Wisconsin Center for Dairy Research report.

Objectives 2.

See Southest Dairy Foods Research Center report.

Objectives 3.

See California Dairy Products Technology Center report.

Objectives 4. (Western Dairy Center)

Microbial populations.

No differences were observed in microbial populations as a functionof sodium gluconate addition (see Figures 1-3).

Sensorry and flavor information was provided by North Carolina SState University. (Tables 2-4.) As the level of sodium gluuconate addition increased, the salty taste of the cheese slightly increased. At 4 months of age, there was no significant difference in any of the other attributes tested.

At 6 months, the control cheese was very slightly more bitter (score = 1.0) than the cheeses with 1.6% and 2.4% sodium gluconate added (score = 0.5) and still slightly more salty.

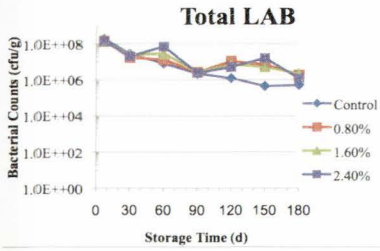

Figure 1. Total numbers of lactic acid bacteria measured in low fat cheese made with 0%, 0.8%, 16% or 2.4% addition of sodium gluconate during salting.

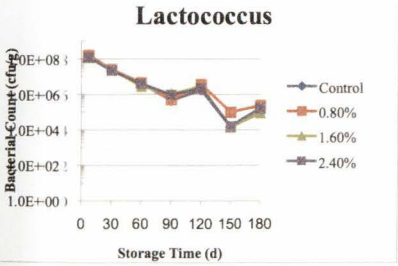

Figure 2. Nuumber of starter culture lactococci measured in low fat cheese madde with 0%, 0.8%, 16% or 2.4% addition of sodium gluconate during salting.

Objective 5.

Samples of cheese, and plate scrapings, were being collected and stored frozen.

Objective 6.

Two vats of low fat cheese were made using the current WDC low fat cheese make procedure, using two levels of sodium gluconate, and the cheese with 0.8% sodium gluconate was selected for submission of 30 lbs of cheese for the DMI consumer testing in November.

NEXT STEPS

Continue storage of cheese and sending samples out for analysis for bacterial populations monthly, furanone content and sensory flavor analysis for a 9 month time point in January. Complete the analysis of sensory and chemical data at the Southeast Dairy Foods Research Center, and then commence writing a manuscript for publication.

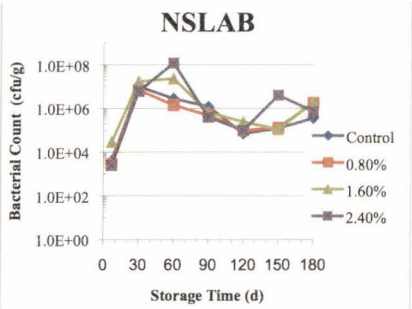

Figure 3. Numbers of nonstarter lactic acid bacteria (NSLAB) measured in low fat cheese made with 0%, 0.8%, 16% or 2.4% addition of sodium gluconate during salting.

Table 1. Mineral analysis of cheeses.

Sodium Gluconate Added (%)	Moisture (%)	Salt (%)	Calcium (%)	Sodium (%)
Replicate 1				
0	53.4	1.70	0.835	0.66
0.8	53.2	1.82	0.805	0.66
1.6	53.6	1.82	0.810	0.72
2.4	54.0	1.86	0.765	0.78
Replicate 2				
0	54.1	1.72	0.900	0.695
0.8	52.3	2.18	0.965	0.900
1.6	52.9	1.98	0.940	0.890
2.4	53.8	1.90	0.915	0.975

Table 2. Sensory means at four months.

trt	control	0.80%	1.6%	2.4%
Cooked/milky	3.0a	3.0a	2.9a	2.8a
whey	2.7a	2.7a	2.7a	2.8a
milkfat	0.8a	0.9a	0.9a	1.0a
sulfur	0.8a	0.9a	1.1a	1.0a
brothy	1.8a	2.0a	2.0a	2.0a
sour	2.6a	2.8a	2.7a	2.6a
bitter	ND	ND	ND	ND
salty	3.2b	3.4ab	3.4ab	3.5a
sweet	1.7a	1.8a	1.7a	1.9a
umami	2.0a	2.1a	2.0a	2.1a
comments	Dirty, burnt rosy, meaty	Dirty, burnt, rosy, meaty	Dirty, burnt, meaty, rosy	Dirty, burnt meaty, rosy

[abc]Means within rows with the same letter were not significantly different, α=0.05

Table 3. Sensory means at six months.

trt	control	0.80%	1.6%	2.4%
Cooked/milky	2.7a	2.9a	2.8a	2.9a
whey	2.8a	2.7a	3.0a	2.8a
milkfat	0.5a	0.5a	0.7a	0.8a
sulfur	1.3a	1.3a	1.4a	1.5a
brothy	2.7a	2.5a	2.7a	2.7a
sour	2.9a	2.9a	2.9a	3.0a
bitter	1.0a	0.8a	0.5b	0.5b
salty	3.3b	3.4ab	3.6a	3.6a
sweet	1.9a	2.0a	2.2a	2.1a
umami	2.5a	2.5a	2.6a	2.5a
comments	Rosy, burnt	Rosy, burnt	Rosy, burnt	Rosy, burnt

[abc]Means within rows with the same letter were not significantly different, α=0.05

Table 4. Mean concentration (ug/kg relative to internal standard) of various important flavor compounds in low fat cheese made without (control) or with 0.8%, 1.6%, or 2.4% sodium gluconate, after 6 months of storage.

trt	control	0.80%	1.6%	2.4%
phenyl ethanal	62.80a	54.87a	41.15a	47.61a
homofuraneol	6.27a	4.29a	3.51b	3.64b
phenyl ethanol	0.59a	0.75a	0.56a	0.95a
sotolone	23.37a	17.27a	16.07a	21.73a
furaneol	3.70a	2.60a	3.43a	3.81a
phenyl acetic acid	11.77a	9.26a	3.73b	6.92b

Influence of salt-in-water content on flavor of full fat and low fat cheddar cheese

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Funded by: Dairy Management Inc., December 2008 - July 2010

ABSTACT

Full fat and low fat cheeses were manufactured at Utah State University with high and low salt-in-water levels. Microbial populations of total lactic acid bacteria, lactococci and lactobacilli were measured at Weber State University. Cheese samples were send to North Carolina State University for descriptive sensory analysis.

BACKGROUND

It is thought that the lower salt-in-water content of low fat cheese is the cause of insufficient control over bacterial growth during storage that then results in development of atypical cheddar cheese flavors, such as burnt brothy flavor. A quick test of whether this is correct is to make full fat and low fat cheeses that have the same salt-in-water content and to compare their microflora and flavor as the cheese ages. Full fat and low fat cheeses will be made in 1500-lb vats at two salt levels: 4.8% salt-in-water and 3.8% salt-in-water. Cheeses will be stored at 6°C and sampled each month for starter and nonstarter lactic acid bacteria. Descriptive flavor analysis will be performed at 2, 5, and 8 months. The experiment will be performed in triplicate.

RESEARCH PLAN

Objective 1.

Manufaacture full fat and low fat cheddar cheeses at both high and low salt levels of 4.75% and 3.50% salt-in-water content in duplicate.

Objective 2.

Store cheeses at 42°F for 8 months.

Objective 3.

Measure die-off of starter lactococci and growth of nonstarter lactic acid bacteria during storage.

Objective 4.

Determine differences in cheese flavor during storage, especially development of burnt brothy flavor.

Objective 5.

Determine if development of burnt brothy flavor in low fat cheese is a function of low salt-in-water content.

Objective 6.

Write and submit results of research for scientific publication.

MATERIALS AND METHODS

Both full fat and low fat cheeses will be made using 1500-lb milk in Scherping enclosed cheese vats with pumping to a drain table for whey drainage. Stirred curd make procedures and the same starter culture will be used. Curd from each make will be divided into two prior to salting with one half receiving the high salt level and the other the low salt level. The curd will then be filled into 20-lb rectangular hoops and pressed overnight. Cheese will be made in duplicate.

- Full Fat:
  - High salt (4.75% s/w): 37.0% moisture, 52% FDB, 1.76% salt, pH 5.15
  - Low salt (3.50% s/w): 37.0% moisture, 52% FDB, 1.30% salt, pH 5.15

Cheese manufacture will follow a make procedure similar to that used in the Systematic Low Fat Cheese Project for full fat cheese using DVS850 starter culture, and designed to produce cheddar cheese with moisture content and pH at the above targets. Cheese curd will be salted at 0.23 lb or 0.16 lb salt per 10 lb curd for high and low salt respectively. Cheese will be pressed overnight at 15 psi.

- Low Fat:
  - High salt (4.75% s/w): 54.0% moisture, 6% fat, 2.55% salt, pH 5.15
  - Low salt (3.50% s/w): 54.0% moisture, 6% fat, 1.90% salt, pH 5.15

Cheese manufacture will follow a make procedure similar to that used in the Systematic Low Fat Cheese Project for low fat cheese using DVS850 starter culture. Milk will be pre-acidified to pH 6.30 prior to ripening, and the curd will be cold water washed designed to produce low fat cheddar cheese with moisture content and pH at the above targets. Cheese curd will be salted at 0.37 lb or 0.27 lb salt per 10 lb curd for high and low salt respectively. Cheese will be pressed overnight at 8 psi.

- Cheese will be sampled at 1 wk and composition determined. Cheese will be cut into 2-lb blocks and stored for 9 months at 42 F.

Cheese will be measured for moisture using vacuum oven, fat by babcock, salt by chloride analysis, protein by combustion N, calcium and other minerals by ICP, and pH by glass electrode.

Bacterial population.

Cheese will be sampled each month (with wk 1 being the initial sample) for bacterial population, pH and water activity.

Cheese will be measured for *Lactococcus* using Elliker agar with aerobic incubation at 30 C for 18 hours, total lactic acid bacteria using MRS dextrose/sorbitol agar with anaerobic incubation at 37 C for 48 h, and *Lactobacillus casei/paracasei* using MRS-Vanomycin agar with anaerobic incubation at 37 C for 48 h.

Cheese flavor.

Cheese will be tested for flavor development at 2, 5 and 8 mo.

Cheese will be sent to North Carolina State University for sensory descriptive flavor analysis as performed during the Low Fat Systematic Project for development of cheese flavor, especially for development of the burnt brothy flavor typical of low fat cheese.

RESULTS AND DISCUSSION

Objectives 1 and 2.

A number of vats of cheese were made, as shown in Table 1, and one more vat of full fat cheese needs to be made to complete Rep C. Slight changes to the manufacturing procedure had to be made to compensate for the effect of salt on whey expulsion and starter cultures when splitting the curd into two portions from the same cheese vat. Cheeses are being sent to Weber State University for microbial analysis and to North Carolina State University for descriptive flavor analysis.

Objective 3.

At normal salt-in-moisture levels in cheese the typical die-off of the starter culture was observed and a gradual increase in nonstarter lactic acid bacteria until the nonstarter became the dominant bacteria. At higher than normal salt-in-moisture levels here was also a suppression of the nonstarter lactobacilli. At lower salt-in-moisture levels (those typical of low fat cheese) there was virtually no suppression of the lactococci and they remained at a high level.

Objective 4.

Most of the sensory flavor analysis has been performed at North Carolina State University.

CONCLUSIONS

Cheeses were made that had the required differential in salt content although there were some variations in salt, moisture content and pH of the cheese. Cheesemaking and storage has been completed, as well as microbial and sensory flavor analysis.

NEXT STEPS

Q4 2009.

Complete the sensory flavor and microbial analysis and perform statistical analysis of data.

Table 1. Cheese composition.

Rep	Cheeese Code	Date Made	Moisture %	Salt %	Fat %	pH	%Salt in Moisture S/(S+M)
A	HHF-LS	1/29/09	38.19	1.48	32.5	5.34	3.73%
A	HHF-HS	1/29/09	36.3	2.08	33	5.57	5.42%
A	LLF-LS	3/5/09	52.56	1.98	7.5	4.95	3.63%
A	LLF-HS	3/5/09	52.47	3.1	7	5.36	5.58%
B	HHF-LS	3/4/09	38.4	1.58	32	5.06	3.95%
B	HHF-HS	3/4/09	36.92	2.52	33	5.26	6.39%
B	LLF-LS	4/1/09	52.85	1.64	6.5	5.19	3.01%
B	LLF-HS	4/1/09	51.83	2.34	6	5.27	4.32%
C	HHF-LS	7/9/09	37.5	1.36	32	5.01	3.50%
C	HHF-HS	7/9/09	35.82	2.08	32	5.09	5.49%
C	LLF-LS	4/8/09	55.1	2	5.5	5.17	3.50%
C	LLF-HS	4/8/09	52.2	2.44	5.5	5.14	4.47%

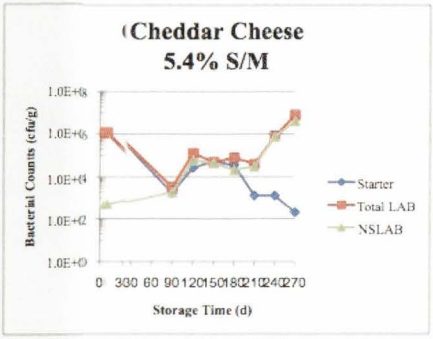

Figure 1. Changes in microbial populations in cheese with a normal salt-in-moisture content (i.e., 5.4%).

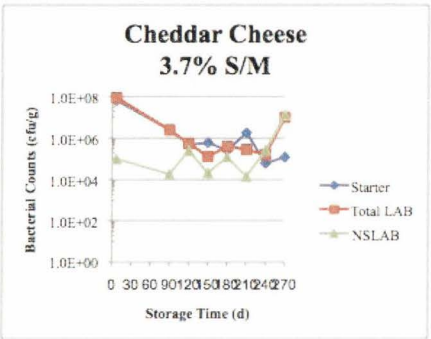

Figure 3. Changes in microbial populations in cheese with a lower than normal salt-in-moisture content (i.e., 3.7%).

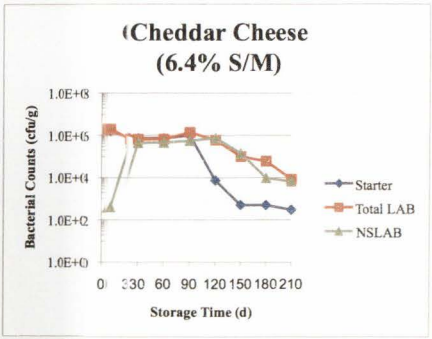

Figure 2. Changes in microbial populations in cheese with a higher than normal salt-in-moisture content (i.e., 6.4%).

Designing filler particles to imitate fat in cheddar cheese

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ABSTACTT

The gooal of this project is to systematically evaluate the application of filler particles for replacement of fat cheddar cheese. Baased on our prior research on cheeses at different fat levels (3 to 33%), cheese firmness increases as fat decreases due to theree being more protein gel network per unit volume. The difference in chewdown properties, such as adhesiveness, smoothnesss and cohesiveness, appears to be related to different fracture patterns in the cheese network due to the absence of fat. This leeads us to hypothesize that the two critical factors required for a successful filler particle will be appropriate phase volume andd a similar effect on the fracture pattern. This project will have two phases. In the first, we will study the effects of different t types and amounts of interacting and non-interacting filler particles on rheological and microstructure of cheese. The goal bbeing to screen treatments making 1 kg batches of cheese. Dr. Vardhanabhuti will be responsible for making (obtaining)) the particles and all rheological and microstructural analysis, while Dr. McMahon will make the cheese. At the end of the fifirst phase, we should be able to select various filler particles that produce the textural properties of full fat cheese. Selected fililler particles will be extensively investigated in the second phase which will include the sensory analysis. At the end of the p project, we should be able to identify the critical properties of filler particles that allow them to function like fat.

BACKGROUND

Introductition.

The DMMI project entitled *"Investigating the filled gel model for tlthe role of fat in cheese"* has been completed and produced ssome unique insight. In order to determine the role of fat i in cheddar cheese texture, a set of cheeses were developed t that ranged in fat content from 3 to 33%. The texture of tlthese cheeses was measured over an aging time of 12 weekk to determine the combined effects of fat and the early evvents in the aging process. The results from the first replicaation will be discussed as a foundation for this proposed pproject.

The firsrst data point at two weeks showed that cheeses with 3 or 8%% fat were much firmer than the other treatments. However, bby 4 weeks, the cheeses were starting to separate based on faat content. This is showing that extremely low fat cheeses s (3 and 8% fat) have different structures from the start, aand that these structure do not change much with aging.:. In contrast, cheeses with ≥ 13% fat undergo structural rearrangement in the first 4 weeks. This is most logically due to a redistribution of water between trapped pockets of whey and the casein gel network to produce a semi-homogeneous casein gel network.

As aging time progressed, differences between almost every fat level appeared such that at 12 weeks there was a general step-wise increase in firmness as the fat content was decreased. An increase in firmness has historically been seen as a problem with low fat cheese texture (Gwartney et al., 2002), and this could be due to changes in the concentration of protein in the gel network or the amount of gel network. The water:protein ratio for the cheeses varied from 1.41 to 1.66 w/w, suggesting that there was minimal difference in the protein concentration of the gel network. ***This would indicate that the texture differences are due to the amount of gel network rather than the inherent strength of the gel network***; and this will be further justified in the discussion of fracture properties.

Firmness is a sensory *first bite term* that is evaluated during the first chew. While it is important to the overall impression of texture, the texture perceived during

chewing, called *chewdown terms*, have been shown to be a major factor differentiating low fat from normal cheeses (DMI Annual Report, Low Fat Natural Cheese Strategic Platform, 2008).

As was seen with sensory firmness, the cheeses started at a similar adhesiveness then aged into structures that showed major differences due to fat level. In this case, a high level of adhesiveness is desired to prevent a "rubber ball-like" texture seen in low fat cheeses where elastic, non-interacting particles are created during chewing. By 12 weeks, the treatments with ≥ 23% fat had a desired level of adhesiveness while the rest remain much lower.

Increasing fat content changed not only the amount of fat observed in the microstructure, but also increased size and altered the shape of fat globules. ***This clearly showed that the choice of particle size used to imitate fat is more complex than just adding particles in the range of milk fat globules (0.1 – 10 μm; Walstra et al, 1999).*** The deviation of a particle from a spherical geometry can be measured by determining the shape factor. A sphere would have a shape factor of 1 and values lower than one indicates the degree of non-spherical shape. There was a general trend of the fat particles becoming more non-spherical as the fat content increased. The *mean fat globule area* is an indication of size and, as observed in Figure 3, area increased with the amount of fat.

One goal of this project was to see if the filled gel model could be used to predict how fat would function in cheddar cheese. This model states that the elastic modulus (G') of a gel filled with particles will increase or decrease due to the relative moduli of the gel network and filler particle (G'_{filler}/ $G'_{gel\ network}$), the amount of filler particles and if the particles interact (active filler) or do not interact (inactive filler) with the gel network (Tolstoguzov and Braudo 1983; van Vliet 1988). Previous research with Gouda cheese has shown that the filled gel model can be used to explain various rheological transitions, especially those associated with fat and temperature changes (Visser, 1991).

The compositions of the cheeses were adjusted such that as fat content was increased (3 to 33%) there was a decrease in protein content (35 to 24%), while the water:protein ratio was targeted to be held constant. This approach allowed for the cheese to be viewed as a filled gel with filler particles (fat) dispersed in a protein network of constant concentration (i.e., constant water:protein ratio). The actual compositions of the cheeses were very close to the target values. While the water:protein ratio varied from 1.42 to 1.72, it did not show a systematic variation and the three cheeses with the highest amount of fat had the same water:protein ratio.

At 10°C, the filler particles are expected to be more solid than liquid and $G'_{filler} > G'_{gel\ network}$. There was an increase in G'_{cheese} as filler particle (fat) percentage increased, and gel network concentration (protein) decreased. This trend shows a reinforcing of the network by the filler particle and suggests that fat is acting as an active filler at 10°C. Rheological properties were much different when the temperature was increased to 25°C; the temperature of cheeses during sensory evaluation. At 25°C, the fat is more liquid than solid and therefore $G'_{filler} < G'_{gel\ network}$ and little effect is seen on the gel network. Similar temperature-dependent trends in G'_{cheese} were observed with Gouda cheese (Visser, 1991).

Changes in G'_{cheese} reflect the overall stiffness of the gel network at deformations that do not cause irreversible changes in the gel structure. Like microscopy that provides visual images of the structure, G'_{cheese} provides a mechanical description of the cheese structure. In contrast, fracture properties (fracture stress and fracture strain) tell us how much force (stress) and deformation is needed to rupture the gel structure. Changes in fracture stress were opposite those of G' (10°C; 1 Hz). The general trend was for fracture stress to decrease with fat content and increase with protein content. This is consistent with the concept that fracture stress represents the network strands that need to be ruptured to fracture through the cheese and that an increase in protein content would increase the network strand density. Note that fracture stress increased most substantially for cheeses with 3 and 8% fat (Figure 8), that had on average higher (1.66) and lower (1.41) water:protein ratios than the other cheese. ***Therefore, it appears that the amount of gel network, instead of the concentration of protein in the gel network, is the critical factor determining fracture properties and sensory firmness. This suggests that low fat cheese with acceptable texture can be made with filler particles that interact (or do not) with the gel network like that of fat, and have the same filler volume.***

There are several key conclusions that can be drawn from our current and past investigations. First, there are two main textural differences between low fat and full fat cheeses. Low fat cheeses tend to have a greater firmness and breakdown into larger, more rubbery, less smooth particles during chewing. The initial firmness can be explained by an increase in the relative amount of protein network as compared to filled fat particles but the differences in chewdown properties (smoothness, adhesiveness, particle size) are more complex. One can envision the chewdown properties being different due to variation in crack propagation through the cheese structure. Three possible fracture paths are: 1) proceeding through the gel network and along the fat particle surface; 2) proceeding through the gel network and through the fat particles and 3) proceeding through the gel network and leaving the fat-gel network interfaces intact. The plausibility of each path may be discussed based on findings in model systems.

Using mixed protein-polysaccharide gels as a model, van den Berg et al. (2007) showed different fracture patterns that were dependent on microstructure. Gels with protein continuous and bicontinuous networks were

perceived as *crumbly*, whereas those with coarse stranded gels were viewed as *spreadable*. Moreover, these textural differences were associated with different crack propagation mechanisms observed by confocal microscopy. In protein continuous structures, those most closely resembling cheese, micro-cracks appeared during deformation (strain of 0.3) before the actual fracture point (fracture strain of 1.04 to 1.15). The main fracture path went through the gel network and the dispersed, fluid hydrocolloid solution particles. This suggests that either path 1 or 2 are logical for cheese, and would depend on the fluid/solid nature of the fat. Therefore, if we knew how to design a low fat cheese microstructure with all the essential physical/chemical elements of a full fat cheese microstructure, there should be little or no textural differences. The questions become what microstructural elements are key to full fat cheese texture and how do we create such structures without fat?

One approach is to use starch granules as fat replacers. The logic underpinning of this approach is that starch can act as an inactive filler (e.g., not interaction between starch granules and the protein matrix), thereby taking up space and, hopefully, acting like fat particles. However, in an imitation cheese, addition of starch cause a major increase in fracture stress and decrease in fracture strain (Noronha et al., 2008). These rheological transitions are indicative of a firmer, more brittle sensory texture. The increase in firmness/toughness was associated with a decrease in water mobility, suggesting that water was partitioning inside of the starch granules and thereby increasing the protein concentration of the gel network. These results show that ability of a filler particle to remove or add water to the protein gel network phase is essential to how they will function.

Besides sensory analysis, an evaluation of the chewing process helps us understand differences among cheeses. We have used three dimensional jaw tracking to measure the chewing process of a line of commercial cheddar cheeses with different fat levels. This brand was picked because it had 50% and 75% reduced fat cheeses, and the 75% reduced fat cheese contained starch. The jaw tracking data showed that as fat was reduced in the cheese, it required more extensive chewing before swallowing. In addition, with the 75% reduced fat cheese, there was at least one intermediate swallow before the final clearing swallow (Figure 9). This showed directly from mastication measurements that as fat is reduced the cheese becomes tougher and requires more extensive chewing before swallowing. While we cannot determine how the added starch is affecting the cheese texture, it is clear that it is not reducing the overall toughness.

Proposal.

Our proposal for the next project is to build on our current findings and start investigating a range of filler particles. The following particles will be investigated:

Polar whey protein isolate gel particles.

Whey proteins can be heated to form polymers then added to calcium chloride solution under shear to produce gel particles of a range of sizes depending on shearing conditions. The particles would be made at the same water activity as the cheese to prevent moisture migration. The water activity of the particles will be adjusted to that of cheese (0.93 to 0.97) by adding sugar alcohols. Alternatively, it may be desirable to cause moisture migration to or from the particles and this can also be done by adjusting particle water activity.

Non-polar whey protein isolate gel particles.

A similar approach as outlined in #3 would be used but glucono-delta-lactone is added to the whey protein polymer solution prior to being added to oil under shear. The protein-polymer solutions will form emulsion droplets in the oil and the glucono-delta-lactone will slowly lower the pH causing gelation of the droplet. The surfaces of these particles are expected to be hydrophobic.

Whey protein-hydrocolloid particles.

Various approaches have been used to form particles with whey protein and hydrocolloids, such as pectin (Bédié et al., 2008). In all cases, the particle properties are adjusted by altering solution conditions (pH, polymer concentration) and protein:polysaccharide ratio. We would produce a range of particles for evaluation.

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RESEARCH PLAN

Objective 1.

Determine how whey protein gel particles alter the rheological and microstructural properties of cheddar cheese.

Objective 2.

Determine how whey protein-polysaccharide particles alter the rheological and microstructural properties of cheddar cheese.

Objective 3.

Determine the sensory textural properties of cheddar cheeses containing particles showing optimal rheological and microstructural results.

Research hypotheses.

1. Low-fat cheese is tough (high firmness) and does not break down like full fat cheese because the reduction of fat coincides with an increase in protein network. Fat particles function by altering the fracture pattern of the cheese network.
2. Filler particles that have the same phase volume as fat will produce the same sensory firmness as a full fat cheese.
3. Filler particles that produce the same fracture pattern as fat will produce the same chewdown sensory properties as full fat cheese.
4. One, or a combination of the following filler particles, will have the characteristics described in hypotheses 2 and 3: polar whey protein isolate gel particles, non-polar whey protein isolate gel particles, or whey protein-hydrocolloid particles.

Project design.

Overall, the project will consist of two approaches. In the first approach, we will study the effects of different types and amounts of interacting and non-interacting filler particles on rheological and microstructure of cheese. The goal being to screen a large number of treatments making 1 kg batches of cheese. At the end of the first phase, we should be able to select various filler particles that produce the textural properties of full fat cheese. Selected filler particles will be extensively investigated in the second phase which will include the sensory analysis and mastication evaluation. At the end of the project, we should be able to identify the critical properties of filler particles that allow them to function like fat. We expect to be able to provide the knowledge needed to make low fat cheese having the texture quality of full fat cheese.

Part I. Screening of filler particles based on textural and microstructural properties.

In the first part of our study, we will investigate different types of interacting and non-interacting filler particles at different fat replacement levels (15 and 27%) in corresponding to reduced fat (18%) and low fat cheese (6%), respectively. The initial screening of particles will be determined over a 2 month storage using rheological analysis and confocal microscopy. Particles that produce textured changes similar to what was observed in the full fat control will be further analyzed in Part II. Treatments in Part I are listed in Table 1.

Table 1. List of treatments.

Treatments no.	Treatments	Particle Replacement Level (%)
1	Full fat control	-
2	18% fat	-
3	6% fat	-
4	Polar polymer 4%	15
5	Polar polymer 10%	15
6	Polar Polymer 4 or 10%	27
7	Non Polar Polymer 4%	15
8	Non Polar Polymer 10%	15
9	Non Polar Polymer 4 or 10%	27
10	WPI+pectin 1	15
11	WPI+pectin 2	15
12	WPI+pectin 3	15

These particles will be made at North Carolina State University and shipped to Utah State University for cheese making. One kilogram of cheese per treatment will be made in duplicate. For each set of experiments (6 cheeses per 2 month storage), full fat cheese will be included as control. The rheological properties and confocal microscopy of cheese samples will be studied. Sampling will be done at 2, 4, 6 and 8 weeks.

Part II. Sensory evaluation of cheese made with select particles.

Filler particles that result in cheese with rheological and microstructural properties similar to full fat cheese will be further investigated (select 3). Some select rheological properties will be evaluated but the main focus will be on sensory analysis. Similar to what is outlined in Part I, each set of experiments will include full fat cheese as a control. Each treatment will be made and analyzed in duplicate (i.e. two replications). Sampling will be done at 2, 8, 12 and 24 weeks.

At the end of the project, we should be able to determine the key properties of filler particles that will provide the overall properties similar to fat in full fat cheese.

MATERIALS AND METHODS

Part I. Screening of filler particles which provide the textural and microstructural properties of cheese similar to full fat cheese.

Preparation of filler particles.

See reports from Southeast Dairy Foods Research Center.

Cheese making and chemical analysis.

Cheese will be made in small stainless steel vats using 40 lb of milk. Stirred curd make procedures will be used based on the full fat, reduced fat and low fat cheeses made for the systematic platform project. The cheese will be pressed into a 3 to 4-lb block overnight, and then vacuum packaged and stored at 6°C. Four vats of cheese will be made simultaneously on each day of manufacture. Duplicate sets of cheese will be made. After 1 wk of storage the cheese will be cut into four pieces. One piece will be used for proximate analysis of moisture (by vacuum oven), fat (by babcock), protein (by N), pH (by glass electrode), salt (by chloride), and calcium (by ICP spectroscopy). The remaining three cheese pieces will then be vacuum packaged, and stored at 6°C.

Cheese will be shipped to NCSU at 2 and 7 wk for rheological screening at 3 and 8 wk and one piece of cheese retained for additional testing/storage if needed.

Rheological analysis.

See reports from Southeast Dairy Foods Research Center.

Confocal microscopy.

See reports from Southeast Dairy Foods Research Center.

Part II. Sensory evaluation of cheese made with select particles.

Filler particles that produce 1 Kg cheeses with rheological and microstructural properties similar to full fat cheeses will be used to make 10 Kg batches of cheese. A few key rheological properties will be measured but the primary focus will be on sensory analysis and mastication evaluation.

Cheese making and analysis.

Cheese will be made in stainless steel vats using 300 lb of milk. Stirred curd make procedures will be used based on the full fat, reduced fat and low fat cheeses made for the systematic platform project. The cheese will be pressed into a 20-lb block overnight and then vacuum packaged and stored at 6°C. Duplicate sets of cheese will be made four weeks apart.

After 1 wk of storage the cheese will be cut into 2-lb and 1-lb blocks. One of the 1-lb blocks of cheese will be sampled for proximate analysis of moisture (by vacuum oven), fat (by babcock), protein (by N), salt (by chloride), ash (by furnace), pH (by glass electrode), calcium (by ICP spectroscopy), soluble calcium and protein hydrolysis (by pH 4.6 soluble N). The other blocks will be vacuum packaged, two 2-lb blocks will be shipped to NCSU for the 2 wk sensory analysis, and the others will be returned for storage at 6°C.

At 8, wk of storage, a 1-lb block of cheese will be tested for soluble calcium (by water extraction and ICP spectroscopy), and protein hydrolysis (pH 4.6 soluble N), pH (by glass electrode).

At 7, 11 and 23 wk, two 2-lb block of cheese will be sent to NCSU for sensory analysis.

Sensory analysis.

Descriptive sensory analysis will be conducted using the methods laid out by Brown et al (2003) and Yates and Drake (2007). Analysis will be conducted using an experienced texture panel consisting of seven panelists with approximately 100 hours of experience in descriptive

texture analysis utilizing the Spectrum method. Samples will be characterized on a 15 point scale anchored on the right by the term "very" and on the left by term "not" using terms from the texture lexicon laid out by Brown et al (2003). Panelists will be given 8 cubes of each cheese, measuring 1.27cm^3, in lidded 4oz. plastic cups labeled with three digit codes. Panelists will be given deionized water to clean their palettes between each sample, and reference cheeses will be made available for each session. The basic terms described in Table 2 will serve as core terms and new ones may be added. Samples will be evaluated in triplicate for each replication. Data will be analyzed by univariate and multivariate statistical analyses using SAS (V 9.1, Cary, NC). Specifically, analysis of variance with means separation will be used to identify differences between treatments and attributes. Principal components analysis will be used to identify gross relationships between multiple treatments and attributes.

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RESULTS AND DISCUSSION

No cheese has been made at this time.

NEXT STEPS

Manufacture cheese after whey protein particles have been made.

At what salt level do consumers notice decreasing salt concentrations and at what concentration is acceptance negatively impacted

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Funded by: Dairy Management Inc., February 2010 - January 2011

ABSTRACT

The role of salt concentration on the flavor profile and consumer acceptance of low fat Cheddar-type cheeses will be investigated. Low fat cheeses at 54% moisture with 0.8% sodium gluconate and 0.7 % salt will be manufactured and aged for 1 month. Following 1 month aging, cheese will then be divided into eight portions and sufficient salt added to produce cheese containing 0.7% (no addition), 1.2%, 1.7%, 2.2%, 2.7%, and 3.25% salt. Two full fat Cheddar cheese with 0.7% salt (and with or without 0.8% sodium gluconate) will also be manufactured and following 1 month aging full fat cheese with 0.7% (no addition), 1.2%, 1.7%, 2.2% salt will be produced as controls. Cheeses will be aged for an additional 3 and 6 months at 6°C. At each subsequent timepoint, a trained descriptive sensory panel will document flavor and texture properties of cheeses and consumer acceptance testing will be conducted. An additional unrelated project objective will be to evaluate volatile compound profiles of selected experimental low fat Cheddar cheeses with promising flavor profiles.

BACKGROUND

This is a follow-up project to initial testing of sensitivity of a trained descriptive panel to salt levels in low fat cheese and that potential impact on perception of other cheese flavors by trained panelists. Preliminary data obtained from a consumer study indicated that acceptability dropped dramatically with reduced salt. (However, the consumer panel was an add-on at a later time since we had additional cheese. Intense flavors (rosy and burnt-brothy flavors) were evident in all cheeses and cheeses at all salt concentrations were rated poorly by consumers which confounded the effect of salt level).

RESEARCH PLAN

Objective 1.

Manufacture low fat cheddar cheese (54% moisture, 6% fat, pH 5.1) with sodium gluconate and full fat cheddar cheese (37% moisture, 33% fat, pH 5.1) with and without sodium gluconate at Utah State University with 0.7% salt content, and begin storage at 38°F. Rep 1 by Jan 31, 2010, Rep 2 by Feb 28, 2010, Rep 3 completed by March 31, 2010.

Objective 2.

Comminute the cheeses after 1 month of storage, add salt and repress to produce low fat cheeses with salt contents of 0.7%, 1.2%, 1.7%, 2.2%, 2.7% and 3.25%, and full fat cheeses with salt contents of 0.7% 1.2%, 1.7% and 2.2% and return to storage at 42°F. Rep 3 completed by April 30, 2010.

Objective 3. (Southeast Dairy Foods Research Center)

Document flavor and texture properties of low and full fat cheeses by descriptive sensory analysis after 3 and 6 months ripening.

Objective 4. (Southeast Dairy Foods Research Center)

Determine consumer acceptance of low and full fat cheeses by descriptive sensory analysis after 3 and 6 months ripening.

Objective 5. (Southeast Dairy Foods Research Center)

Document volatile compound properties of selected low fat Cheddar cheeses.

MATERIALS AND METHODS

Objective 1.

Cheese manufacture.

Fresh cow's milk (~pH 6.7) was obtained from Utah State University's Caine Dairy Research and Teaching Center (Wellsville, UT) and standardized and pasteurized (73°C for 15 s) in the Gary Haight Richardson Dairy Products Laboratory to a protein to fat ratio 4.8 (nominally ~0.6% fat). At the same time as 700 kg of milk at about 20°C was pumped into a Tetra Scherping horizontal cheese vats (Tetra Pak Cheese & Powder Systems, Inc., Winsted, MN), ~270 kg of milk was filled into an open vat with both vats being treated in a comparable manner and then the curd combined prior to draining in the drain table. For the 700-kg portion of milk, inject ~8 L of L-lactic acid (diluted 1:16 with deionized water) using a peristaltic pump to acidify the milk to pH 6.20. Then heat the milk to 32°C and add 120 g of frozen pellets of DVS 850 *Lactococcus lactis* ssp. *lactis/cremoris* starter culture (Chr. Hansen Inc., Milwaukee, WI). Then add 44 ml of single strength annatto color (DSM Food Specialties USA Inc., Eagleville, PA), 120 g of titanium dioxide emulsion (Roha USA LLC., St. Louis, MO), and after 45 min of ripening add ~40 ml of double strength chymosin rennet (Maxiren, ~650 International milk clotting units/ml, DSM Food Specialties USA), stirred for 2 min, and allow the milk to coagulate without stirring until a firm set is reached (~20 min). Cut the curd at 10 revolutions per minute (rpm) for 1 min, then at 14 rpm for 1 min, then reduce the agitator speed to 5 rpm (while keeping in the cutting direction) and continue stirring the curd while gradually increasing the speed to 9 rpm over 8 min. Continue stirring the curd for another ~65 min then pump the curd and whey into a drain table (Kusel Equipment Co., Watertown, WI) with partial whey drainage and continued stirring until a curd pH of 5.95 to 6.00, and then drain all the whey. Dry stir the curd until pH 5.50 and then wash the curd with 10°C-water to lower curd temperature to 22°C (~0.4 kg water per kg of curd). After 10 min, drain the water, then apply salt and sodium gluconate to the curd at a rate of 24 g salt and 8 g sodium gluconate per kg of curd using 3 applications spaced 5 min apart. Then fill 12.5 kg of curd into sanitized and cheese cloth lined, rectangular stainless steel hoops and press the cheese at ~55 kPa overnight at room temperature (~20°C). Then vacuum package in oxygen-barrier plastic bags and store at 3°C. After ~5 d of storage, the cheese blocks will be sampled for analysis of pH by glass electrode, moisture by microwave oven, fat by babcock and salt content by chloride analysis.

Objective 2.

Cheese comminuting and salt addtion.

The 10-kg blocks of cheese will be removed from storage after 15 d of storage, and allowed to come to room temperature (~22°C) and then cut into pieces about 5 cm in size and then comminuted by passing through a Urschel Comitrol 1700 (Urschel Laboratories, Inc., Valparaiso, IN) fitted with a 1.5-mm cutting head. Batches of 10 kg of comminuted low fat cheese (with nominal salt content of 0.70%) will be placed into a bowl chopper (Model VCN25; Hobart Corp., Troy, OH) along with sufficient salt to produce cheese with 0.7%, 1.0%, 1.2%, 1.4%, 1.6%, 1.8%, 2.0%, or 2.2% salt, and mixed for 30 s. The cheese-salt mixture will then be filled into sanitized and cheese cloth lined, rectangular stainless steel hoops and pressed at ~55 kPa overnight at room temperature (~20°C). The full fat cheese (with nominal salt content of 0.7%) will be treated the same way so as to produce cheese with 0.7% and 1.8% salt. After pressing the cheese will be vacuum packaged and stored at 6°C for 4 wk and then cut into 1-kg blocks, vacuum packaged and labeled, randomized, and returned to storage at 6°C. A sample from each 10-kg block will be collected, and the cheeses analyzed for salt (by chloride analysis), sodium and other minerals (by ICP spectropscopy), moisture (by vacuum oven) and pH (by glass electrode). Approximately 4 kg of cheese will be shipped to North Carolina State University for sensory evaluation when the cheese is 12 and 25 wk of age.

RESULTS AND DISCUSSION

Objective 1.

Replicate 1.

Low fat cheese was made on February 17, 2010 and full fat cheese on February 18, 2010 by the WDC Dairy Technology Innovation Laboratory. For low fat cheese, 6 blocks were salted to obtain a salt of 0.7% and the other one was targeted at 2.0% salt, all blocks had 0.8% Sodium Gluconate added. For full fat cheese , 8 blocks were made, all containing 0.7% salt with 4 blocks made with 0.8% so-

dium gluconate added. (Tables 1-2.)

Replicate 2.

Low fat cheese was made on March 3, 2010 and full fat cheese on March 4, 2010 by the WDC Dairy Technology Innovation Laborratory. For low fat cheese, 6 blocks were salted to obtain a salt of 0.7% and the other one was targeted at 2.0% salt, all blocks had 0.8% Sodium Gluconate added. For full fat cheese , 8 blocks were made, all containing 0.7% salt with 4 blocks made with 0.8% sodium gluconate added. (Tables 3--4.)

Objective 2.

Replicate 1.

Cheese was comminuted and salted on March 22, 2010.

CONCLUSIONS

Q1 2010.

Two replicates of cheese were successfully manufactured. Cheese from Replicate 1 was comminuted, salted and repressed.

NEXT STEPS

Q1 2010.

Objective 1.

The third replicate of cheese is scheduled to be made on April 7 and 8, 2010. Which will then complete objective 1.

Objective 2.

Cheese from replicate 2 and replicate 3 will be comminuted, salted, and returned to storage.

Table 1. Low fat cheese composition (Rep 1).

Cheese Manufacture			Cheese Composition			
Cheese Block	*Salt Added (%)*	*Sodium Gluconate Added (%)*	*Moisture (%)*	*Fat (%)*	*Salt (%)*	*pH*
1	0.7	0.8	52.9	6.0	0.67	5.31
2	0.7	0.8	53.0	6.0	0.67	5.30
3	0.7	0.8	52.7	6.0	0.64	5.28
4	0.7	0.8	52.4	6.0	0.68	5.29
5	0.7	0.8	52.1	6.0	0.65	5.28
6	0.7	0.8	52.5	6.0	0.67	5.28
7	2.0	0.8	53.4	6.0	1.99	5.30

Table 2. Full fat cheese composition (Rep 1).

Cheese Manufacture			Cheese Composition			
Cheese Block	*Salt Added (%)*	*Sodium Gluconate Added (%)*	*Moisture (%)*	*Fat (%)*	*Salt (%)*	*pH*
1	0.7	0	35.43	35	0.76	5.23
2	0.7	0	36.09	35	0.64	5.17
3	0.7	0	35.87	35	0.76	5.18
4	0.7	0	35.75	35	0.67	5.20
5	0.7	0.8	36.17	35	0.75	5.23
6	0.7	0.8	35.52	35	0.76	5.23
7	0.7	0.8	35.52	35	0.61	5.23
8	0.7	0.8	34.94	35	0.67	5.24

Table 3. Low fat cheese composition (Rep 2).

Cheese Manufacture			Cheese Composition			
Cheese Block	*Salt Added (%)*	*Sodium Gluconate Added (%)*	*Moisture (%)*	*Fat (%)*	*Salt (%)*	*pH*
1	0.7	0.8	52.8	5.0	0.69	5.14
2	0.7	0.8	52.6	5.0	0.71	5.16
3	0.7	0.8	52.4	5.0	0.72	5.19
4	0.7	0.8	52.5	5.0	0.69	5.18
5	0.7	0.8	52.2	5.0	0.68	5.17
6	0.7	0.8	52.2	5.0	0.72	5.18
7	2.0	0.8				

Table 4. Full fat cheese composition (Rep 2).

Cheese Manufacture			Cheese Composition			
Cheese Block	*Salt Added (%)*	*Sodium Gluconate Added (%)*	*Moisture (%)*	*Fat (%)*	*Salt (%)*	*pH*
1	0.7	0	36.2	34	0.68	5.17
2	0.7	0	36.3	34	0.66	5.17
3	0.7	0	36.4	34	0.72	5.18
4	0.7	0	36.2	34	0.61	5.18
5	0.7	0.8	36.0	34	0.76	5.27
6	0.7	0.8	35.4	34	0.68	5.25
7	0.7	0.8	35.8	34	0.72	5.24
8	0.7	0.8	35.9	34	0.56	5.18

DTIL Projects Completed in 2009

A [Dairy Technology and Innovation Laboratory (DTIL) at the Western Dairy Center

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Funded by: Dairy Management Inc., September 2007 - December 2009

ABSTRACT

The Dairy Technology Innovation Laboratory (DTIL) was established at the Western Dairy Center to provide support to DairyManagement Inc. and the dairy industry in the development of new technologies, investigate the feasibility of producing value added dairy products, gather scientific information on dairy food systems and resolve manufacturing problem.

The research projects were conducted under the various objectives within the DTIL. For clarity all projects are listed individually in this report.

In the first two years of operation the DTIL completed the following projects:

1. Develop a a method for adding functional or nutritional components to reduced-fat Cheddar cheese curd.
2. Evaluate the flavor of omega-3 fortified cheese by descriptive and consumer taste panels.
3. Investigate methods for incorporating omega-3 fatty acids in cheese milk to improve retention in curd and minimize loss in whey.
4. Evaluate the flavor of whey obtained from cheese fortified with omeg-3 fatty acids.
5. Investigate methods for incorporating vitamin D in cheese.
6. Evaluate the flavor of vitamin D fortified cheese by descriptive and consumer taste panels.
7. Develop emulsions containing vitamin D to improve retention in cheese curd.
8. Evaluate and improve plating techniques for the enumeration of probiotic bacteria in cheese.
9. Develop techniques using qPCR to enumerate probiotic bacteria in cheese.
10. Determine the survival of probiotic bacteria in low-, reduced-, and full-fat Cheddar cheese during aging by plating on selective media and qPCR techniques.
11. Evaluate consumer acceptance of milk pasteurized by HTST, UHT and electrical resistive heating techniques.
12. Enrichingg low-fat cheese with four different dietary fibers.

RESEARCH PLAN

Objective 1.

Develop three value-added cheese products.

Objective 2.

Explore the development of 5-7 additional value-added cheese products.

Objective 3.

Review the status of shelf-stable milk, value-added cheese, and value-added milk technologies.

Objective 4.

Explore methods for production of value-added milk, including microfiltration and lactose reduction.

Objective 5.

Partitioning of omega-3 fatty acids in cheese curd and whey.

Objective 6.

Vitamin D fortified cheese.

Objective 7.

Development Cheddar cheese containing probiotics.

SUMMARY OF PROJECT OUTCOMES

Project 1. A method was developed to add functional or nutritional components to cheese by grinding the cheese curd, mixing in the component of interest, and pressing ground curd into a block.

Project 2. Omega-3 fortified cheese was made using the method developed in Project 1. A trained taste panel detected a fishy flavor in Cheddar cheese fortified with omega-3 fatty acids. A consumer taste panel rated the omega-3 fortified cheese less acceptable than non-fortified cheese.

Project 3. Homogenizing omega-3 fatty acids in a portion of the cheese milk or cream was the best method for incorporating omega-e fatty acids in cheese.

Project 4. After three months of storage, a slight fishy flavor developed in whey obtained form cheese fortified with omega-3 fatty acids.

Project 5. A method was developed to fortify cheese with vitamin-D.

Project 6. Fortifying cheese with vitamin-D did not impact cheese flavor.

Project 7. Dairy based emulsions of vitamin-D were developed which improved retention of vitamin-D in cheese.

Project 8. Selective media for the enumeration of probiotic bacteria in cheese were improved to reduce the interference of non-starter lactic acid bacteria.

Project 9. A technique was developed and proven for the enumeration of probiotic bacteria in cheese.

Project 10. Full-, reduced-, and low-fat cheeses were made containing probiotic cheese. Seven commercially available strains were tested and found to survive to 270 days of aging.

Project 11. Three methods of producing shelf stable milk, direct steam, indirect plate, and eectrical resistive heating were compared with standard HTST pasteurization. This project is ongoing.

Project 12. Low-fat Cheddar cheese enriched with 5% fiber had improved textural properties and comminuted cheeses had higher cohesiveness than the non-comminuted control, which was due to the rearrangements of cheese particulates making it more malleable. Better performance of cheese was observed when fiber added with equal amount of water than fiber alone. Moreover, adding fibers did not impact cheese flavor. Out of 4 types of dietary fibers tested in this study, inulin and pectin had promising results while polydextrose and resistant starch had poor appeal.

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DTIL Project 1:
Develop a method for adding functional or nutritional components to reduced-fat Cheddar cheese curd

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

Cheese is potentially a good vehicle for providing added nutrients such as omega-3 fatty acids, vitamins, fiber, probiotics, and prebiotics to the diet. Addition of such nutrients will add value to cheese. This project investigated methods of efficiently adding compounds to cheese while minimizing inclusion in whey.

MATERIALS AND METHODS

Cheese manufacturing method.

All reduced fat cheeses were made using the standard USU cheese make procedure which consists of adding mesophilic cultures and rennet to milk containing 0.6 % fat at 31°C, cutting the curd, cooking to 35.5 °C for one hour and 15 minutes, draining the whey at pH 6.25, adding enough wash water at 18°C to bring the curd to 29°C, washing for 10 minutes, draining the wash water and stirring the curd until the pH drops to 5.9. Three cheese-making replicates were conducted.

Curd grinding method.

In order to determine the optimal curd grinding time, 13.6 kg of salted reduced-fat cheese curd was ground (Hobart, model VCM 25) for 15, 30 or 60 seconds. Five grams of Solkaflock was added to 500 g of curd, mixed by hand, and passed through USA Standard sieves with 12.5, 9.5, 6.3, 4.0, 2.0 and 1.0 mm openings. The amount of curd retained by each sieve was determined by weight.

Moisture retention.

Cheese curd from four different cheeses, ranging form 44.5% to 46.5% moisture, were ground for 15, 30, or 45 seconds. Six pounds of ground curd was placed in circular hoops and pressed overnight at 55.1. kPa. Proximate analysis was preformed on the cheese at 5 days of age. The cheeses were weighed before and after pressing to determine moisture loss during press.

Confocal microscopy.

Cold cheese, 3°C, was sliced using a Spencer microtome into sections 1mm X 10mm X 10mm. The sections were immersed into 0.025% Nile Red (Sigma) in Dimethyl Sulfoxide (DMSO) for 1 minute, rinsed in distilled water for 1 minute, and placed on microscope slides, 3" x 1" x 1.0 mm (Fisher Scientific). 0.2 ml of 0.1% flourothio-isocyinide (FTIC) in 50% acetone $_{Aq}$ was pipetted onto the surface of the cheese section, allowed to stand at 20°C for 3 minutes, and covered with 22 X 50mm micro cover glass (VWR). Glycerol jelly was pipetted between the micro cover glass and the slide, trapping the cheese section, and creating an airtight seal around the cheese.

The completed preparation was imaged with the BioRad LSCM (BioRad, NY, NY), using an Argon-Krypton laser. Images were captured digitally, and analyzed using Adobe Photoshop and Imaris BitPlane software.

RESULTS AND DISCUSSION

The size distribution of curd particles ground for 15, 30 and 45 seconds is shown in Figure 1.1. The experimental control was non-ground curd. The mode for particle size decreased from 4.0 mm for the control, to 1.0 mm for 30 seconds of grinding. There was no decrease in curd size distribution by increasing the grinding time to 45 seconds.

Moisture retention in the cheese increased with increasing grinding time. Increases were greater with

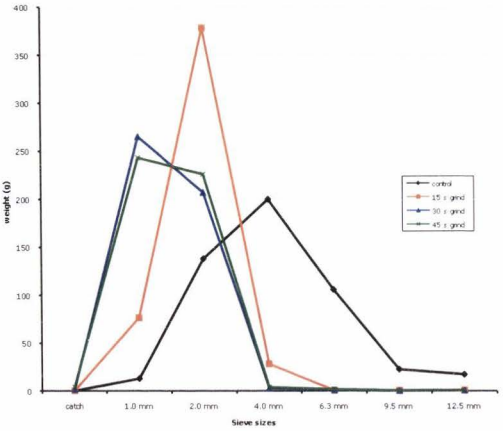

Figure 1.1. Size distribution of reduced-fat Cheddar cheese curd, ground for 0, 15, 30 and 45 seconds.

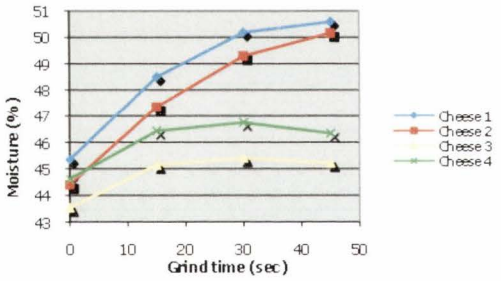

Figure 1.2. Moisture retention in ground Cheddar cheese curd.

increasing initial moisture content (Figure 1.2).

The effect of grinding time on the cohesiveness of the pressed curd blocks, as a function of storage time is shown in Figure 1.3. The ground cheeses were less cohesive than the non-ground control, however there was little difference in the cohesiveness of cheese with different grinding times.

The effect of press time and pressure on the cohesiveness of ground reduced-fat Cheddar cheese is shown in Figure 1.4. The cohesiveness of the cheese increased from one to 30 days of age for all press times and pressures. The cohesiveness of the cheese decreased from 30 to 60 days of age.

Distribution of fat in the ground and non-ground cheese is shown in Figures 1.5 and 1.6 respectively. In these micrographs, the fat appears red, and the protein appears green to brown. The curd particle interface appears as light green-yellow. As seen in Figure 1.5, the fat released upon grinding is trapped between the curd particles. This provides more space between the particles to entrap and retain moisture. Fat between the curd particles also limits the ability of the protein to knit together, resulting in a less cohesive cheese as demonstrated in Figure 1.3.

CONCLUSIONS

Day ingredients can be added to ground cheese or cheese curd and the ground cheese press onto blocks. The resulting cheese contains more moisture and is less cohesive than traditionally pressed cheese curd.

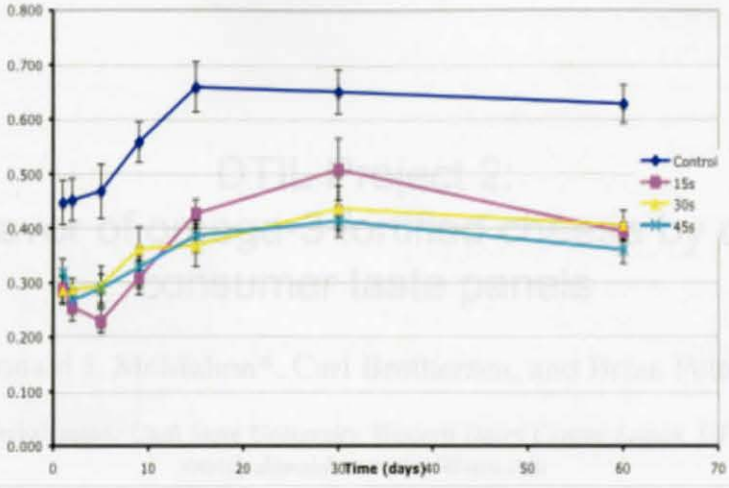

Figure 1.3. Cohesiveness of ground and non-ground reduced-fat Cheddar cheese curd as a function of age of cheese.

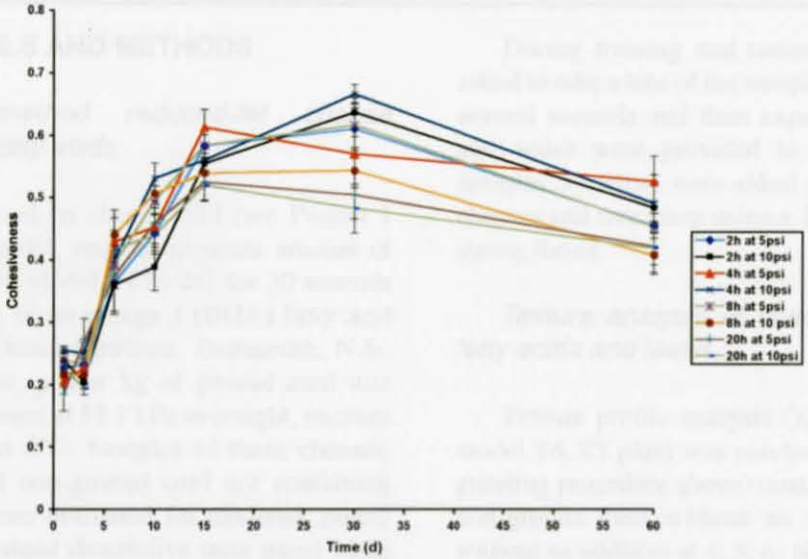

Figure 1.4. Cohesiveness of ground reduced-fat Cheddar cheese as a function of press time, pressure, and age of the cheese.

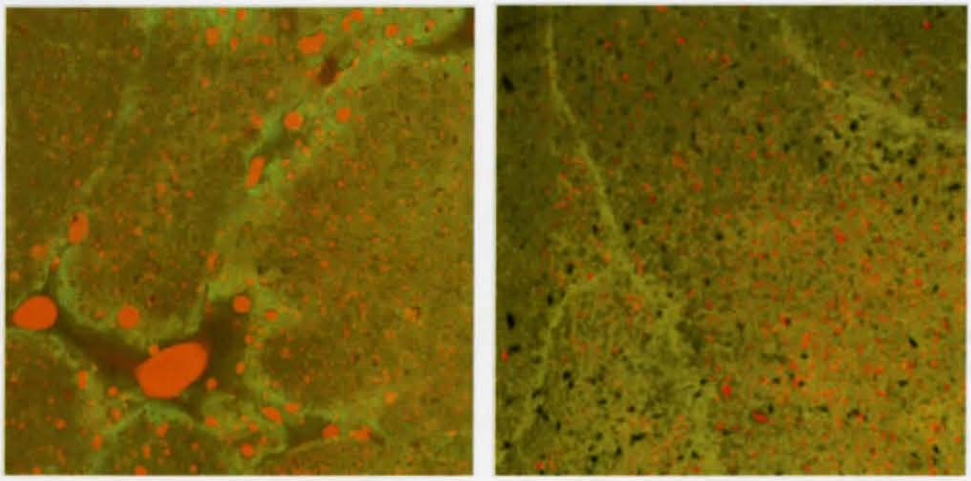

Figure 1.5 (Left). Confocal micrograph of ground cheese curd in which the fat appears red and the protein appears green.
Figure 1.6 (Right). Confocal micrograph of non-ground cheese curd in which the fat appears red and the protein appears green.

DTIL Project 2:
Evaaluate the flavor of omega-3 fortified cheese by descriptive and consumer taste panels

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Funded by: Dairy Managment Inc., September 2007 - December 2009

MATERIALS AND METHODS

Manufacturing method reduced-fat cheese containining omega-3 fatty acids.

Sixteteen kg of reduced-fat cheese curd (see Project 1 for cheesesemaking methods), and the requisite amount of salt was s ground (Hobart, model VCM 25) for 30 seconds with 50,), 100, or 200 g of an omega-3 (DHA) fatty acid preparatition (MEG-3, Ocean Nutrition, Dartmouth, N.S., Canada).). Two and three quarter kg of ground curd was placed in n hoops and pressed at 55.1 kPa overnight, vacuum packageded, and stored at 8°C. Samples of these cheeses, along wivith ground and non-ground curd not containing omega-3 3 fatty acids, were evaluated for oxidized, rancid and fishyy flavor by a trained descriptive taste panel when the cheesse was 1, 7, 30, 90, and 180 days of age.

Sensnsory evaluation.

The e attributes of cheeses formulated without and with the additition of different amounts of DHA were evaluated using delescriptive techniques. Thirteen panelists were trained i in the identification and quantification of specific sensory a attributes that can be related to flavor defects in omega-3 3 fortified cheeses. The attributes chosen were: oxidized d, rancid and fishy. Panelists received 10 hours of training g prior to the testing period and 1 hour per week during tc testing. For the training sessions, cheeses were made to) reflect specific attributes. Samples used to train panelists s in the oxidized flavor were made by mixing 5 mL of a 1% s solution of $CuSO_4$ in 1.4 kg of cheese. For training in the rarancid flavor, feta cheese was added. Lastly, fresh samples o of cheeses formulated with the addition of 3.7, 7.3 and 14.7 7 mg of DHA per g of cheese were used for training in the fisishy flavor.

During training and tasting sessions, panelists were asked to take a bite of the sample, keep it in their mouth for several seconds and then expectorate. Unsalted crackers and water were provided to rinse the palate between samples. Panelists were asked to identify the flavor in the cheeses and rate them using a 1, no flavor, to 5, extremely strong flavor.

Texture analysis of cheese containing omega-3 fatty acids and inulin.

Texture profile analysis (Texture Technologies Corp. model TA.XT.plus) was conducted on ground cheese (see grinding procedure above) containing omega-3 fatty acids, non-ground curd without an additive and ground curd without an additive at 1, 3, 6, 10, 15, and 30 days of age.

RESULTS AND DISCUSSION

Flavor analysis of reduced-fat cheese containing omega-3 fatty acids.

Figures 2.1-2.3 show the intensity of the three flavor attributes tested for the three levels of DHA (omega-3 fatty acids) added, and the control cheese. Data shown in theses plots are the average of the three replicates. It can be observed from these figures that DHA added at these levels imparted a strong flavor in the cheese. The highest ratings observed in the cheeses during the entire experiment (180 days) for oxidized, rancid and fishy were 0.7, 0.9 and 1.2, respectively. Considering that a rating of 0 means no flavor was detected, and a rating of 1 means slight flavor, we can conclude that the addition of DHA did not affect in great proportion the flavor of the fortified cheese. Initially, the experiment was designed using two controls (stirred and ground). No significant differences were observed between

the sensory ratings of the two controls and therefore only the ground control values are reported in Figure 2.1.

No significant differences were found between the oxidized and rancid attributes of the different cheeses. However, a significant increase in the intensity of these attributes was observed after 90 days of aging ($p < 0.05$). The fishy flavor was significantly different between samples and also storage time. Cheeses with 3.7 mg of DHA/g of cheese was not significantly different from the control at any time during the experiment. When the DHA content increased to 7.3 mg of DHA/g of cheese, the fishy flavor was significantly different from the control after 1 day of storage, but the fishy flavor intensity decreased after 7 days of storage resulting in no significant differences from the control. The same behavior was observed for the cheeses with 14.7 mg of DHA/g of cheese, with the difference that the fishy flavor intensity significantly decreased after 90 days of storage showing no significant differences with the control. Table 2.1 summarizes the significant differences between the cheeses and their storage time.

The cohesiveness of the cheese with added omega-3 fatty acids, the non-ground control and the ground control is shown in Figure 2.4. The ground cheeses were significantly less cohesive than the non-ground control. The cheese containing omega-3 fatty acids were the least cohesive at 60 days of age. The reduction in cohesiveness of ground cheese to which DHA was added, was similar to the decreased in cohesiveness of ground cheese with no additive as noted in Project 1, Figure 1.3.

CONCLUSIONS

Omega-3 fatty acids can be added to ground, reduced-fat Cheddar cheese with little change in flavor or texture of the repressed cheese.

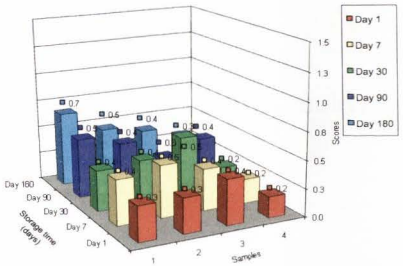

Figure 2.1 Oxidized flavor intensity scores of cheese fortified with DHA and non-fortified controls. Sample 1 contains no added omega-3 fatty acids and samples 2, 3 and 4 contain 3.7, 7.3 and 14.7 mg of DHA/g of cheese, respectively.

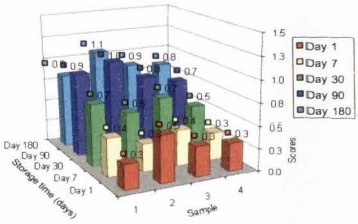

Figure 2.2. Rancid flavor intensity scores of cheese fortified with DHA and non-fortified controls. Sample 1 contains no added omega-3 fatty acids and samples 2, 3 and 4 contain 3.7, 7.3 and 14.7 mg of DHA/g of cheese, respectively.

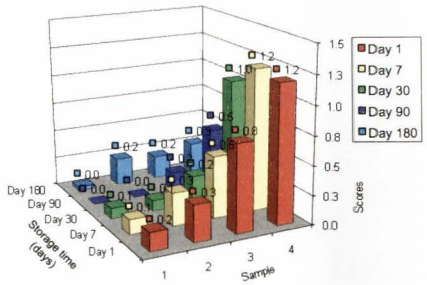

Figure 2.3. Fishy flavor intensity scores of cheese fortified with DHA and non-fortified controls. Sample 1 contains no added omega-3 fatty acids and samples 2, 3 and 4 contain 3.7, 7.3 and 14.7 mg of DHA/g of cheese, respectively.

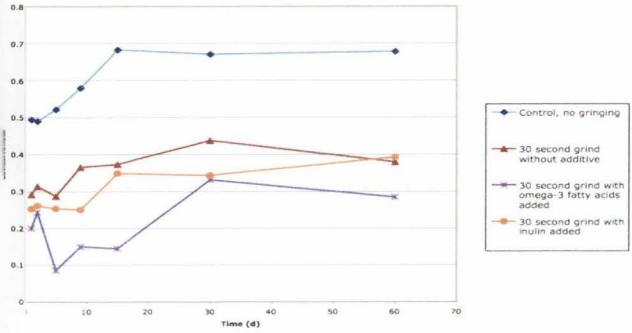

Figure 2.4. Cohesiveness values for treated and non-treated cheeses.

Table 2.1. Significan differences between samples and age time. Sample 1 is the Control and samples 2, 3, and 4 are 3.7, 7.3, and 14.7 mg of DHA/g of cheese, respectively. * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.0001$

		1				7				30				90				180			
AGE (days)	**Sample**	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	1	0.2		*	***				***				***								
	2		0.3		***				***				**								
	3	*		0.8		*				*	*			*	*	*		*			
	4	***	***		1.2	***	***	*		***	***	***		***	***	***	*	***	***	***	***
7	1			*	***	0.1			***				***								
	2				***		0.3		***				**								
	3				*			0.5	*												
	4	***	***			***	***	*	1.2	***	***	***		***	***	***	*	***	***	***	***
30	1			*	***				***	0.1			***								
	2			*	***				***		0.1		***								
	3				***				***			0.2	**								
	4	***	**			***	**			***	***	**	1.0	***	***	***		***	**	**	**
90	1			*	***				***				***	0.0							
	2			*	***				***				***		0.0						
	3			*	***				***				***			0.2					
	4				*				*								0.5				
180	1			*	***				***				***					0.0			
	2				***				***				**						0.2		
	3				***				***				**							0.2	
	4				***				***				**								0.3

DTIL Project 3:
Investigate methods for incorporating omega-3 fatty acids in cheese milk to improve retention in curd and minimize loss in whey

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

Currently there is interest in adding omega-3 fatty acids to cheese to produce a more healthful product. Commercial preparations of omega-3 fatty acids are available in two physical states, liquid and encapsulated powder; and two sources, plant and animal. There are also two methods of incorporating omega-3 fatty acids into the cheese, adding it to the cheese milk, and adding it to the finished curd.

This project will investigate how liquid omega-3 fatty acids from two different suppliers, representing animal and plant sources, partition between the curd and whey, when incorporated in the cheese milk using different techniques.

MATERIALS AND METHODS

Step A. Adding Omega-3 oils to milk at start of cheesemaking.

Cheese was made with 10-L of pasteurized whole milk. Sufficient omega-3 (O-3) fatty acids were added to the milk before renneting (a 80% yield assumed) to give at least 32 mg of DHA/EPA omega-3 fatty acids per ounce of cheese using one the following methods of incorporation.

1. Mixing liquid omega-3 oil in a portion of the milk.
2. Homogenizing liquid omega-3 oil in a portion of the milk.
3. Mixing liquid omega-3 oil in cream and adding to the cheese milk.
4. Homogenizing liquid omega-3 oil in a portion of the cream.
5. Mixed encapsulated omega-3 oils into milk.

The experimental cheeses were made in duplicate. Omega-3 oil sourced from algae (Martek Biosciences Corporation, Columbia, MD, 21045) and fish (Ocean Nutrition, Dartmouth, N.S., Canada) sources were used. The cheese curd was packed in round plastic hoops and pressed at 103.4 kPa overnight into approximately 1 kg blocks. Whey was collected and weighed during cheesemaking and during pressing.

A portion of the whey was frozen and stored for analysis. Cheese was vacuum packaged and stored at 6°C. A sample of each cheese was frozen and stored for later analysis. The omega-3 fatty acid content of the cheese curd, the pressed cheese, whey obtained during draining, and the whey obtained during pressing was determined using GC methods. The partitioning of omega-3 fatty acids between whey and cheese was determined.

Step B. Homoginization of omega-3 fatty acids in a portion of cheese milk and cream.

After the data from Step A was analyzed, a new homogenization regimen was developed in order to improve the omega-3 fatty acid retention in the curd. The requisite amount of O-3 was homogenized in cheese milk, cream containing 12% fat, and cream containing 24% fat. These homogenized treatments then made up 10% of the fat in the cheese milk. Cheese was than made as outlined in Step A.

Step C. Adding Omega-3 oil to cheese curd at end of cheesemaking.

Cheese was made from 600 lb of milk and the curd divided into ten portions. Sufficient omega-3 fatty acids was added to cheese curd (a 100% yield assumed) to give at least 32 mg of DHA/EPA omega-3 fatty acids per ounce of cheese using different methods of incorporation along with

a control cheese with no omega-3 oil added (in duplicate):

1. Mixing liquid omega-3 oil with salt and adding to curd.
2. Mixed encapsulated omega-3 oils with salt and adding to curd.

Omega-3 oil sourced from algae and fish origins was used. The cheese curd was packed in round plastic hoops and pressed into 2.25 kg block at 103.4 kPa overnight. Whey was collected and weighed during cheese pressing.

A portion of the whey was frozen and stored for later analysis. Cheese was vacuum packaged and stored at 6°C. A cheese sample was frozen for later analysis. The omega-3 fatty acid content of the cheese curd, the pressed cheese, whey obtained during draining, and the whey obtained during pressing was determined using GC methods. The partitioning of omega-3 fatty acids between whey and cheese was determined.

RESULTS AND DISCUSSION

Recovery of omega-e fatty acids (O-3) in cheese curd is shown in Figure 3.1. In general, the animal sourced O-3s were retained in the curd better than the plant sourced.

Recovery of O-3 in cheese curd is shown in Figure 3.2.

CONCLUSIONS

Retention of Omega-3 oils in cheese curd can be improved by homogenizing the oil in a portion of the cheese milk or cream.

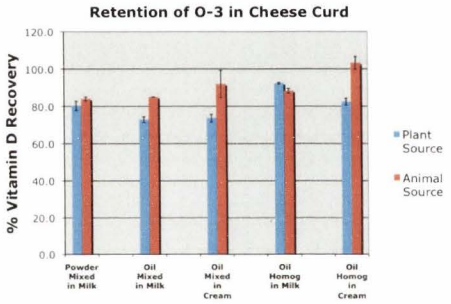

Figure 3.1. Retention of omega-3 fatty acids (vegetable and animal source) in cheese curd as a percent of the total omega-3 added to the cheese milk. Treatment 1: Encapsulated omega-3 powder added to the cheese milk. Treatment 2: Omega-3 oil added to the cheese milk. Treatment 3: Omega-3 oil mixed in cream and added to cheese milk. Treatment 4: Omega-3 oil homogenized in milk and added to cheese milk. Treatment 5: Omega-3 oil homogenized in cream and added to cheese milk.

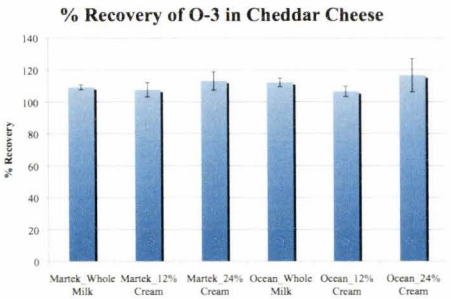

Figure 3.2. Recovery of omega-3 fatty acids in cheese curd when added as homogenized portions of milk, 12% fat cream, and 24% fat cream, constituting10% of total fat.

DTIL Project 4:
Evaluate the flavor whey obtained from cheese fortified with omeg-3 fatty acid by descriptive taste panels

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

Fortifying cheese by adding omega-3 fatty acids to cheeese milk will result in the inclusion of some omega-3 fatitty acids in the whey. This may impart a flavor defect to the whey. This project investigated the flavor of dried whhey obtained from the manufacture of omega-3 fatty acid fortified cheese.

MATERIALS AND METHODS

Whey was collected from cheese made by homog enizing omega-3 fatty acids in 10% of the cheese miilk. The whey was divided into two portions, the fat was sepparated from one portion, and the fat retained in the other portions These were then concentrated by reverse osmosis and dried. The dried whey from each fat treatment was divvided into two portions, one stored at room temperature and one stored at 4C. Whey samples were reconstituted to 10% solids and analyzed for flavor by descriptive taste panel at 1, 3, 6, and 9 months of age.

RESULTS AND DISCUSSION

Descriptive taste panel scores for the reconstituted whey are shown in Figures 4.1, 4.2, 4.3 and 4.4 for whey samples at 1, 3, 6, and 9 months of age respectively. The flavors perceived significantly differently across whey samples and varied across storage time, with fishy flavor being perceived higher in omega-3 fatty acid whey only after 6 mo of storage. Conversely lactone/fatty acid and oxidized flavors were perceived higher in omega-3 fatty acid whey at 1 and 3 mo of storage.

CONCLUSIONS

There was no significant difference in flavor of whey from cheese fortified with omega-3 fatty acids and non-fortified cheese.

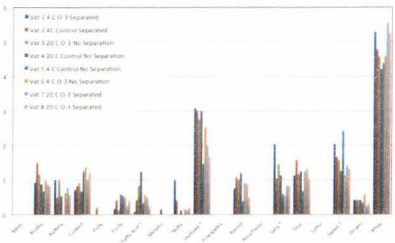

Figure 4.1. Descriptive taste panel scores of dried whey from Cheddar cheeses manufactured with added omega-3 fatty acids after 1 mo storage. Asterisk (*) denotes flavors that were perceived significantly ($p<0.01$) differently across samples.

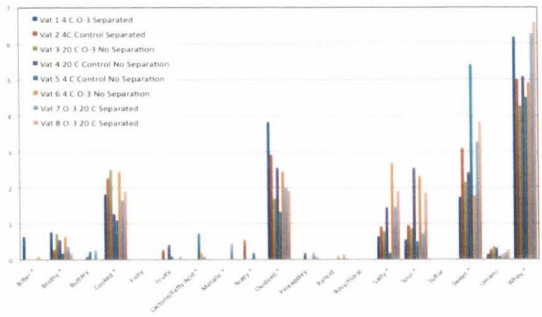

Figure 4.2. Descriptive taste panel scores of dried whey from Cheddar cheeses manufactured with added omega-3 fatty acids after 3 mo storage. Asterisk (*) denotes flavors that wee perceived significantly ($p<0.01$) differently across samples.

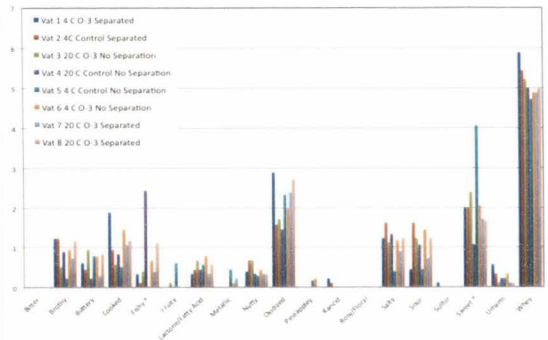

Figure 4.3. Descriptive taste panel scores of dried whey from Cheddar cheeses manufactured with added omega-3 fatty acids after 6 mo storage. Asterisk (*) denotes flavors that wee perceived significantly ($p<0.01$) differently across samples.

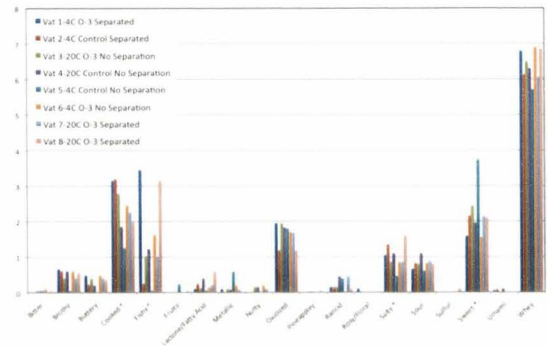

Figure 4.4. Descriptive taste panel scores of dried whey from Cheddar cheeses manufactured with added omega-3 fatty acids after 9 mo storage. Asterisk (*) denotes flavors that wee perceived significantly ($p<0.01$) differently across samples.

DTIL Project 5:
Investigate methods for incorporating vitamin D in cheese

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

It is anticipated that FDA will increase the daily dietary Vitamin D recommendation for humans from 400 to 1000 IU. Cheese may be a good vehicle for increasing Vitamin D consumption. However, there is a potential for Vitamin D to contribute off flavors to cheese and whey, especially oxidized flavors. Data on the flavor profile of Vitamin D fortified cheese is not available.

In previous studies, Vitamin D retention in cheese ranged from 40-60%, depending on the vitamin preparation. More efficient techniques for adding Vitamin D to cheese milk need to be developed to improve Vitamin D retention in the cheese and reduce losses in the whey.

MATERIALS AND METHODS

Development of cheesemaking procedures with 10 L of milk.

Four cheesemaking trials were conducted to determine procedures to make cheese with 10 kg of whole milk in 22 cm X 35 cm X 22 cm containers. The containers were placed in a water bath to control the temperature of the milk. The pasteurized milk was tempered to 31°C (88°F) inoculated with 1.9 g of frozen culture (DVS 850, Chr. Hansen, Milwaukee, WI); ripened for 30 minutes; set with 0.75 ml double strength rennet (DSM Food Specialties, Parsippany NJ); cut with 1.5 cm knives; cooked to 39°C (102.5°F) for 50 minutes; the whey was drained; the curd was allowed to mat; the mats were maintained at 35°C and turned every 15 minutes until the pH reached 5.3; the mats were cut in to pieces approximately 2 cm X 2 cm X 6 cm; 29.5 g salt was added in three applications, five minutes apart; the curd was packed into cylindrical hoops and pressed overnight at 68.9 kPa (10 psi). Proximate analysis was conducted on the cheese at 5 days of age.

Step A. Evaluate the retention of vitamin D in cheese curd.

Trials using the procedure listed above were conduced to evaluate the procedures for adding vitamin D to Cheddar cheese. The six methods of addition were: 1) homogenizing the requisite amount of commercially available emulsion of vitamin D (VitaSystems VS-AD200, Continental Custom Ingredients, Inc.) in milk and adding 100 mL of the this fortified milk to 10 kg of cheese milk; 2) adding the Vitamin D emulsion directly to the cheese milk without homogenization; 3) homogenizing the requisite amount of commercially available encapsulated vitamin D in powdered form (BASF, Mount Olive, NJ) in milk and adding 100 mL of the this fortified milk to 10 kg of cheese milk; 4) adding the encapsulated Vitamin D powder directly to the cheese milk without homogenization; 5) homogenizing the requisite amount of commercially available vitamin D oil (BASF, Mount Olive, NJ) in milk and adding 100 mL of the this fortified milk to 10 kg of cheese milk; and 6) adding the Vitamin D oil directly to the cheese milk without homogenization. The content vitamin D content of the cheese milk was 8,000 IU/10 L. Vitamin D content in the cheese curd and whey was determined using the AOAC official method 952.29 by O'Neal Scientific, St. Louis, MO. Duplicate vats of each treatment were made and means reported.

Step B. Evaluate the vitamin D retention using the two best methods from Step A using regular cheesemaking procedures.

Duplicate trials were conducted using the cheese make procedures listed above with 113 kg (250 lbs) of whole milk in open vats. The curd was cut with 1 cm knives. The curd packed into 20 pound Wilson hoops and pressed overnight. The cheese was sampled for proximate and vitamin D analysis at 5 days of age. The vitamin D treatments were

adding the powdered and emulsified forms directly to the cheese milk.

Step C. Determine the distribution of vitamin D in cheese curd when added with salt.

Cheese was made as described in Step B without adding vitamin D to the milk. Two vats of cheese were made wherein the curd was matted, Cheddared, milled, and Vitamin D powder was added with salt. The curd packed into 29 kg Wilson hoops and pressed overnight. The cheese was sampled for proximate and vitamin D analysis at 5 days of age.

RESULTS AND DISCUSSION

Step A. Evaluate the retention of vitamin D in cheese curd.

Figure 5.1 shows the amount of Vitamin D retained in the curd using oil, powdered, and emulsified preparations with the homogenized and non-homogenized treatments. The initial amount of vitamin D added to the cheese milk was 8,000 IU. Composition of the cheeses is shown in Table 5.1.

Adding Vitamin D in powdered form resulted in the greatest retention in the curd, while adding the Vitamin D oil directly into the cheese milk without homogenization resulted in the least retention.

Step B. Evaluate the vitamin D retention using the two best methods from Step A using regular cheesemaking procedures.

The average recovery of vitamin D in curd, when added as a powder or emulsification, is shown in Figure 5.2. Recovery was best with the emulsified form of vitamin D 8% and 14% lost in the whey for the emulsion and powdered forms respectively. Composition of theese cheeses is shown in Table 5.2.

The recovery of vitamin D in cheddared and stirrred curd cheese, when added in powdered from with saltt is shown in Figure 5.3. There was no significant differernce in recovery of vitamin D between the two treatmernts. Recovery of vitamin D when added with salt was muuch lower than when added to the cheese milk. Composition of these cheeses is shown in Table 5.3.

CONCLUSIONS

Encapsulated vitamin D in encapsulated form resultted in the best retention in cheese curd.

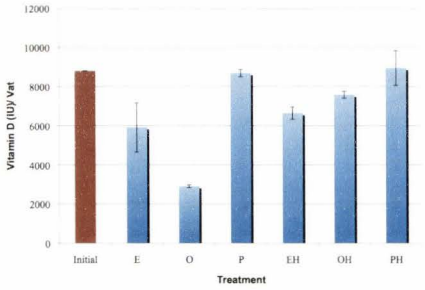

Figure 5.1. Recovery of vitamin D in cheese curd with six treatments: E – emulsion, non-homogenized; O – oil, non-homogenized; P – powder, non-homogenized; EH – emulsion homogenized; OH – oil homogenized; PH – powder homogenized.

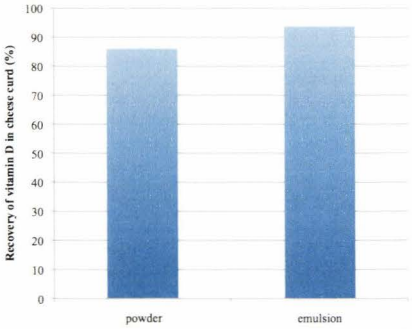

Figure 5.2. Percent recover of vitamin D in cheese curd when added in powdered and emulsified form.

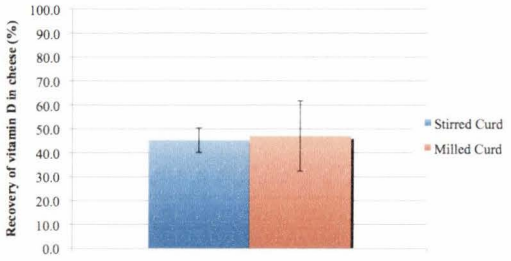

Figure 5.3. Retention of vitamin D in cheese when added to curd with salt.

Table 5.1. Composition of cheeses fortified with vitamin D emulsion, oil, and powder, with homogenization and non-homogenization treatments.

Treatment	Moisture (%)	Fat (%)	pH
Vitamin D emulsion, non-homogenized, Rep 1	38.40	31.5	4.98
Vitamin D emulsion, non-homogenized, Rep 2	38.65	33	4.92
Vitamin D emulsion homogenized, Rep 1	38.30	33	5.02
Vitamin D emulsion homogenized, Rep 2	38.74	31	4.97
Vitamin D powder, non-homogenized, Rep 1	38.50	33	4.95
Vitamin D powder, non-homogenized, Rep 2	39.44	31	494
Vitamin D powder, homogenized, Rep 1	38.74	33	4.95
Vitamin D powder, homogenized, Rep 2	39.51	30	4.98
Vitamin D oil, non-homogenized, Rep 1	38.22	31.5	4.94
Vitamin D oil, non-homogenized, Rep 2	39.37	32.5	4.96
Vitamin D oil, homogenized, Rep 1	37.80	33	5.03
Vitamin D oil, homogenized, Rep 2	39.29	31	4.96

Table 5.2. Composition of cheeses cheese fortified with powdered or emulsified vitamin D.

Treatment	Moisture (%)	Fat (%)	pH
Vitamin D emulsion, Rep 1	38.3	32	4.86
Vitamin D emulsion, Rep 2	38.9	32	4.79
Vitamin D powder, Rep 1	37.9	32	4.95
Vitamin D powder, Rep 2	38.4	32	4.83

Table 5.3. Composition of cheese fortified with vitamin D by addition with salt.

Treatment	Moisture (%)	Fat (%)	pH
Milled curd, Rep 1	37.80	33.0	5.16
Milled curd, Rep 2	37.83	33.0	5.12
Stirred curd, Rep 1	36.88	33.0	5.35
Stirred curd, Rep 2	37.01	33.0	5.45

DTIL Project 6:
Evaluate the flavor of vitamin D fortified cheese by descriptive and consumer taste panels

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

It is anticipated that FDA will increase the daily dietary Vitamin D recommendation for humans from 400 to 1000 IU. Cheese may be a good vehicle for increasing Vitamin D consumption. However, there is a potential for Vitamin D to contribute off flavors to cheese and whey, especially oxidized flavors. Data on the flavor profile of Vitamin D fortified cheese is not available. This project evaluated the flavor of vitamin D fortified cheese at 3, 6, and 9 months of age.

MATERIALS AND METHODS

Powdered vitamin D was added to cheese milk to obtain final fortification levels of 200 and 400 IU/serving. Cheese was made from 113 kg of whole milk as described in Project 5. A control cheese was made without vitamin D fortification. Cheeses were stored at 5.5°C until sampled for analysis. Proximate analysis was conducted on the cheese at 5 days of age. Consumer taste panels were conducted on all cheeses at 3 and 6 months of age. Descriptive taste panels were conducted on all cheeses at 3, 6, and 9 months of age. The cheeses were analyzed for vitamin D content at 0, 3, 6, and 9 months of age.

RESULTS AND DISCUSSION

Chemical analysis of vitamin D in cheese curd and whey showed that up to 90% of vitamin D added to milk was retained in cheese curd at both levels of fortification, while the remaining 10% was found in whey (data not shown). Stability of Vitamin D3 in Cheddar cheese during ageing was verified by chemical analysis of vitamin D3 levels at 0, 3, 6, and 9 mo for Cheddar cheese fortified at 200 and 400 IU/serving. Vitamin D3 content did not change significantly for both fortification levels from 0 to 9 months (Fig. 6.1). Since vitamin D levels were stable across ageing Cheddar cheese appeared to be a suitable vehicle for consistent delivery of vitamin D.

A consumer preference taste panel was conducted on vitamin D-fortified Cheddar cheese at 3, 6, and 9 mo. Even at 3 mo there was no significant difference ($p > 0.05$) in mean liking scores between the control and fortified cheeses (Fig. 6.2). Further consumer preference taste panels conducted on 6 mo and 9 mo old fortified Cheddar cheese verified the lack of any significant difference ($p > 0.05$) in scores between the unfortified and fortified cheeses.

Descriptive taste panels were conducted at 3, 6, and 9 mo for Cheddar Cheese supplemented with Vitamin D at 200 IU/serving and 400 IU/serving (Fig. 6.3-6.5). Among all attributes tested bitter, salty, and sour flavors were significantly ($p<0.05$) differently perceived in the 3 mo old cheese; whereas bitter, buttery, metallic, sour, sweet, and umami flavors were significantly ($p<0.05$) different at 6 mo and only sour flavor was significantly ($p<0.05$) different at 9 mo. Perception of buttery flavor decreased with increasing vitamin D levels in cheese at 6 mo. However considering that this difference was not noted at 3 or 9 mo, the correlation at 6 mo is unlikely to be attributable to vitamin D levels. Notably none of the other flavor differences were correlated to change in vitamin D concentrations, suggesting that these flavor differences are probably not linked with the addition of vitamin D to cheese. In summary differences found in Cheddar cheese flavor perception during ageing are likely not linked to vitamin D addition.

A consumer preference taste panel was conducted on Cheddar cheese fortified at 200 IU/serving, and 400 IU/serving, and a non-fortified control cheese at three (Figure 6.6) and six (Figure 6.7) months of age. The cheeses were scored on a nine point hedonic scale: 1) Dislike extremely, 2) Dislike very much, 3) Dislike moderately, 4) Dislike slightly, 5) Neigher like nor dislike, 6) Like slightly, 7)

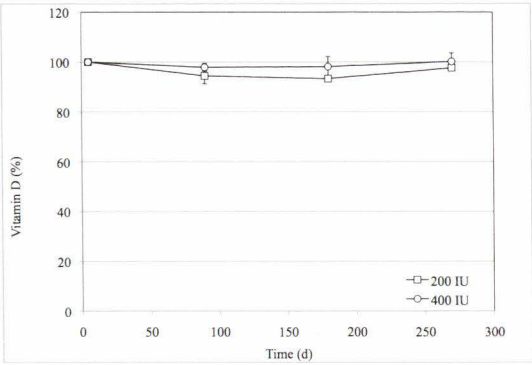

Figure 6.1. Retention of Vitamin D (% of level at 5 d) in Cheddar Cheese during ageing.

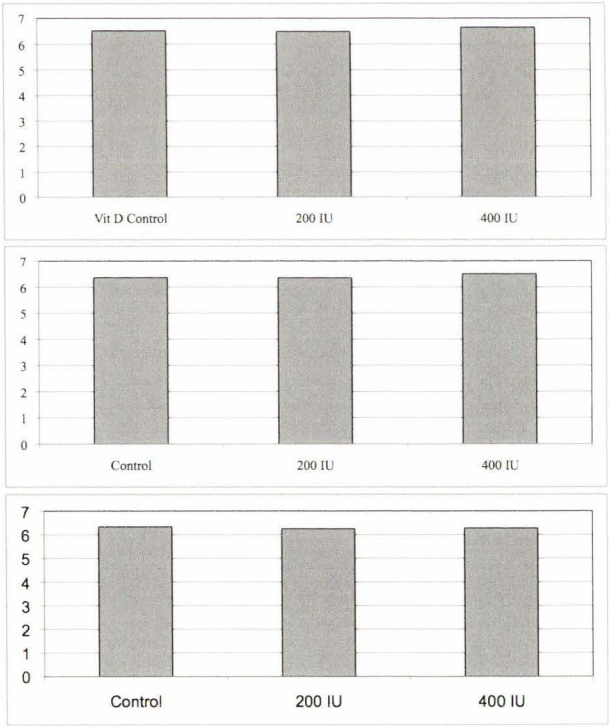

Figure 6.2. Mean liking scores (y axis) of a consumer preference taste panel for Cheddar cheese fortified with Vitamin D at 200 IU/serving, 400 IU/serving and the non-fortified control cheese (x axis) at 3 mo (top panel), 6 mo (middle panel), and 9 mo (bottom panel).

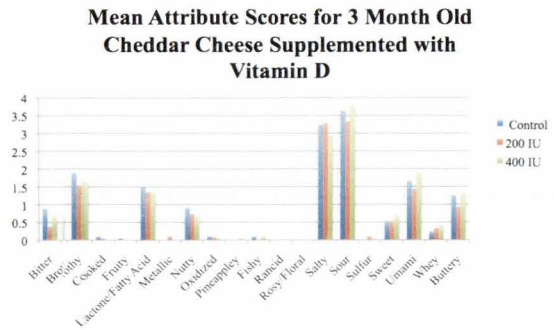

Figure 63.3 Mean attribute scores for 3 month old Cheddar cheese supplemented with Vitamin D at 200 IU/serving and 400 IU/serving.

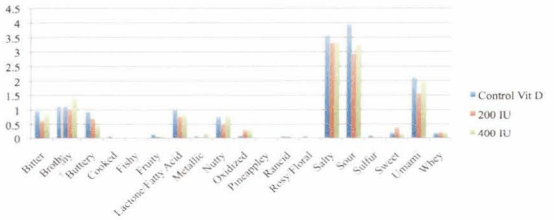

Figure 63.4. Mean attribute scores for 6 month old Cheddar cheese supplemented with Vitamin D at 200 IU/serving and 400 IU/serving.

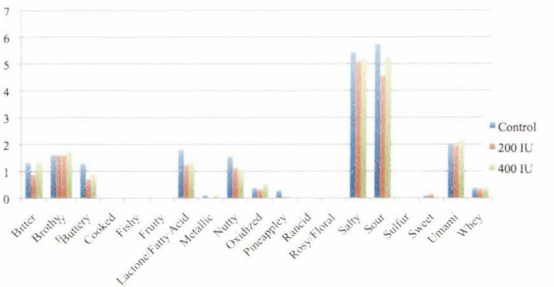

Figure 63.5. Mean attribute scores for 9 month old Cheddar cheese supplemented with Vitamin D at 200 IU/serving and 400 IU/serving.

Like moderately, 8) Like very much, 9) Like extremely. The mean liking scores are shown in Figures 6.6-6.7. There were no significant difference in scores between the control and fortified cheeses.

Descriptive taste panels were conducted at 3, 6 and 9 months on Cheddar cheeses supplemented with Vitamin D at 200 IU/serving and 400 IU/serving and a non-fortified control cheese. There were no significant differences between vitamin D treatments for any attributes tested in the 3 month old cheese. Figure 6.3 and 6.4 show the mean attribute scores for 3 month old and 6 month old cheese respectively.

CONCLUSIONS

Fortification of Cheddar cheese with vitamin D3 improves its nutritional quality. However the flavor of Cheddar cheese is a complex attribute that arises from the myriad compounds found in the cheese matrix produced by bacterial metabolism. The addition of a novel component may alter this perception and the vitamin may be susceptible to bacterial degradation. We found that vitamin D3 is stable in Cheddar cheese aged for 9 mo and at all stages of cheese ageing the flavor perception of fortified cheeses was similar to unfortified cheese. Hence the addition of Vitamin D does not impact the flavor of Cheddar cheese.

Bacterial cultures used in this study are listed in Table 1. Starter and probiotic adjunct cultures were obtained from commercial sources, while representative NSLAB cultures came from the LAB Culture Collection at Utah State University and as gifts from other university researchers. Lactococcal cultures were grown in M17 lactose (M17-L) broth (Difco, Becton, Dickson and Co., Sparks, MD), *Bifidobacterium* strains were maintained in MRS Broth (Criterion, Hardy Diagnostics, Santa Maria, CA) supplemented with 0.5% cysteine (Fisher Scientific, Fair Lawn, NJ), while all other LAB cultures were propagated in MRS broth. *Lactococcus* was incubated at 30°C and the rest of the cultures were grown at 37°C.

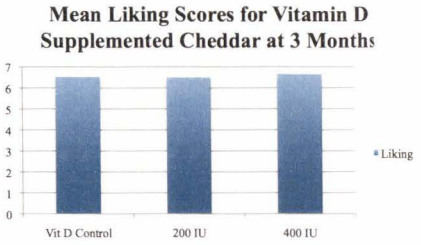

Figure 6.6. Mean liking scores of a consumer preference taste panel for three-month old Cheddar cheese fortified with Vitamin D at 200 IU/serving, 400 IU/serving and the non-fortified control cheese.

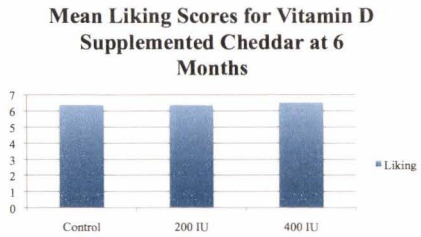

Figure 6.7. Mean flavor attribute scores for 3 mo old Cheddar cheese fortified with Vitamin D at 0 (control), 200, and 400 IU/serving. Significantly different ($p \leq 0.05$) attibutes are indicated by a *.

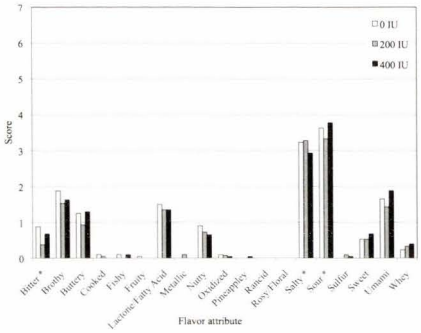

Figure 6.8.

DTIL Project 7: Develop emulsions containing vitamin D to improve retention in cheese curd

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

It is anticipated that FDA will increase the daily dietary Vitamin D recommendation for humans from 400 to 1000 IU. Cheese may be a good vehicle for increasing Vitamin D consumption. In previous studies, Vitamin D retention in cheese ranged from 40-60%, depending on the vitamin preparation. More efficient techniques for adding Vitamin D to cheese milk need to be developed to improve Vitamin D retention in the cheese and reduce losses in the whey. This project will develop dairy-based vitamin D emulsions in order to improve the retention in curd.

MATERIALS AND METHODS

Emulsions were formulated using Vitamin D_3 as the oil phase and a 2% casein protein as an emulsifier. The final pH of the emulsion were maintained at 7.0 using buffer phosphate to avoid Vitamin decomposition. The variables studied were:

Casein type and concentration.
- Sodium caseinate
- Calcium caseinate
- Skim milk powder
- Whey protein concentrate

Homogenization condition.
- High shear (HS)
- High pressure homogenization – very low pressure (VLPH)
- High pressure homogenization (HPH)

Oil content in the emulsion.
- 1%
- 5%
- 10%

Measurements.

Physicochemical stability. The physicochemical stability of the emulsions were determined using a vertical scan macroscopic analyzer (TurbiScan MA 2000). TurbiScan consists of a reading head moving along a flat-bottomed cylindrical cell while scanning the entire sample height. The reading head consists of a pulsed near-infrared light source and two synchronous detectors. Only the backscattering (BS) detector, which receives the light backscattered by the product (135 °), was used due to the emulsion being opaque. The reading head acquires BS data every 40 μm to a maximum height of 80 mm. The profile obtained characterizes the sample's homogeneity, particle concentration, and mean diameter. The parameters are represented by a curve showing the percentage of BS light as a function of the sample height in mm. The acquisition along the product is repeated with programmable frequency obtaining a superimposition of sample fingerprints, which characterize the stability or instability of the sample (e.g., the more identical the readings, the more stable the system). After forming the emulsions, they were placed in an assay tube and held at room temperature. BS measurements were taken as a function of time until the emulsions were significantly unstable. Emulsions' destabilization kinetics were measured by calculating the variation in BS as a function of time at half the maximum of the BS peak value with respect to the initial reading.

Droplet size distribution. Droplet size distributions for all the emulsions were determined using a Beckman Coulter particle characterization equipment (LS20 Version 3.19, Beckman Coulter Inc.). Isolated droplets were measured with this equipment as evidenced by the lack of flocculation observed in the emulsions when observed under a microscope (data not shown).

Retention of the Vitamin D. The retention of Vitamin

Table 7.1. Emulsifiers used for the retention experiments in vitamin D fortified cheese. Skim milk povder (SMP), whey protein concentrate (WPC80), calcium (CaCas) and sodium (NaCas) caseinate.

Emulsifier (E)	Protein (%)	Amount of E added	Source
SMP	36	5.57	
WPC80	80	2.5	Grande
CaCas	94.2	2.12	Erie Foods International, Inc. (Rochelle, IL)
NaCas	94	2.13	Erie Foods International, Inc. (Rochelle, IL)

Table 7.2. Characteristics of the materials used as oil phase.

Oil	Company
Soybean Oil (SBO)	Bunge Limited: White Plains, NY.
Vitamin D (vitD)	BASF Corp: Florham Park, NJ
Pre-emulsified vitD (control)	Continental Custom Ingredients, Inc.: Chicago, IL.

D into cheese curd was determined on a laboratory scale by adding the emulsion to milk and performing a curd syneresis experiment. Appropriate amounts of the Vitamin D emulsion was added to 10 g of milk weighed into centrifuge tubes and heated to 35°C. Then 0.02 ml of diluted rennet plus 0.2 g glucono-delta-lactone was added and the milk incubated at 35°C for 30 min. After coagulation, two perpendicular cuts were made in the curd and the samples centrifuged at 250 x g at 25 °C for 20 min. After centrifugation, the volume of released whey was measured and expressed as a percentage of total moisture (g) initially present in the sample, and the Vitamin D content of the whey measured. Partitioning of the Vitamin D between the curd and the whey was calculated and used to predict its retention during cheese making.

Statistical analysis. Experiments were performed in duplicate or triplicate as necessary. Data reported are the mean and standard deviation values calculated from the replicates. Significant differences were analyzed using a Two- or One-way ANOVA test, as appropriate, and a Bonferroni post-test ($\alpha = 0.05$). Statistical analysis was performed using Graph Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Vitamin D determination. Vitamin D content in the cheese curd and whey was determined using the AOAC official method 952.29.

Emulsion formulation.

Five percent oil-in-water emulsions were formulated using vitamin D and soybean oil (SBO) as the oil phase, and different emulsifiers as stabilizers. Skim milk powder (SMP), whey protein concentrate (WPC80), calcium (CaCas) and sodium (NaCas) caseinate were used as emulsifiers to give 2% protein in the emulsion. Table 7.1 presents a summary of the emulsifiers used, their amounts and the sources of these ingredients. For the oil phase, vitamin D was mixed with SBO in a 50:50 ratio. A control was performed using a pre-emulsified vitamin D source. Details about these ingredients are presented in Table 7.2.

The emulsifiers were dissolved in a 0.01M Na_2HPO_4-$7H_2O$ to obtain a final concentration of 2% in protein. The two phases (oil and water) were pre-homogenized using high shear force for 1 min at 18,000 rpm, and then homogenized with a microfluidizer at ~2500psi.

Bench-top cheese.

Bench-top cheeses were formulated by adding the emulsions described above to pasteurized (72 °C 15 s) skim and whole milks (Gary H. Richardson Dairy Products Lab, Utah State University, Logan, UT). For each sample, 200 μl of the vitamin D emulsions or 125 μl of the pre-emulsified vitamin D were added to the milk to obtain approximately 250 IU per gram of curd. These fortified milks were used to make the bench-top cheese. Two-hundred ml of each fortified milk treatment were prepared in triplicate in 250 ml polycarbonate Nalgene centrifuge bottles. The fortified milk was then heated in a waterbath to 72° C, after which they were cooled to 35 °C. Glucono-delta-lactone and double strength rennet (Maxiren DS, DSM Food Specialties, Parsippany , NJ) diluted to 1.8 rennet units per ml with cold water were added to each mixture at 2% w/v and 0.2% v/v respectively. Mixtures were then incubated 30 min at 35°C to allow coagulum formation. Following incubation, coagulum was cut and centrifuged at 250 x g at 25 °C for 30 min. Syneresis was measured immediately following centrifugation and again after 30 min by volume of decanted whey. Percent curd was calculated for each by finding the difference in volume between the initial 200 ml and decanted whey and then dividing that difference by 200 ml. Observations were made and recorded on both curd and whey properties; 100 g of whey and over 5 g of curd from each treatment was retained and frozen for further analysis.

RESULTS AND DISCUSSION

Droplet size and standard deviations for the four

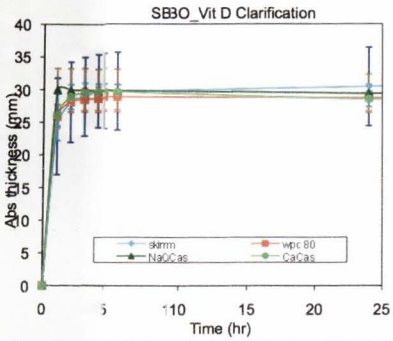

Figure 7.1. Detabilizationn kinetics (clarification) of Vitamin D emulsions formiated with i different emulsifiers.

Table 7.3. Droplet size of the emulsions at time zero.

Emulsifier	$D_{(3,2)}$ (µm)	SD
WPC	3.360	0.040
Skim milk	1.258	0.085
NaCas	4.454	1.062
CaCas	6.153	0.234

Table 7.4. Droplet size (D3,2) of emulsions formulated with different emulsifiers.

D(3,2)	Average	std dev
CaCas	0.713	0.068
NaCas	0.730	0.094
WPC80	0.733	0.033
SMP	0.718	0.037

Table 7.5. \olume obf whey (mL), weight of curd (g), moisture content (%), and curd yield (%) for the bench-top cheeses.

SKIM	Volurne (mL)	Std E	Curd (g)	std E	Moisture (%)	std E	Curd (%)	std E
SMP	1448.0	4.7	40.7	2.5	76.2	0.8	26.0	2.4
WPC80	1443.7	10.2	47.3	6.5	75.6	1.8	28.2	5.1
NaCas	1449.3	2.4	44.2	1.8	76.7	0.4	25.3	1.2
CaCas	1555.2	2.5	37.5	2.2	75.3	0.6	22.4	1.3
Control	1447.7	3.8	45.8	5.2	77.3	1.3	26.2	1.9
WHOLE	**Voblume**	**Std E**	**Curd (g)**	**std E**	**Moisture (%)**	**std E**	**Curd (%)**	**std E**
SMP	1550.7	5.6	46.0	5.7	67.6	2.0	24.7	2.1
WPC80	1557.0	2.0	41.3	0.6	65.4	1.4	21.5	5.3
NaCas	1553.3	0.9	44.0	2.3	67.8	0.4	23.3	0.9
CaCas	1444.7	1.5	48.5	1.5	68.7	1.3	27.7	2.6
Control	1449.0	2.5	49.4	4.4	69.5	1.4	25.5	2.2

different emulsions are shhown in Table 7.3. Stability of the emulsions as deermined by vertical scattering turbiddity is shown in Figure 7.1 Enmulsions were very unstable, with separation occuring withhin two minutes.

Droplet size summaary of emulsions.

After formuation, thhe droplet size distribution of the emulsions was ceterminee using Beckman backscattering equipment. Tabe 7.4 shoows the average droplet size of all emulsions expreised as D(3,2) values.

From Table 7.4 it can be observed that different emulsifiers did rot affect t the droplet size of the emulsions under the proceising conndditions used. Figure 7.2 shows the stability of the emnulsions as the thickness of the separating layer as a funnction of time. All the emulsions were destabilizel throughh a creaming phenomenon when stored at 5C. From Figuure 6.2 it can be observed that all emulsions showed good s stability for the first 6 hours after which they slowly destabbilized with time. After 24 hours a creaming layer of approximately 1 mm was observed. When emulsions were stored for 7 days, the creaming layer increased to 3-5 mm. After 24 hours the most stable emulsion was the one formulated with SMP as emulsifier and the least stable was the one formulated with WPC80.

Bench-top curd.

The volume of whey, the weight of curd, the moisture content of the curd, and the percent of curd yield was calculated. Results are presented in Table 7.5. The amount of whey obtained represented approximately 74-80% of the initial volume of milk with the curd equaling 20-26% of the initial volulme.

The Vitamin D content present in the whey and curd obtained from the bench-top cheeses was determined by a private laboratory (O'Neil). Table 7.6 presents the values obtained, while Table 7.7 present the percentage of vitamin D that was retained by the crud and the percent lost in the whey.

CONCLUSIONS

Dairy protein based emulsions of vitamin D were developed with improved the retention of vitamin D in cheese curd over commercially available emulsions.

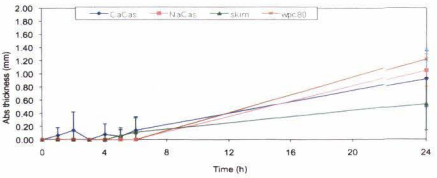

Figure 7.2. Physicochemical stability of emulsions formulateted with SMP, WPC80, CaCas and NaCas as emulsifiers.

Table 7.6 Vitamin D content of the curd and whey obtained from the bench-top cheeses. The expected vitamin D content in the curd is 250 IU per g of curd.

Whey				
Whole milk	**Average IU/g**	**std dev**	**Ave % of IU/g**	**std % of IU/g**
Ctrl	1.31	0.37	0.24	0.15
SMP	0.37	0.04	0.19	0.02
WPC80	0.56	0.05	0.18	0.02
NaCas	0.46	0.01	0.24	0.01
CaCas	0.62	0.19	0.32	0.08
Skim milk				
Ctrl	0.94	0.13	0.49	0.05
SMP	1.29	0.35	0.54	0.14
WPC80	1.32	0.65	0.37	0.26
NaCas	1.04	0.20	0.35	0.08
CaCas	0.86	0.07	0.35	0.03
Curd				
Whole milk	**Average IU/g**	**std dev**	**Ave % of IU/g**	**std % of IU/g**
Ctrl	57.00	6.28	21.95	2.51
SMP	66.10	5.87	31.23	2.35
WPC80	66.30	8.45	28.21	3.38
NaCas	76.83	2.88	33.84	1.15
CaCas	73.47	5.00	25.01	2.00
Skim milk				
Ctrl	55.55	6.07	17.99	2.43
SMP	58.70	39.96	44.59	15.99
WPC80	92.57	15.53	38.73	6.21
NaCas	97.00	25.22	41.36	10.09
CaCas	114.33	4.46	42.39	1.78

Table 7.7. Percentage of recovery of vitamin D in the curd and whey.

	% Whey recovery		**% Curd recovery**	
Whole milk	**Average**	**std E**	**Average**	**std E**
Ctrl	2.4	0.4	97.6	0.4
SMP	0.5	0.0	99.5	0.0
WPC80	0.8	0.1	99.2	0.1
NaCas	0.5	0.0	99.5	0.0
CaCas	1.0	0.2	99.0	0.2
Skim milk	**Average**	**std E**	**Average**	**std E**
Ctrl	2.1	0.3	97.9	0.3
SMP	1.2	0.1	98.8	0.1
WPC80	1.3	0.3	98.7	0.3
NaCas	1.0	0.0	99.0	0.0
CaCas	0.8	0.1	99.2	0.1

DTIL Project 8:
Evaluate and improve plating techniques for the enumeration of probiotic bacteria in cheese

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MATERIALS AND METHODS

Thhe objectives of this project were to determine the effectiviveness of selective media for enumerating probiotic LAB such as *Lb. acidophilus*, *Bifidobacterium* and *Lb. caasei*, when they are used as adjunct cultures in the manufacture of cheese, and do develop media which will result i in more accurate counts of the bacteria of interest by eliminaating the growth of competing organisam. A number of pubblished media were initially screened for their ability to diffeferentiate between selected LAB genera and species (unpubblished data) and 6 selected for cheese microflora enumeeration.

Baacterial cultures and propagation.

Baacterial cultures used in this study are listed in Table 8.1. Starter and probiotic adjunct cultures were obtainced from commercial sources, while representative NSLAAB cultures came from the LAB Culture Collection at Utaah State University and as gifts from other university researcchers. Lactococcal cultures were grown in M17 lactosee (M17-L) broth (Difco, Becton, Dickson and Co., Sparks.s, MD), *Bifidobacterium* strains were maintained in MRS I Broth (Criterion, Hardy Diagnostics, Santa Maria, CA) ssupplemented with 0.5% cysteine (Fisher Scientific, Fair LLawn, NJ), while all other LAB cultures were propagggated in MRS broth. *Lactococcus* was incubated at 30ºC aand the rest of the cultures were grown at 37 C.

Teesting of enumeration media.

Sixix media were selected for enumeration of specific bacteririal types in probiotic cheese as listed in Table 8.2, along with their incubation parameters. Two base media were uused, M17-L and Lactobacilli MRS with a number of additives including lactose (Fisher Scientific), sorbitol (Acros Organics, Morris Plains, NJ), vancomycin (Sigma-Aldrich Inc., St. Louis, MO), bromocresol green (J.T. Baker Chemical Co., Phillipsburg, NJ).

Each culture was grown for 24 h in the appropriate media at the optimum incubation temperature. Cultures were diluted using 9-ml dilution blanks containing sterile 0.1% (wt/vol) peptone phosphate buffer. Each dilution was plated in triplicate by using the pour plate method with each of the selected media. *Lactococcus* cultures were incubated at 30 °C aerobically. *Lactobacillus* and *Bifidobacterium* strains were incubated in gaspaks (Oxoid....) at 37 °C. *Lactobacillus* strains were also incubated anaerobically at several temperatures (37, 45, and 50 °C) to determine the effect of incubation temperature on growth. M17-Lplates were examined at 24 and 48 h while all other media were examined at 48 and 72 h. In addition to examining all culture strains used in the manufacture of probiotic-enhanced cheese, a number of representative NSLAB strains were also tested on all 6 media. Lactococcal media was incubated aerobically at 30ºC, *Lb. acidophilus* media was incubated at 45ºC in a gaspak, with all other media were incubated at 37ºC in a gaspak.

RESULTS AND DISCUSSION

From the initial screening of selective media, it was observed that adding 10 mg/g sorbitol to MRS agar that was incubated anaerobically at 37 °C for 48 h, provided the highest overall bacterial counts for the starter and adjunct cultures used in this study. This media (designated MRS+S) was subsequently used to obtain the total LAB count in the cheese samples. All of the bacterial strains grew on media developed particularly for their enumeration and fit the expected profile of growth or inhibition (Table 8.4), but these media were not exclusionary.

Table 8.1. Bacterial cultures used in this study.

Culture	Genus/Species	Function	Source
DVS850	*Lc.*[1] *lactis ssp. lactis*	Starter	Chr. Hansen[a]
LH32	*Lb.*[2] *helveticus*	Flavor	Chr. Hansen
LA-5	*Lb. acidophilus*	Probiotic	Chr. Hansen
L 10	*Lb. acidophilus*	Probiotic	DSM[b]
Bif-6	*Bf.*[3] *lactis*	Probiotic	Cargill[c]
BB-12	*Bf. lactis*	Probiotic	Chr. Hansen
CRL 431	*Lb. casei*	Probiotic	Chr. Hansen
L26	*Lb. casei*	Probiotic	DSM
F19	*Lb. casei/paracasei*	Probiotic	Medipharm[d]
367	*Lb. brevis*	NSLAB[4]	USU[e]
1364P	*Lb. brevis*	NSLAB[4]	USU
M36	*Lb. casei*	NSLAB	USU
37	*Lb. casei*	NSLAB	
25598	*Lb. casei ssp. pseudoplantarum*	NSLAB	
LiLa	*Lb. paracasei*	NSLAB	
14931	*Lb. fermentum*	NSLAB	
14957	*Lb. rhamnosis*	NSLAB	
7469	*Lb. rhamnosis*	NSLAB	

[1]*Lactococcus*, [2]*Lactobacillus*, [3]*Bifidobacterium*, [4]Nonstarter lactic acid bacteria
[a]Chr. Hansen Inc., Milwaukee, WI.
[b]DSM Food Specialties USA Inc., Eagleville, PA.
[c]Cargill Inc., Waukesha, WI
[d]Medipham USA, Des Moines, IA
[e]Utah State University Lactic Acid Bacteria Culture Collection, courtesy of Prof. J. Broadbent, 8700 Old Main Hill, Utah State University, Logan, UT 84322.

For example, growth of *Lb. helveticus*, which is commonly used as a flavor adjunct in Cheddar cheese to prevent bitterness, was unattenuated on media designed for *Lb. acidophilus* in which sorbitol was the sole sugar constituent (MRS-S) and incubated at 45 °C. This was expected given the close relationship between these two species. All three of the *Lb. casei* and *Lb. paracasei* adjunct cultures similarly grew on the MRS-NNLP media that had been proposed as selective for bifodobacteria, although usually as very small colonies. One strain of *Lb. casei/paracasei* also grew on the MRS-S media (Table 8.3).

The MRS+S media gave higher bacterial counts at all test periods even above *Lb. casei* specific media in aged cheese indicating it could be used to obtain the total LAB count for cheese. It is known that NSLAB can grow on M17-L media making it difficult to count starter culture populations during cheese storage (Cogan et al., 2002), however when M17-L media was analyzed by 24 h it could be used for lactococcal enumeration. The *Lb. casei/paracasei* cultures also grew on the M17-L media but much more slowly, and could be disregarded at 24 h as pinpoint colonies. However, with longer growth times the colonies continued to grow and were indistinguishable from the lactococcal colonies. When a representative collection of NSLAB strains was grown on the selective enumeration media this general phenomenon was also observed with a variety of *Lb. casei*, *Lb. paracasei*, *Lb. fermentus*, and *Lb. rhamnosis* strains capable of forming colonies on both the *Bifidobacterium* and *Lb. acidophilus* media after 48 h of incubation (Table 8.4).

CONCLUSIONS

Published methods for enumeration bacteria in cheese by plating on selective media were found to be limited to young cheese before the growth of high numbers of nonstarter lactic acid bacteria that have the ability to grow on the media used in this study. Enumeration of lactococci, bifidobacteria, or *Lactobacillus acidophilus* in aged cheese is inaccurate due to the lack of selectivity of the media,. Modifications were made to the media to improve accuracy during the first four month of cheese age, and prior to the propagation of non-starter lactic acid bacteria.

Table 8.2. Agaar media used to determine specific lactic acid bacteria (LAB) in cheese.

Media	Incubation °C	Incubation *Oxygen*	Presumptive LAB Selection	Reference
M17-L[1]	30	aerobic	*Lactococcus*	
MRS+S[2]	37	anaerobic	Total LAB	unpublished data
MRS-S[3]	45	anaerobic	*Lb. acidophilus*	Dave and Shah (1996)
RCA-BV[4]	37	anaerobic	*Lb. casei*	Darukaradhya et al. (2006)
MRS-V[5]	37	anaerobic	*Lb. casei*	Tharmaraj and Shah (2003)
MRS-NNLP[6]	37	anaerobic	*Bifidobacterium*	Laroia and Martin (1991)

[1]M17-Lactose
[2]MRS media with a added sorbitol
[3]MRS media with s sorbitol substituted for dextrose.
[4]Reinforced clostridiidial agar with added bromocresol green and vancomycin.
[5]MRS media with a added vancomycin
[6]MRS media with a added cysteine and antibiotic mixture consiting of nalidixic acid, neomycine sulfate, lithium chloride and paromoycine sulfaifate.

Table 8.3. Grcrowth of cultures[1] added during cheesemaking on selective enumeration media[2].

Cultures	Media M17-L	MRS+S	MRS-V	MRS-NNLP	MRS-S	RCA-BV
DVS850	2.2×10^{9}	1.9×10^{9}	$<10^{4}$	$<10^{4}$	$<10^{4}$	$<10^{4}$
LH 32	$<10^{4}$	5.1×10^{7}	$<10^{4}$	$<10^{4}$	5.1×10^{7}	$<10^{4}$
LA 5	$<10^{4}$	9.2×10^{7}	$<10^{4}$	$<10^{4}$	8.0×10^{8}	9.2×10^{7}
L 10	$<10^{4}$	1.1×10^{9}	$<10^{4}$	7.0×10^{8}	9.0×10^{8}	
L10	$<10^{4}$	1.8×10^{9}	$<10^{4}$	2.0×10^{9}	3.4×10^{9}	$<10^{4}$
L 26	$<10^{4}$	2.4×10^{9}	2.2×10^{9}	3.5×10^{9}	$<10^{4}$	1.9×10^{9}
CRL431	$<10^{4}$	2.2×10^{9}	2.2×10^{9}	2.6×10^{9}	$<10^{4}$	2.4×10^{9}
F-19	$<10^{4}$	6.0×10^{8}	1.2×10^{9}	2.0×10^{9}	5.0×10^{8}	8.0×10^{8}
Bif 6	$<10^{4}$	1.4×10^{9}	$<10^{4}$	8.0×10^{8}	$<10^{4}$	$<10^{4}$
BB 12	$<10^{4}$	1.8×10^{9}	$<10^{4}$	9.0×10^{8}	$<10^{4}$	$<10^{4}$

[1]See Table 1 for c culture descriptions.
[2]See Table 2 for m media descriptions and incubation conditions.

Table 8.4. Grcowth of nonstarter lactic acid type bacterial strains[1] on presumptive selective media[2].

Strains	Media M17-L	MRS+S	MRS-V	MRS-NNLP	MRS-S	RCA-BV
367	5.5×10^{9}	ND	1.0×10^{9}	$<10^{2}$	$<10^{2}$	7.1×10^{9}
1364P	$>10^{7}$	2.0×10^{9}	1.7×10^{9}	$<10^{2}$	$<10^{2}$	1.5×10^{9}
M 36	$>10^{7}$	2.7×10^{9}	3.1×10^{9}	3.3×10^{9}	2.2×10^{9}	3.3×10^{9}
M36	3.1×10^{9}	ND	2.4×10^{9}	1.9×10^{9}	1.0×10^{7}	2.4×10^{9}
37	2.5×10^{9}	ND	2.7×10^{9}	2.5×10^{9}	1.0×10^{7}	2.6×10^{9}
25598	1.1×10^{9}	ND	1.2×10^{9}	1.1×10^{9}	1.1×10^{9}	8.6×10^{8}
LiLa	2.9×10^{9}	ND	2.4×10^{9}	1.0×10^{7}	$<10^{2}$	2.6×10^{9}
14931	$<10^{2}$	1.5×10^{9}	1.6×10^{9}	$>1.0 \times 10^{7}$	1.6×10^{9}	1.9×10^{9}
14957	$>10^{7}$	2.0×10^{9}	1.9×10^{9}	1.9×10^{9}	1.9×10^{9}	2.0×10^{9}
7469	$>10^{7}$	6.0×10^{8}	6.0×10^{8}	$> 10^{7}$	7.0×10^{8}	7.0×10^{8}

[1]See Table 1 for ciculture descriptions.
[2]See Table 2 for mmedia descriptions.

DTIL Project 9: Develop techniques using qPCR to enumerate probiotic bacteria in cheese

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Funded by: Dairy Management Inc., September 2007 - December 2009

MATERIALS AND METHODS

Extraction of genomic DNA from cheese.

Total genomic DNA was extracted from cheese using the TRizolLS reagent (Invitrogen, Carlsbad, CA). Grated cheese (0.25 g) was suspended in TrizolLS (500 μL) along with glass beads (0.3 g, size 0.1 mm; sterile acid washed; BioSpec Products Inc., Bartlesville, OK). The cheese suspension was disrupted on a Mini-Beadbeater (BioSpec Products) for 30 s to lyse bacteria and shaken with chloroform (200 μL) for 30 s to allow phase separation. The top aqueous phase was removed after centrifugation (12,000 × g for 15 min at room temperature) and DNA was precipitated from the organic phase with 100% ethanol for 3 min. The DNA was collected by centrifugation (2,000 × g for 5 min at 4°C), washed twice with 0.1M sodium citrate in 10% ethanol (30 min at room temperature), once with 75% ethanol (15 min at room temperature), pelleted, air-dried, and resuspended in autoclaved ddH_2O.

Quantitative PCR.

Bacterial levels in cheese were determined by quantitative PCR (qPCR). All bacterial primers were selected from previous studies that designed genus- and species-specific primers for the starter and probiotic organisms (Table 9.1). Briefly, 1μL of the total DNA

Table 9.1. Sequences of primers used for genus- or species-specific 16s ribosomal gene qPCR and DNA amplification for phylogenetic array analysis.

Organism	Specificity	Primer sequence	Application	Reference
Bifidobacterium lactis	Species	F: 5' - GTGGAGACACGTTTCCC - 3'	qPCR	(Ventura et al., 2001)
		R: 5' - CACACACACAATCAATAC - 3'	qPCR	
Lactobacillus acidophilus	Species	F: 5' - GAGGCAGCAGTAGGGAATCTTC - 3'	qPCR	(Delroisse et al., 2008)
		R: 5' - GGCCAGTTACTACCTCTATCCTTCTTC - 3'	qPCR	
Lactobacillus casei/paracasei	Species	F: 5' - GCACCGAGATTCAACATGG - 3'	qPCR	(Byun et al., 2004)
		R: 5' - GGTTCTTGGATYTATGCGGTATTAG - 3'	qPCR	
Bifidobacterium	Genus	F: 5' - CTCCTGGAAACGGGTGG - 3'	qPCR	(Matsuki et al., 2004)
		R: 5' - GGTGTTCTTCCCGATATCTACA - 3'	qPCR	
Lactobacillus	Genus	F: 5' - TGGAAACAGRTGCTAATACCG - 3'	qPCR	(Byun et al., 2004)
		R: 5' - GTCCATTGTGGAAGATTCCC - 3'	qPCR	
Bacterial 16s rDNA	Universal	F: 5'-AGAGTTTGATCCTGGCTCAG-3'	Phylochip	(Brodie et al., 2007)
		R: 5'-ACGGCTACCTTGTTAGCACTT-3'	Phylochip	

extracted from cheese was used in a 25μL reaction that included qPCR master Mix (HotStart-IT® SYBR® Green, USB Corp., Cleveland, OH) and 10 pmol of genus- and species-specific primers targeting the 16s ribosomal gene (Table 2). The qPCR was performed on a DNA Engine OPTICON2 (Bio-Rad Labs Inc., Hercules, CA) with initial enzyme activation at 95°C for 5 min, followed by 40 cycles of: denaturation at 95°C for 15 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min.

RESULTS AND DISCUSSION

All bacterial primers were selected from previous studies that designed genus- and species-specific primers for the starter and probiotic organisms (Table 9.1). Briefly, 1μL of the total DNA extracted from cheese was used in a 25μL reaction that included qPCR master Mix (HotStart-IT® SYBR® Green, USB Corp., Cleveland, OH) and 10 pmol of genus- and species-specific primers targeting the 16s ribosomal gene (Table 9.1). The qPCR was performed on a DNA Engine OPTICON2 (Bio-Rad Labs Inc., Hercules, CA) with initial enzyme activation at 95°C for 5 min, followed by 40 cycles of: denaturation at 95°C for 15 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min.

Standard curves were created to count different bacterial populations (Fig. 9.1). Bacterial plate counts on MRS agar for lactobacilli and bifidobacteria (30-37°C, anaerobic) were compared with threshold cycle times obtained at different cell numbers (Ct) to create the standard curves.

CONCLUSIONS

qPCR methods for enumerating probiotic bacteria in cheese were developed and tested.

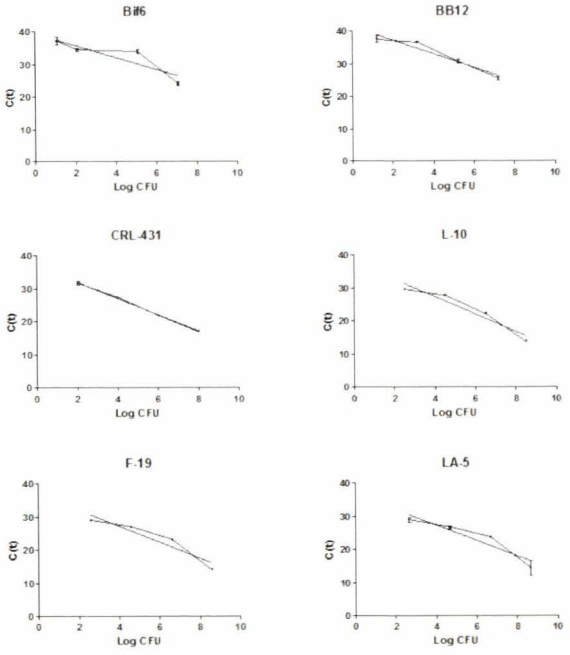

Figure 9.1 Standard curves created for qPCR analysis of 6 different probiotic bacteria. Threshold cycle times (C(t), y-axis) were compared to plate counts (log_{10}CFU, x-axis).

DTIL Project 10:
Determine the survival of probiotic bacteria in low-, reduced-, and full-fat Cheddar cheese during aging by plating on selective media and qPCR techniques

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MATERIALS AND METHODS

Cheese manufacture.

Fresh cow's milk was obtained from the George B. Caine Dairy Research and Teaching Center (Wellsville, UT) then standardized to protein to fat ratios of 0.83, 1.9 and 5.0 for making full fat (FF), 50% reduced fat (RF) and low fat (LF) cheeses, respectively, and pasteurized at 73°C for 15 s in the Gary Haight Richardson Dairy Products Laboratory at Utah State University. Aliquots of 136 kg of milk were pumped into open stainless steel vats with the milk for making the LF cheese cooled to 10°C and acidified to pH 6.4 with vinegar, while for the RF and FF cheeses the milk was warmed to the set temperature and cheese manufacturing followed as outlined in Table 10.1.

The same starter culture (see Table 10.2) was used for all cheeses and the adjunct probiotic cultures (see Table 10.2) were added to the milk at a rate of ~4 x 10^9 cells/kg which was calculated to yield 1 x 10^9 cells per 28-g serving of resultant cheese. Each probiotic culture was used 2 times.

After ripening, the cheese was renneted and the resultant curd cut with wire harps, the curd was allowed to heal for a few minutes then manually stirred and heated prior to draining. For the FF cheese, the curd was allowed to matt together then cut into slabs, cheddared and milled, while for the RF and LF cheeses the curd was washed (using about 15 kg of cool water and held in the water for 10 min) and manually stirred to prevent matting. When the curd reached the desired pH the curd was salted using 3 applications at 5-min intervals, then filled into a cheese clothlined stainless steel hoop and pressed overnight into a nominal 10-kg block. Each cheese then was cut into ~ 1-kg blocks that were vacuumed packaged and stored at 6°C.

Cheese analysis.

Proximate composition of the cheeses was determined after ~5 d as described by McMahon et al. (2009). Moisture content was by weight loss using a microwave oven, fat content by a modified Babcock method, salt by chloride measurement, and pH by glass electrode. Samples of cheese were shipped to Weber State University (Ogden, UT) for microbial analysis at 1 wk, 1, 2, 3, 4, 5 and 6 months for enumeration of bacteria by plating technique as described in Project 8 above. Cheese samples were sent to The Center for Integrated Biosystems, Utah State University (Logan, UT) for enumeration of bacteria by qPCR technique, as described in Project 9 above.

RESULTS AND DISCUSSION

Enumeration by plating technique.

In the full fat Cheddar cheese there was a general trend of decreasing lactococci counts and increasing NSLAB counts over storage time (Table 10.3). This is the generally recognized trend in changes in cheese microflora during the initial few months of storage of Cheddar cheese. Considerable variations in this trend have been observed depending on the autolytic behavior of the lactococcal starter culture, their salt-sensitivity and salt content of the cheese, cheese storage temperature, and the difficulty in differentiating between NSLAB and lactobacilli added as adjunct cultures (Shakeel-ur-Rehman et al., 2008). By counting colonies growing on M17-L agar by 24 h, the confounding effect of the NSLAB population was removed and the lactococci counts decreased from a level of 1 x 10^7 cfu/g at d 7, to 5 x 10^2 cfu/g after 180 d or storage

Table 10.1. Cheese manufacturing procedures[1] used for making full fat (FF), 50% reduced fat (RF) and low fat (LF) cheeses using 136 kg standardized milk.

Cheesemaking Steps	FF	RF	LF
Milk pH[2]	6.65	6.65	6.40
Starter culture, g	17	30	26
Ripening time, min	45	45	45
Set Temperature, °C	31	31	32
Calcium chloride[3], ml	18	0	0
Annatto[4], ml	12	12	18
Rennet[5], ml	12	10	12
Set-to-Cut time, min	30	30	25
Curd size	small	large	large
Set-to-Start of Cook time, min	65	60	35
Cooking Temperature (time), °C (min)	39 (35)	36 (15)	38 (15)
Set-to-Drain time[1], min	135	130	75
Drain pH	6.30	6.35	6.00
Wash water temperature, °C	NA	18	18
Curd wash temperature, °C	NA	29	27
Set-to-Salt time[1], min	275	210	190
Salting pH	5.40	5.90	5.90
Salt, g/kg curd	27[6]	25	26
Pressing, kPa	100	100	55

[1]Typical times with some variation based on rate of acid development.
[2]Milk for making LF cheese was pre-acidified prior to renneting with distilled white vinegar (Heinz North America, Pittsburgh, PA)
[3]35% calcium chloride solution from
[4]Double strength chymosin (Maxiren, ~650 International milk clotting units/ml, DSM Food Specialties USA Inc, Eagleville, PA).
[5]Single trength annatto solution (DSM Food Specialties USA Inc, Eagleville, PA).
[6]Approximate value for FF cheese, salt actually added at a rate of 2.95 g/kg milk.

Table 10.2. Bacterial cultures used in this study.

Culture	Genus/Species	Function	Source
DVS850	*Lc.*[1] *lactis* ssp. *lactis*	Starter	Chr. Hansen[a]
LH32	*Lb.*[2] *helveticus*	Flavor	Chr. Hansen
LA-5	*Lb. acidophilus*	Probiotic	Chr. Hansen
L 10	*Lb. acidophilus*	Probiotic	DSM[b]
Bif-6	*Bf.*[3] *lactis*	Probiotic	Cargill[c]
BB-12	*Bf. lactis*	Probiotic	Chr. Hansen
CRL 431	*Lb. casei*	Probiotic	Chr. Hansen
L26	*Lb. casei*	Probiotic	DSM
F19	*Lb. casei/paracasei*	Probiotic	Medipharm[d]

[1]*Lactococcus*, [2]*Lactobacillus*, [3]*Bifidobacterium*, [4]Nonstarter lactic acid bacteria
[a]Chr. Hansen Inc., Milwaukee, WI.
[b]DSM Food Specialties USA Inc., Eagleville, PA.
[c]Cargill.....
[d]Medipharm.....

Table 10.3. Microbiological analysis of Cheddar cheese[1] during 180 d storage at 6 °C using presumptive selective media[2] for enumerating various lactic acid bacteria.

	Media					
Time (d)	M17-L	MRS+S	MRS-V	MRS-NNLP	MRS-S	RCA-BV
7	1.0×10^7	2.1×10^7	3.1×10^5	1.7×10^2	1.3×10^4	6.0×10^5
30	1.4×10^6	9.0×10^5	2.0×10^4	$< 10^2$	8.2×10^2	1.3×10^4
60	5.9×10^5	7.3×10^6	5.9×10^5	$< 10^2$	6.3×10^3	6.4×10^5
90	1.4×10^4	1.7×10^6	6.0×10^6	2.6×10^5	1.7×10^6	3.0×10^6
120	1.4×10^2	1.3×10^7	1.3×10^7	4.0×10^6	4.8×10^6	1.5×10^7
180	5.0×10^1	3.9×10^7	4.2×10^7	8.0×10^6	4.5×10^7	4.1×10^7

[1]See Table 10.5 for cheese composition. [2]See Table 8.2 for media description.

even in the presence of NSLAB population of 4 x 10^7 cfu/g. The NSLAB population present in the full fat Cheddar cheese not onlygrew on the 2 media specifically used for enumerating *Lb casei/paracasei* (i.e., MRS-V and RCA-BV) but also grew to the same level (4 x 10^7) on MRS-NNLP (supposedly specific for bifidobacteria) and to 8 x 10^6 on MRS-S incubated at 45 °C for *Lb. acidophilus*.

In the low fat cheese, the lactococci stayed at higher level (10^5 cfu/g through 180 d storage while the NSLAB grew to 10^6 within 30 d of storage and were >10^7 cfu/g after 90 d (Table 10.4). The reason for this difference has not been determned. Salt levels in the full fat cheeses were much lower than normal (Table 10.5), and those in the low fat cheeses sligitly higher than normal, so salt-in-water concentrations for all the cheeses were in a similar range. There were also differences in NSLAB populations in the cheese between replicate batches of cheese, suggesting that different vats of cheese received differing inoculations of NSLAB during cheese manufacture. Nevertheless, it was apparent that NSLABs can grow on media that have been proposed as being selective for bifidobacteria and *Lb. acidophilus*.

Given the confounding nature of NSLAB growth on media selective for probiotic adjunct cultures it is advantageous to compare bacterial counts between media selective for *Lb. casei* that are used for enumerating NSLABs (MRS-V and RA-BV), and media used for enumerating bifidobacteria (MRS-NNLP) or *Lb. acidophilus* (MRS-S). The time point at which no clear differentiation could be made between the bacterial counts on the media was termed the crossover point. Illustrative of this concept is a comparison of bacterial counts on selective enumeration media for full fat cheese with a *Bifidobacterium* adjunct culture (Table 10.6). Up to 120 d, the bacterial counts on the MRS-NNLP media were at least one log higher than the MRS-V (or RCA-BV) media, but at 180 d the counts were nearly the same for both media. In low fat cheese supplemented with the same *Bifidobacteria* adjunct culture, the time that MRS-NNLP counts became obscured by NSLAB occurred earlier at 90 d (Table 10.7).

The same phenomenon occurred in full fat cheese supplemented with *Lb. acidophilus* adjunct culture, with MRS-S counts being much higher than counts on MRS-V (or RCA-BV) media until d 180. A composite table of crossover times for the probiotic culture adjuncts by cheese type shows a general trend that as cheese was made with less fat, the ability to confirm the presence of the adjunct culture in the cheese by using selective media decreased with time (Table 10.8).

In cheese made with *Lb. casei/paracasei* adjunct cultures, the bacterial counts on the MRS-V and RCA-BV media remained much higher than on any other selective media (Table 10.9). As shown in Table 4, the particular culture also grows unattenuated on both MRS-NNLP and MRS-S media. Initial total LAB counts of this cheese were almost 10^8 cfu/g with similar counts on the MRS-V and RCA-BV media, with perhaps a half log less on the MRS-NNLP and MRS-S media. Thus, the adjunct *Lb. casei/paracasei* culture was the initial dominant LAB species in the cheese (lactococcal counts on the M17-L media were only 10^6 cfu/g). During storage of the cheese, total LAB and MRS-V and RCA-BV counts remained constant at 10^8 cfu/g implying *Lb. casei/paracasei* culture was still present at high numbers. However, counts on MRS-NNLP media consistently decreased to 10^3 cfu/g over 180 d while counts on MRS-S media decreased to low numbers over 60 d then increased back up to 10^7 cfu/g by 180 d. This suggests that the *Lb. casei/paracasei* adjunct culture had probably died off during storage and was replaced by a growing NSLAB population that did not have the ability to grow on the MRS-NNLP media but could grow on the MRS-S media.

Enumeration by qPCR technique.

Probiotic bifidobacteria were initially added to Cheddar cheese at 2.5X10^6 CFU/gm cheese and survived the aging process with a small population reduction after 280 days (Tables 10.9-10.12). Added Lactobacillus acidophilus populations (10^7 CFU/gm cheese) and NSLAB populations (10^8 CFU/gm cheese) increased significantly ($p<0.05$) by 10 to 100-fold during the same time. The increases in probiotic populations over time were independent of fat level in cheese. Probiotic addition also did not modify Cheddar cheese flavor compared to cheese made without probiotics. In conclusion, probiotic bacteria are capable of surviving throughout the cheese-making and ageing process, indicating that they can be delivered to the consumer via hard cheeses even after extended ageing without altering cheese acceptability.

There are no significant differences between any of the probiotic cheeses for any flavor attributes for the reduced-fat cheese at six months of age. At nine month of age only nutty flavor for CRL-431, rosy/floral for L-26, and sour for LA-5, L-26 and BB-12. The only significant differences in the flavors of the low-fat cheese at six months of age were rancid for L-26. In the full-fat cheese at six months of age, the only flavors showing significant difference were lactone/fatty acid in CRL-431.

CONCLUSIONS

Cheese fortified with seven commercially available strains of probiotic bacteria were made. Survival of these strains in cheese was monitored for 9 mo of aging by both plating on differential media and qPCR techniques.

There is moderate agreement in the enumeration of the probiotic cultures between the qPCR technique utilizing the specific-specific primers and the plating technique. The addition of probiotics does not impact the flavor of the cheese.

Table 10.4. Microbiological analysis of low fat Cheddar cheese[1] during 180 d storage at 6 °C using presumptive selective media[2] for enumerating various lactic acid bacteria.

	Media					
Time (d)	**M17-L**	**MRS+S**	**MRS-V**	**MRS-NNLP**	**MRS-S**	**RCA-BV**
7	4.4×10^{6}	4.1×10^{6}	2.3×10^{3}	5.0×10^{2}	$< 10^{2}$	1.0×10^{2}
30	5.7×10^{5}	2.7×10^{6}	2.4×10^{6}	7.0×10^{5}	1.0×10^{6}	2.4×10^{6}
60	6.5×10^{4}	1.1×10^{7}	4.0×10^{7}	5.6×10^{6}	4.0×10^{3}	5.0×10^{7}
90	2.7×10^{5}	7.1×10^{7}	7.4×10^{7}	1.2×10^{5}	2.5×10^{7}	6.7×10^{7}
120	1.9×10^{5}	5.7×10^{8}	5.4×10^{7}	4.9×10^{7}	2.1×10^{8}	5.6×10^{8}
180	2.9×10	1.0×10	8.5×10	4.5×10	1.2×10	1.1×10

[1]See Table 10.5 for cheese composition. [2]See Table 8.2 for media description.

Table 10.5. Average cheese composition (and range).

Cheese	Moisture (%)	Fat (%)	pH	Salt (%)	S/W[1] (%)
Full Fat	38.8 (36 to 41)	31.5 (31 to 33)	4.99 (4.8 to 5.3)	1.22 (1.0 to 1.5)	3.17 (2.3 to 4.0)
Reduced Fat	46.0 (43 to 49)	17.0 (16 to 19)	5.32 (5.1 to 5.6)	1.93 (1.4 to 2.2)	4.19 (2.9 to 4.8)
Low Fat	50.5 (49 to 52)	7.5 (7 to 8)	5.35 (5.1 to 5.5)	2.04 (1.7 to 2.3)	4.03 (3.4 to 4.6)

[1]Salt divided by moisture content expressed as percent.

Table 10.6. Microbiological analysis of Cheddar cheese[1] made with added Bifidobacteria adjunct culture Bif-6[2] during 180 d storage at 6 °C using presumptive selective media[3] for enumerating various lactic acid bacteria.

	Media[3]					
Time (d)	**M17-L**	**MRS+S**	**MRS-V**	**MRS-NNLP**	**MRS-S**	**RCA-BV**
7	6.7×10^{7}	4.8×10^{8}	4.4×10^{5}	3.3×10^{8}	3.7×10^{5}	5.7×10^{5}
30	4.2×10^{6}	3.1×10^{8}	9.6×10^{6}	1.9×10^{8}	2.9×10^{3}	8.6×10^{6}
60	2.8×10^{5}	3.1×10^{8}	3.2×10^{7}	1.6×10^{7}	5.5×10^{7}	3.7×10^{7}
90	3.2×10^{5}	5.2×10^{8}	1.2×10^{7}	7.6×10^{8}	1.7×10^{7}	1.2×10^{7}
120	5.0×10^{4}	4.1×10^{8}	2.2×10^{8}	1.9×10^{8}	1.2×10^{8}	2.0×10^{8}
180	3.8×10	1.5×10	1.5×10	6.8×10	2.0×10	1.3×10

[1]See Table 10.5 for cheese composition. [2]See Table 8.1 for description of cultures. [3]See Table 8.2 for media description.

Table 10.7. Microbiological analysis of low fat Cheddar cheese[1] made with added Bifidobacteria adjunct culture Bif-6[2] during 180 d storage at 6 °C using presumptive selective media[3] for enumerating various lactic acid bacteria.

	Media					
Time (d)	**M17-L**	**MRS+S**	**MRS-V**	**MRS-NNLP**	**MRS-S**	**RCA-BV**
7	1.4×10^{8}	2.5×10^{7}	1.3×10^{7}	1.6×10^{4}	2.4×10^{4}	1.4×10^{8}
30	3.5×10^{7}	1.0×10^{7}	2.1×10^{7}	6.0×10^{6}	7.0×10^{6}	3.5×10^{7}
60	5.3×10^{8}	4.1×10^{8}	2.0×10^{7}	1.1×10^{5}	6.2×10^{8}	5.3×10^{8}
90	1.8×10^{8}	1.5×10^{8}	8.9×10^{4}	1.5×10^{7}	1.6×10^{8}	1.8×10^{8}
120	1.3×10^{8}	1.5×10^{8}	9.0×10^{7}	4.7×10^{5}	2.0×10^{8}	1.3×10^{8}
180	2.3×10	1.9×10	8.6×10	6.7×10	2.1×10	2.3×10

[1]See Table 10.5 for cheese composition. [2]See Table 8.1 for description of cultures. [3]See Table 8.2 for media description.

Table 10.8. Crossover times in full fat, reduced fat and low fat Cheddar cheeses[1] when nonstarter lactic acid bacteria microbial population enumerated on MRS-V[2] is equal has equal numbers as the population enumerated on media specific for various types of probiotic bacteria[3].

Probiotic Culture[3]	Time (months)		
	Full Fat Cheese[1]	**Reduced Fat Cheese[1]**	**Low Fat Cheese[1]**
L-10	3	3 to 4	2 to 3
LA-5	6	2 to 3	3
BB-12	3 to 4	2 to 3	3 to 4
Bif-6	4 to 6	3	2
F-19	6	6	6
CRL-431	6	6	6
L-26	6	6	6

[1]See Table 8.3 for cheese composition. [2]See Table 8.2 for media description. [3]See Table 8.1 for description of cultures.

Table 10.9. Plate counts and quantitative PCR enumeration (cfu/gm) of LAB and probiotic bacteria in full fat Cheddar cheese.

Organism	Strain	AGE	Plate counts (cfu/gm)					qPCR (cfu/gm)		
			Lactococcus	Total LAB	L. casei	Bifidobacterium	L. acidophilus	L. casei	NSLAB	Probiotic
Control	-	1	2.4E+07	3.8E+07	7.5E+05	8.6E+01	6.5E+03	7.5E+05	2.8E+08	-
		30	1.5E+07	1.3E+07	6.0E+06	6.0E+04	4.9E+02	7.0E+06	8.9E+07	-
		60	3.1E+06	5.4E+06	3.8E+06	4.8E+05	3.9E+03	4.8E+06	8.1E+07	-
		90	2.3E+05	2.2E+07	2.5E+07	1.4E+05	3.5E+05	1.9E+07	1.6E+08	-
		120	1.1E+05	7.3E+06	7.3E+06	2.0E+06	2.4E+06	1.1E+07	3.8E+07	-
		180	6.3E+03	2.4E+07	2.6E+07	4.0E+06	2.3E+07	2.7E+07	1.1E+08	-
		270	6.0E+07	6.4E+07	5.2E+07	1.6E+07	4.0E+06	6.8E+07	6.7E+08	-
L. acidophilus	L-10	1	2.5E+07	5.2E+07	5.2E+04	1.0E+05	6.7E+06	1.6E+05	7.2E+08	7.3E+06
		30	1.5E+06	9.0E+06	2.0E+06	1.8E+05	1.6E+06	9.5E+05	1.2E+08	4.1E+06
		60	2.1E+05	8.0E+06	3.8E+06	3.0E+05	3.1E+06	3.0E+06	7.1E+07	2.6E+06
		90	1.4E+04	2.3E+07	1.8E+07	3.3E+06	9.6E+06	1.8E+07	2.9E+08	1.6E+07
		120	4.4E+04	1.5E+07	1.1E+06	3.7E+06	9.0E+06	8.0E+06	6.9E+07	1.2E+06
		180	1.0E+04	2.9E+07	2.9E+07	6.2E+06	2.4E+07	5.0E+07	1.8E+08	9.7E+05
		270	1.6E+04	1.6E+07	1.4E+07	1.1E+07	1.2E+04	1.5E+07	2.5E+09	1.7E+07
L. acidophilus	LA5	1	3.3E+07	1.8E+08	3.9E+05	3.7E+04	1.0E+08	5.0E+04	7.0E+08	1.5E+06
		30	1.1E+07	1.2E+08	2.1E+06	1.6E+05	4.7E+07	2.9E+06	1.6E+08	4.9E+05
		60	1.4E+06	1.8E+08	2.6E+06	2.1E+05	1.5E+08	1.1E+06	2.0E+08	1.3E+06
		90	2.3E+05	8.1E+07	3.6E+06	1.8E+05	7.6E+07	2.6E+06	2.1E+09	4.4E+06
		120	5.9E+04	5.0E+07	7.4E+05	5.2E+05	5.1E+07	2.0E+06	2.2E+08	1.3E+05
		180	1.3E+04	6.0E+07	1.0E+07	8.1E+06	6.6E+07	3.2E+07	1.7E+08	1.1E+04
		270	3.2E+05	1.0E+07	7.0E+06	4.8E+06	3.5E+05	7.9E+06	4.3E+08	7.7E+05
L. casei	CRL-431	1	2.8E+08	5.2E+08	6.1E+08	5.2E+07	2.8E+04	4.2E+08	4.2E+08	3.2E+06
		30	1.4E+04	6.9E+08	6.7E+08	2.6E+05	4.4E+03	5.4E+08	9.2E+07	5.5E+05
		60	7.4E+03	1.2E+09	1.2E+09	1.4E+08	5.6E+04	9.2E+08	2.0E+08	1.1E+06
		90	7.0E+02	6.6E+08	7.6E+08	2.5E+06	2.2E+08	6.7E+08	8.7E+07	7.0E+05
		120	1.7E+03	7.4E+08	4.1E+09	4.4E+06	2.8E+06	6.5E+08	1.3E+08	3.0E+05
		180	1.0E+00	7.4E+08	4.2E+09	1.4E+07	1.3E+08	6.7E+08	6.6E+08	1.6E+06
		270	2.7E+03	3.4E+08	3.6E+08	1.1E+08	3.0E+03	3.4E+08	6.9E+08	1.3E+07
L. casei	L-26	1	2.8E+07	6.9E+08	5.7E+08	5.7E+08	3.4E+08	4.5E+08	7.7E+08	1.1E+07
		30	1.2E+05	7.6E+08	5.2E+08	3.6E+08	7.7E+07	6.7E+08	2.3E+08	3.0E+06
		60	1.3E+04	8.4E+08	8.1E+08	4.1E+08	2.4E+08	7.3E+08	3.3E+08	8.1E+06
		90	4.1E+03	6.2E+08	5.4E+08	7.5E+08	6.4E+08	4.6E+08	2.6E+08	8.8E+06
		120	2.6E+03	8.1E+08	7.4E+08	5.3E+08	6.2E+08	6.6E+08	2.6E+08	5.5E+06
		180	1.2E+05	6.5E+08	7.0E+08	2.5E+08	4.3E+08	6.2E+08	1.2E+09	5.4E+06
		270	9.0E+06	3.8E+08	4.0E+08	3.7E+07	1.1E+08	4.3E+08	4.4E+09	1.8E+08
L. paracasei	F-19	1	4.4E+07	6.1E+08	6.1E+08	3.0E+08	9.0E+08	5.0E+08	2.8E+09	3.1E+07
		30	1.1E+06	8.1E+08	9.3E+08	1.9E+07	9.0E+05	7.4E+08	1.6E+08	3.7E+05
		60	6.0E+06	9.6E+08	9.0E+08	3.9E+08	1.7E+05	1.0E+09	4.5E+08	1.4E+07
		90	1.8E+04	7.6E+08	6.9E+08	3.0E+08	3.6E+08	5.7E+08	6.5E+08	1.2E+06
		120	1.2E+05	9.6E+08	7.7E+08	3.7E+08	6.3E+08	8.4E+08	1.2E+09	8.7E+06
		180	1.0E+00	7.0E+08	6.9E+08	3.1E+06	6.6E+08	6.6E+08	8.0E+08	1.2E+07
		270	3.0E+03	5.3E+08	5.1E+08	1.3E+08	2.0E+06	5.5E+08	4.4E+09	4.6E+07
Bifidobacterium	Bif-6	1	9.4E+07	4.6E+08	7.2E+06	2.8E+08	1.9E+05	8.8E+06	3.2E+05	8.3E+06
		30	2.3E+07	1.8E+08	2.0E+07	1.9E+08	1.5E+05	1.7E+07	6.8E+04	1.2E+06
		60	4.4E+06	2.9E+08	2.6E+07	1.4E+08	5.8E+03	1.4E+07	1.4E+04	1.5E+07
		90	4.2E+05	4.4E+08	1.1E+07	1.3E+08	8.6E+06	1.4E+07	4.9E+04	3.3E+07
		120	2.9E+05	2.4E+08	3.5E+07	1.6E+08	6.3E+06	3.5E+07	3.0E+04	1.1E+08
		180	2.3E+04	1.2E+08	1.1E+08	3.4E+08	1.2E+08	1.1E+08	1.4E+04	1.3E+05
		270	3.5E+07	1.0E+08	9.7E+07	2.2E+07	4.2E+07	8.7E+07	1.6E+05	6.8E+03
Bifidobacterium	BB-12	1	8.8E+07	2.4E+08	1.9E+07	8.5E+07	2.4E+04	2.8E+07	6.2E+05	2.2E+07
		30	3.3E+07	3.4E+08	2.1E+07	7.0E+07	7.1E+03	2.3E+07	7.7E+04	1.3E+06
		60	1.9E+07	3.1E+08	1.4E+07	1.7E+08	7.7E+05	2.1E+07	2.0E+04	3.7E+07
		90	1.5E+05	1.4E+08	3.2E+07	3.5E+07	1.1E+06	3.3E+07	5.1E+04	5.2E+07
		120	4.3E+05	1.6E+08	2.3E+07	6.0E+07	2.5E+06	2.9E+07	2.9E+04	1.3E+07
		180	3.8E+04	5.0E+07	5.2E+07	3.5E+07	3.8E+07	5.3E+07	5.4E+04	7.2E+06
		270	3.3E+04	5.2E+07	4.8E+07	8.8E+06	1.7E+07	5.9E+07	7.5E+04	6.8E+05

Table 10.10. Plate counts and quantitative PCR enumeration (cfu/gm) of LAB and probiotic bacteria in reduced fat Cheddar cheese.

Organism	Strain	AGE	Plate counts (cfu/gm)					qPCR (cfu/g)		
			Lactococcus	Total LAB	L. casei	Bifidobacterium	L. acidophilus	L. casei	NSLAB	Probiotic
	Control	1	4.8E+07	4.2E+07	2.6E+03	1.0E+00	3.3E+02	1.0E+00	-	-
		30	1.1E+07	7.5E+06	5.7E+06	9.1E+01	3.5E+02	7.0E+06	-	-
		60	2.6E+06	4.1E+07	4.6E+07	2.9E+02	9.1E+03	3.9E+07	4.6E+07	-
		90	2.0E+05	3.9E+07	3.4E+07	1.7E+04	3.5E+02	4.0E+07	2.0E+07	-
		120	1.5E+07	2.5E+07	2.8E+05	2.9E+03	1.0E+00	1.4E+07	3.7E+07	-
		180	3.2E+06	1.9E+07	2.0E+07	7.5E+02	2.0E+02	1.4E+07	2.6E+07	-
		270	9.1E+05	7.5E+06	6.5E+06	4.5E+03	6.0E+02	4.3E+06	3.8E+07	-
L. acidophilus	L-10	1	2.6E+07	2.9E+07	9.2E+04	1.5E+04	9.4E+06	9.5E+04	1.2E+09	4.5E+04
		30	3.3E+07	3.2E+07	1.7E+07	2.5E+06	1.3E+07	1.8E+07	7.2E+07	7.1E+06
		60	8.7E+05	1.4E+07	1.1E+07	2.5E+06	3.1E+06	6.5E+06	5.9E+08	3.0E+07
		90	3.1E+05	5.0E+07	4.9E+07	7.5E+06	7.0E+06	1.4E+08	5.9E+08	6.3E+06
		120	6.9E+06	6.1E+07	5.0E+07	7.3E+06	5.0E+06	4.4E+07	3.2E+08	3.0E+07
		180	4.7E+05	1.3E+08	1.3E+08	5.0E+06	8.0E+06	1.4E+08	3.2E+07	1.2E+06
		270	2.6E+07	6.2E+07	7.0E+07	1.7E+07	7.5E+06	6.5E+07	1.4E+08	1.0E+07
L. acidophilus	LA5	1	6.5E+06	4.2E+07	5.3E+04	2.5E+03	3.7E+07	3.3E+03	7.9E+08	2.2E+05
		30	4.3E+06	2.2E+07	5.3E+05	6.5E+04	1.9E+07	5.0E+05	1.0E+08	4.5E+06
		60	2.8E+06	8.9E+07	5.6E+07	1.1E+06	2.4E+07	4.4E+07	3.9E+08	1.7E+06
		90	1.3E+05	1.6E+08	2.1E+08	1.3E+08	1.7E+08	1.6E+08	3.9E+08	7.2E+05
		120	4.4E+07	1.2E+08	1.4E+08	3.0E+07	3.0E+07	1.4E+08	1.5E+08	1.1E+06
		180	2.6E+04	1.6E+08	6.5E+07	5.0E+05	1.2E+07	1.9E+08	4.5E+07	8.0E+05
		270	8.5E+06	1.2E+08	9.8E+07	2.1E+07	1.6E+07	1.0E+08	9.8E+07	2.7E+06
L. casei	CRL-431	1	4.8E+07	1.6E+08	1.8E+08	1.4E+08	9.6E+03	1.3E+08	6.3E+08	5.4E+04
		30	5.6E+04	2.6E+08	2.4E+08	1.9E+06	3.0E+02	2.4E+08	1.1E+08	2.1E+06
		60	1.3E+04	1.8E+08	2.0E+08	7.2E+05	1.8E+05	1.6E+08	8.1E+07	3.4E+06
		90	2.7E+03	1.7E+08	1.3E+08	6.2E+04	2.8E+05	1.8E+08	8.1E+07	8.2E+05
		120	4.4E+07	2.3E+08	2.8E+08	2.1E+06	5.5E+05	2.1E+08	5.0E+07	6.2E+05
		180	4.1E+03	2.8E+08	2.7E+08	2.5E+03	2.4E+03	2.6E+08	1.6E+08	7.4E+05
		270	8.0E+06	3.5E+08	2.6E+08	2.5E+05	2.3E+03	2.2E+08	9.1E+07	5.0E+05
L. casei	L-26	1	4.6E+06	2.0E+08	2.2E+08	1.6E+08	4.0E+07	3.1E+08	1.1E+08	5.5E+05
		30	5.6E+07	6.8E+07	2.7E+08	2.5E+08	6.5E+07	3.6E+08	2.0E+08	7.5E+06
		60	7.5E+02	3.1E+08	3.0E+08	2.2E+08	2.3E+08	2.6E+08	1.4E+08	2.3E+06
		90	4.8E+03	3.0E+08	3.1E+08	1.8E+08	1.5E+08	2.7E+08	1.4E+08	3.0E+06
		120	1.8E+08	4.0E+08	3.7E+08	1.7E+08	1.5E+06	3.4E+08	4.2E+08	2.7E+07
		180	5.5E+05	4.0E+08	3.7E+08	3.0E+07	1.2E+08	4.5E+08	1.7E+08	7.9E+05
		270	1.3E+05	2.8E+08	2.3E+08	1.4E+07	5.1E+06	2.7E+08	1.9E+08	4.0E+06
L. paracasei	F-19	1	7.7E+06	1.3E+08	1.2E+08	1.4E+04	1.8E+07	1.2E+08	5.5E+08	3.4E+04
		30	5.7E+05	2.3E+08	1.9E+08	2.4E+07	2.5E+07	1.2E+08	9.1E+07	2.7E+04
		60	1.2E+05	2.1E+08	2.1E+08	4.9E+07	9.1E+07	2.5E+08	8.8E+08	1.6E+06
		90	1.7E+05	2.4E+08	2.3E+08	5.7E+07	8.0E+07	2.2E+08	8.8E+08	1.3E+05
		120	2.6E+05	3.9E+08	2.7E+08	7.1E+07	1.4E+08	3.1E+08	2.2E+07	5.8E+05
		180	6.9E+03	4.3E+08	3.2E+08	3.6E+06	3.2E+07	3.2E+08	3.2E+07	7.0E+05
		270	3.7E+05	3.7E+08	3.1E+08	1.3E+05	1.8E+05	4.5E+08	9.5E+07	7.4E+04
Bifidobacterium	Bif-6	1	1.2E+07	6.7E+07	4.0E+06	3.9E+07	2.0E+02	1.5E+06	1.1E+06	2.2E+06
		30	9.0E+07	1.1E+08	6.5E+07	3.5E+07	2.8E+03	2.6E+08	2.3E+06	5.0E+04
		60	2.8E+05	1.3E+08	8.5E+07	3.7E+07	3.5E+02	9.5E+07	7.5E+05	1.6E+06
		90	1.3E+05	1.6E+08	1.1E+08	2.7E+07	5.0E+06	1.0E+08	7.5E+05	1.5E+05
		120	7.6E+05	1.3E+08	1.3E+08	4.0E+07	6.0E+06	1.4E+08	9.4E+05	2.1E+06
		180	6.2E+04	1.8E+08	1.4E+08	8.5E+06	5.5E+04	1.3E+08	1.5E+04	1.1E+06
		270	2.9E+07	2.1E+08	2.1E+08	4.0E+07	2.9E+07	1.8E+08	2.8E+04	3.5E+07
Bifidobacterium	BB-12	1	1.2E+07	1.2E+08	1.4E+04	6.9E+07	6.3E+03	1.8E+04	7.0E+05	2.3E+04
		30	1.9E+06	2.6E+09	2.5E+08	2.1E+07	3.5E+06	6.3E+07	4.0E+04	1.4E+05
		60	1.1E+05	1.5E+08	1.5E+08	2.4E+07	9.5E+07	1.3E+08	3.8E+04	1.0E+05
		90	3.0E+04	1.8E+08	1.8E+08	1.3E+07	3.7E+05	1.8E+08	3.8E+04	2.2E+03
		120	7.6E+05	2.0E+08	1.8E+08	5.0E+07	5.5E+04	1.7E+08	6.3E+03	8.3E+03
		180	1.9E+04	1.1E+08	1.2E+08	6.7E+03	3.1E+07	1.1E+08	4.1E+01	3.3E+03
		270	5.0E+05	1.5E+08	1.5E+08	2.6E+03	3.3E+05	1.7E+08	2.8E+04	6.2E+06

Table 10.11. Plate counts and quantitative PCR enumeration (cfu/gm) of LAB and probiotic bacteria in low fat Cheddar cheese.

Organism	Strain	AGE	Plate counts (cfu/g)					qPCR (cfu/g)	
			Lactococcus	Total LAB	L. casei	Bifidobacterium	L. acidophilus	L. casei	NSLAB	Probiotic
Control	-	1	2.0E+07	8.6E+06	7.2E+03	3.2E+02	5.5E+01	7.6E+03	1.6E+08	-
		30	5.8E+06	5.9E+06	2.7E+06	3.5E+05	5.1E+05	2.6E+06	1.9E+08	-
		60	4.7E+05	6.7E+06	2.4E+06	2.8E+05	2.0E+06	3.0E+06	4.6E+07	-
		90	4.1E+06	2.9E+08	3.2E+08	6.0E+05	2.5E+03	3.5E+07	2.4E+07	-
		120	2.4E+05	2.9E+07	2.7E+07	4.9E+05	2.1E+07	2.8E+07	3.3E+07	-
		180	1.9E+05	5.0E+07	4.2E+07	4.5E+07	1.2E+08	5.5E+07	5.0E+07	-
		270	1.2E+04	6.5E+07	6.0E+07	2.0E+06	1.9E+04	4.2E+07	1.8E+08	-
L. acidophilus	L-10	1	2.6E+06	2.7E+06	2.7E+03	1.3E+03	8.8E+05	4.1E+02	1.1E+08	9.0E+06
		30	4.0E+06	8.5E+06	5.5E+06	1.5E+06	7.6E+05	4.4E+06	1.7E+08	5.8E+06
		60	6.5E+05	1.2E+07	2.6E+06	5.6E+06	4.1E+05	8.1E+06	1.0E+07	1.4E+06
		90	3.5E+05	7.1E+07	5.5E+07	2.6E+06	1.3E+05	4.8E+07	1.6E+07	5.5E+06
		120	4.3E+07	3.9E+07	4.6E+07	1.3E+07	3.2E+07	3.5E+07	7.0E+07	6.9E+06
		180	8.5E+07	8.6E+07	7.1E+07	3.6E+07	9.6E+07	9.1E+07	7.7E+07	4.2E+06
		270	4.3E+05	3.2E+07	3.6E+07	1.6E+07	2.4E+07	4.0E+07	1.8E+07	2.4E+05
L. acidophilus	LA5	1	5.9E+06	4.8E+07	5.5E+06	6.6E+04	2.6E+07	5.5E+06	3.5E+08	2.1E+07
		30	8.0E+07	1.3E+08	8.2E+07	1.8E+06	2.4E+07	8.7E+07	4.9E+08	5.9E+06
		60	4.1E+05	8.9E+07	8.0E+07	2.8E+06	1.9E+07	6.5E+07	4.3E+08	2.6E+06
		90	3.2E+07	5.6E+07	5.1E+07	2.3E+06	3.9E+05	5.6E+07	3.3E+07	3.1E+05
		120	4.0E+06	8.9E+07	6.2E+07	3.6E+06	1.4E+07	6.4E+07	1.5E+08	2.7E+05
		180	4.8E+07	9.7E+07	1.1E+08	1.2E+07	1.1E+07	1.2E+08	1.1E+08	1.7E+05
		270	8.0E+06	7.7E+07	7.0E+07	2.7E+07	3.1E+06	6.0E+07	4.8E+08	1.4E+06
L. casei	CRL-431	1	4.8E+05	1.7E+08	1.6E+08	5.1E+05	1.2E+03	1.5E+08	9.1E+07	5.9E+05
		30	1.1E+08	1.9E+08	2.1E+08	6.9E+06	1.5E+04	2.2E+08	2.1E+08	2.1E+06
		60	3.1E+04	2.2E+08	2.2E+08	3.7E+05	3.6E+02	2.3E+08	9.0E+07	2.0E+06
		90	3.9E+07	1.7E+08	1.9E+08	1.0E+05	3.1E+02	1.3E+08	3.9E+07	1.2E+06
		120	8.1E+04	2.2E+08	2.2E+08	6.0E+04	2.6E+03	1.9E+08	6.0E+07	1.0E+06
		180	1.3E+08	1.9E+08	1.9E+08	4.8E+05	5.5E+04	2.5E+08	1.1E+08	4.2E+06
		270	1.1E+07	1.9E+08	1.4E+08	9.5E+06	7.9E+05	1.6E+08	6.3E+08	4.2E+07
L. casei	L-26	1	1.3E+06	2.3E+08	2.1E+08	1.6E+08	1.9E+06	2.4E+08	4.7E+08	6.2E+06
		30	1.4E+06	2.6E+08	3.5E+08	1.7E+08	5.2E+06	2.5E+08	2.8E+08	1.0E+07
		60	3.4E+04	2.1E+08	2.3E+08	1.1E+08	1.5E+08	2.2E+08	4.4E+08	1.3E+07
		90	9.5E+07	2.5E+08	2.9E+08	1.5E+08	1.5E+06	3.0E+08	1.2E+08	7.3E+06
		120	1.6E+08	2.6E+08	2.4E+08	1.4E+08	1.1E+08	2.5E+08	4.0E+07	3.3E+05
		180	3.2E+08	4.9E+08	4.9E+08	3.7E+08	4.6E+08	5.0E+08	9.8E+07	1.1E+06
		270	1.8E+08	2.3E+08	2.0E+08	9.6E+07	7.5E+06	1.6E+08	7.2E+08	2.2E+07
L. paracasei	F-19	1	3.7E+06	4.0E+08	2.3E+08	5.0E+07	2.2E+08	2.4E+08	4.2E+07	8.4E+06
		30	3.2E+06	2.5E+08	2.4E+08	5.8E+07	1.1E+08	2.8E+08	2.4E+07	1.1E+07
		60	1.9E+05	3.5E+08	3.6E+08	3.3E+07	4.3E+05	3.0E+08	5.0E+08	7.9E+06
		90	1.8E+06	3.7E+08	3.9E+08	8.9E+04	1.2E+07	3.3E+08	1.0E+07	1.2E+05
		120	2.6E+04	4.6E+08	4.6E+08	5.6E+05	6.4E+05	5.2E+08	2.8E+08	1.7E+06
		180	2.7E+08	4.0E+08	3.6E+08	8.8E+07	2.3E+08	3.0E+08	1.5E+08	3.6E+06
		270	1.1E+07	1.0E+08	1.0E+08	8.4E+06	2.6E+05	7.4E+07	1.4E+09	4.3E+07
Bifidobacterium	Bif-6	1	1.5E+08	1.9E+08	1.5E+08	2.0E+07	1.3E+04	1.3E+08	6.4E+05	8.4E+06
		30	1.4E+08	1.7E+08	1.7E+08	3.5E+07	3.0E+06	1.7E+08	3.3E+05	1.1E+06
		60	4.1E+05	1.2E+08	1.1E+08	1.0E+07	5.5E+04	1.2E+08	9.3E+04	6.2E+06
		90	8.5E+07	1.8E+08	1.5E+08	5.9E+07	7.5E+04	1.8E+08	1.3E+05	7.5E+06
		120	2.5E+05	1.5E+08	1.6E+08	2.5E+06	4.7E+07	1.8E+08	3.6E+04	7.1E+03
		180	1.2E+08	1.7E+08	1.9E+08	4.7E+07	6.7E+05	1.8E+08	1.1E+05	2.0E+05
		270	5.5E+06	8.2E+07	9.0E+07	1.7E+07	1.8E+05	7.7E+07	4.5E+04	3.1E+03
Bifidobacterium	BB-12	1	1.0E+07	5.1E+07	3.7E+05	9.5E+06	1.2E+03	4.6E+05	2.6E+06	2.7E+06
		30	1.1E+07	5.5E+07	2.9E+05	2.4E+07	1.0E+05	2.6E+05	2.7E+06	3.1E+05
		60	1.6E+06	2.8E+07	4.4E+05	1.4E+07	2.5E+02	1.1E+06	1.2E+05	9.5E+06
		90	1.1E+06	2.1E+07	1.2E+06	1.7E+07	1.5E+03	1.1E+06	8.8E+03	1.6E+06
		120	9.4E+05	3.0E+07	3.0E+05	7.0E+06	4.3E+03	4.1E+05	2.8E+04	2.7E+03
		180	2.2E+06	1.3E+07	2.3E+06	4.0E+06	2.5E+04	1.8E+06	5.8E+04	7.9E+04
		270	1.0E+04	2.4E+07	1.6E+07	5.0E+06	1.6E+05	2.4E+07	3.8E+04	1.2E+03

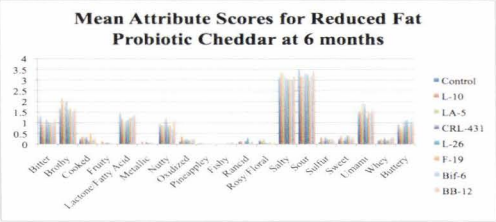

Figure 10.1. Mean flavor attribute scores from descriptive taste panel analysis of reduced-fat Cheddar cheese at 6 months of age for all probiotic strains.

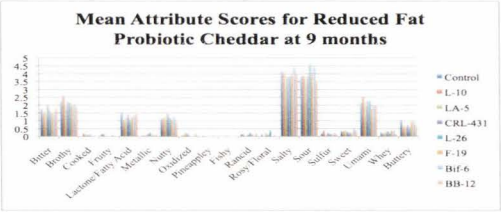

Figure 10.2. Mean flavor attribute scores from descriptive taste panel analysis of reduced-fat Cheddar cheese at 9 months of age for all probiotic strains.

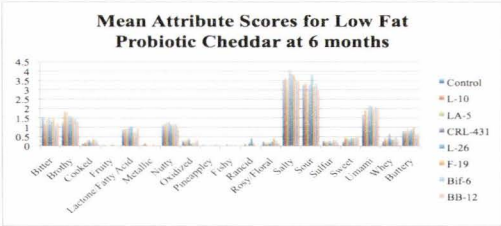

Figure 10.3. Mean flavor attribute scores from descriptive taste panel analysis of low-fat Cheddar cheese at 6 months of age for all probiotic strains.

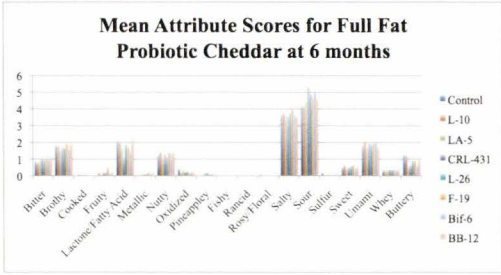

Figure 10.4. Mean flavor attribute scores from descriptive taste panel analysis of full-fat Cheddar cheese at 6 months of age for all probiotic strains.

DTIL Project 11:
Evalaluate consumer acceptance of milk pasteurized by HTST, UHT and electical resistive heating techniques

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Funded by: Dairy Management Inc., September 2007 - December 2009

BACKGROUND

Previoious work at the WDC investigated electrical resistive l heating a method of pasteurizing milk. This method utiutilizes electrical resistance to generate heat, which reduces ththe time to bring the product to pasteurization temperatuure, and time to cool the product. This project evaluated d the consumer acceptance of milk processed by electricical resistive heating along with commercially available h HTST and UHT processed milk.

MATERIALS AND METHODS

Three e types of fluid milk treatments, high temperature short timeie (HTST) pasteurization, ultra high temperature (UHT) papasteurization, and electrical resistive (ohmic) heating, wwith both 0 and 2% fat ,were evaluated by a consumer tr taste panel on a nine point hedonic scale

Commmercially available skim and 2% fat milk was purchased d for the HTST treatment. Commercially available skim and 2 2% fat UHT milk (Gossner Foods, Logan, UT) was purchchased for the UHT treatment. Commercially available s skim and 2% fat milk was purchased and process by ohmic h heating at 145°C for 4 seconds.

A consnsumer taste panel consisting of 115 randomly recruited p panelists was conducted at the Sensory Evaluation Laboratoryry (Utah State University, Logan UT) on a nine point hedolonic scale: 1) Dislike extremely, 2) Dislike very much, 3) L Dislike moderately, 4) Dislike slightly, 5) Neither like nor dislike, 6) Like slightly, 7) Like moderately, 8) Like very v much, 9) Like extremely.

RESULTS AND DISCUSSION

There was no significant difference in liking score between the HTST and the ohmic treated milks (Table 11.1). The skim milk with these two treatments was like significantly less than the 2% milk. There was no significant difference in liking between the UHT skim and 2% milk. The liking scores for UHT milk were significantly lower than the milk receiving the other heat treatments.

A large proportion of the panelists were not consumers of milk of various fat content (Table 11.1).

CONCLUSIONS

There was no significant difference in consumer acceptance of milk pasteurized by HTST and electrical restive heating techniques. A commercially available UHT processed milk was significantly less acceptable.

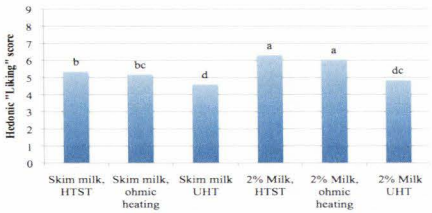

Figure 11.1. Average liking scores of consumer taste panel on a nine point hedonic scale: 1) Dislike extremely, 2) Dislike very much, 3) Dislike moderately, 4) Dislike slightly, 5) Neither like nor dislike, 6) Like slightly, 7) Like moderately, 8) Like very much, 9) Like extremely. Letter superscripts indicate significant difference at p = 0.0001.

Table 11.1. Milk consumption frequency of consumer taste panelists.

Whole Milk		
	Frequency	Percent
Never	64	55.7
At least once a month	17	14.8
At least once a week	9	7.8
At least once a day	25	21.7
Skim Milk		
	Frequency	Percent
Never	53	46.1
At least once a month	20	17.4
At least once a week	16	13.9
At least once a day	26	22.6
2% Milk		
	Frequency	Percent
Never	35	30.4
At least once a month	31	27.0
At least once a week	28	24.3
At least once a day	21	18.3
1% Milk		
	Frequency	Percent
Never	43	37.4
At least once a month	34	29.6
At least once a week	14	12.2
At least once a day	24	20.9

DTIL Project 12:
Enriching low-fat cheese with four different dietary fibers

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Funded by: Dairy Management Inc., September 2007 - December 2009

MATERIALS AND METHODS

Loow-fat Cheddar cheese at 15 days of age was comminnuted to 1.5 mm particle size and divided into none portionns. Inulin, pectin, polydextrose, and resistant starch were eaach added to a portion of the cheese at the rate of 50 g/kg chheese. The fibers were each added to a portion of the cheese : along with 50 g water/kg cheese. The comminuted portionns were then repressed individually in cheese molds, vacuumm packaged and stored at 4°C until analyzed. Texture profile : analysis was executed at 90 and 210 d of storage. Descripptive flavor analysis was performed at 210 d, which i involved flavor profiling of all cheese samples and comparring them with full fat Cheddar cheese. Chewiness attributtes of the cheese samples were evaluated using a chewdoown method by sensory focus group in which the numberr of bites were counted before swallowing.

RESULTS AND DISCUSSION

Fiboer Content in finished low fat cheeses are summarized in Tablde 12.1. No whey expulsion from repressed cheese ensuredd 100% fiber retention except for polydextrose with water wwhich had 0.5% of whey expulsion.

Texxtural properties of enriched low fat cheese evaluated at 90 annd 210 days of storage are summarized in Figures 12.1-122.3. Hardness for inulin (46 ± 2 N) and pectin (55 ± 3 N) chheeses were significantly lower than non-repressed control cheeses (80 ± 2 N) and chewiness was also significcantly reduced from 44 N to 12 N. When added with water, ccheese mixed with inulin or pectin resulted in better knittingg and uniform mixing of cheese particulates which was conmfirmed by increased cohesiveness from 48 to 65% for inuldin with water and 50% for pectin with water.

Hanrdness and cohesiveness were also evaluated at 7 monthss (~210 d) and summarized in Figures 12.4 and 12.5. To deteermine the overall impact of fibers on cheese flavors, descriptive flavor analysis was conducted by a trained panel comprising of 9 panelists. There was no impact on cheese flavors observed when enriched with dietary fibers as shown in Table 12.2.

CONCLUSIONS

In conclusion, low fat Cheddar cheese enriched with 5% fiber had improved textural properties and comminuted cheeses had higher cohesiveness than the non-comminuted control, which was due to the rearrangements of cheese particulates making it more malleable. Better performance of cheese was observed when fiber added with equal amount of water than fiber alone. Moreover, adding fibers did not impact cheese flavor. Out of 4 types of dietary fibers tested in this study, inulin and pectin had promising results while polydextrose and resistant starch had poor appeal.

Table 12.1. Composition of low-fat control cheese and cheese with 5% dietary fibers added with or without 5% additional water.

Sample	Moisture	Ash	Fat	Protein	Salt	Carbs*
Unground control	50.9	4.5	5.5	38.4	1.7	**0.6**
Ground control	51.8	4.3	5.5	38.3	1.7	**0.1**
Inulin	48.8	4.9	4.5	37.3	1.6	**4.5**
Inulin + water	51.6	4.1	5.0	35.5	1.8	**3.8**
Pectin	48.9	4.6	5.5	35.4	1.8	**5.6**
Pectin + water	51.2	4.7	5.5	34.4	1.8	**4.1**
Polydex	49.0	3.8	5.5	38.0	1.8	**3.7**
Polydex + water	51.8	4.3	5.5	34.7	1.7	**3.6**
RS	50.3	3.8	5.5	36.7	1.7	**3.7**
RS + water	52.2	7.2	5.5	31.3	1.8	**3.9**

*Carbs = Total carbohydrate calculated by difference.

Figure 12.1. Hardness of control and fiber enriched cheeses at 3 months (90 d) of storage.

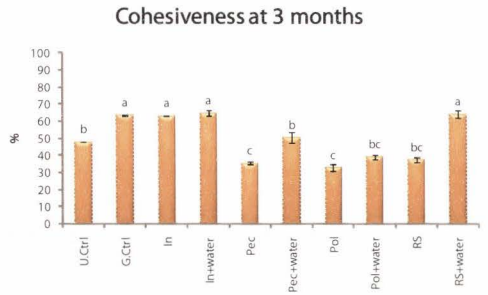

Figure 12.2. Cohesiveness of control and fiber enriched cheeses at 3 months (90 d) of storage.

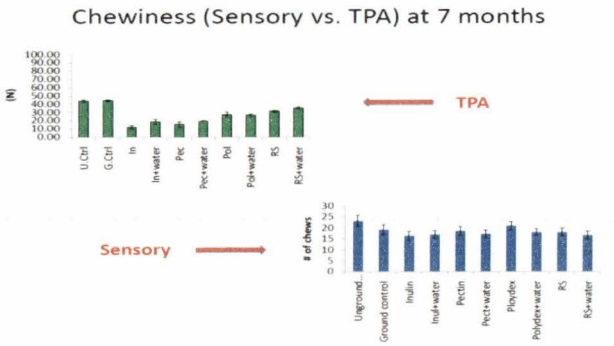

Figure 12.3. Comparison of chewiness property of control and fiber enriched cheeses using TPA and sensory focus group.

Figure 12.4. Hardness of control and fiber enriched cheeses at 7 months (210 d) of storage.

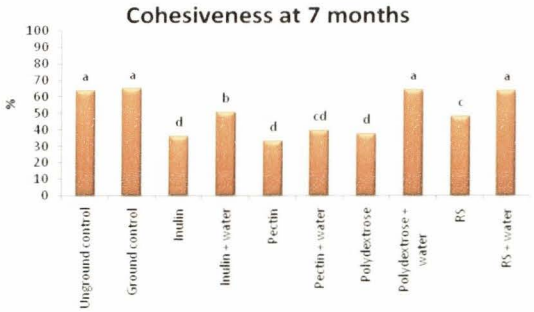

Figure 12.5. Chewiness of control and fiber enriched cheeses at 7 months (210 d) of storage.

Table 12.2. Descriptive analysis of fiber enriched cheeses flavor comparing with commercial medium aged full fat cheese.

Attribute	Commercial Medium	Unground Control	Ground Control	Cheese with Inulin	Cheese with Inulin + 5% Water	Cheese with Pectin	Cheese with Pectin + 5% Water	Cheese with Polydextrose	Cheese with Polydextrose + 5% Water	Cheese with resistant starch	Cheese with resistant starch + 5% water	P-Value
Bitter	0.8	1.1	0.5	0.7	1.0	0.4	0.8	0.7	0.7	0.7	0.3	NS
Brothy	0.9	0.7	1.2	0.8	1.1	1.1	1.0	1.2	1.2	1.1	1.3	NS
Buttery	2.0[a]	0.3[b]	0.5[b]	0.6[b]	0.4[b]	0.6[b]	0.6[b]	0.7[b]	0.8[b]	0.4[b]	0.6[b]	0.0043
Lactone/ Fatty Acid	1.9[a]	0.4[b]	0.2[b]	0.5[b]	0.4[b]	0.4[b]	0.6[b]	0.4[b]	0.4[b]	0.4[b]	0.4[b]	0.0138
Nutty	0.6	0.3	0.4	0.8	0.4	0.7	0.8	0.6	0.3	0.2	0.3	NS
Oxidized	0.0	0.0	0.0	0.2	0.2	0.0	0.2	0.2	0.0	0.0	0.2	NS
Salty	4.0	3.5	4.3	4.3	3.4	4.1	4.1	3.6	3.8	3.9	3.1	NS
Sour	4.5	3.4	4.0	4.3	3.9	4.7	3.4	3.4	3.7	4.0	3.1	NS
Sweet	0.1	0.1	0.1	0.3	0.2	0.2	0.3	0.3	0.0	0.1	0.2	NS
Umani	1.6	1.2	1.2	1.5	1.7	1.7	1.4	1.6	0.9	1.3	1.1	NS
Whey	0.4	0.2	0.3	0.3	0.2	0.4	0.3	0.2	0.2	0.1	0.3	NS

DTIL Projects for 2010

Manufacture reduced sodium cheddar cheeses for USDA

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Funded by: Dairy Management Inc., February 2010 - December 2010

BACKGROUND

DMII has arranged a study to be conducted at USDA Eastern Regional Research Center, Pennsylvania, PA to study survival at two storage temperatures (6 and 12°C) for 3 target pathogens (*L. monocytogenes*, *E. coli*, and *S. aureus*). The cheeses for this study are to be manufactured by the Dairy Technology Innovation Laboratory at the Western Dairy Center.

RESEARCH PLAN

Objecttive 1.

Manufacture (in 2 replicates) cheddar cheeses with salt reductions of 0%, 25%, 45%, and 60%, based on a referencce salt level of 1.8% (w/w), and the cheeses having similar moisture and fat contents, and similar pH.

Objecttive 2.

Send 15 kg of each cheese to USDA ERRS on the second day after day of manufacture of the cheese (cheese to arrivve at USDA ERRS on a Thursday), and store the remaining 15-kg at 6°C for 3 months and then ship to USDA ERRS.

MATERIALS AND METHODS

Objecttive 1.

For Rep 1, on a Monday, cheeses with target salt contents of 1.80% ± 0.05% and 1.35% ± 0.05% will be made, and the next day (Tuesday) cheeses with target salt contents of 1.00% ± 0.05% and 0.70% ± 0.05% will be made.

1. Cheddar cheese will be made according to procedures developed by the DTIL for making cheeses with different salt levels.
2. Pasteurized milk (700 kg) will be ripened with starter culture (no adjunct) and coagulated in an enclosed vat, and then the curd and whey pumped into 2 small vats so that the curd is evenly shared between vats (some whey will need to be drained from each vat so the curd/whey volume in each vat is ~250 L).
3. The curd and whey will be cooked to the appropriate temperature (lower salt contents required higher cooking temperatures), stirred for the appropriate time and then the whey drained.
4. The drained curd will be stirred for the appropriate time (lower salt contents require longer stir times) and the cheese packed and allowed to cheddar.
5. Then the cheese will be milled, and divided into three (3) ~12-kg portions, placed in plastic tubs and salted with the appropriate amount of salt.
6. The salted curd will be placed into SS hoops and pressed overnight.

For Rep 2, the order of cheesemaking will be reversed.

Proposed dates of manufacture are:

- Rep 1: Jan 25 and 26th with arrival at ERRC by Jan 28, 2010.
- Rep 2: Feb 8 and 9th with arrival at ERRC by Feb 11, 2010.

Objective 2.

1. After approximately 16 hours of pressing, the cheese block will be removed from the press, de-hooped, and the block cut along its short side (11 inch) so as to obtain 2 slabs of equal

1. width). Then from each slab from each block remove 1 cm from one outside edge and then cut a 2-cm piece for chemical analysis. This sample will be packaged and sealed to prevent moisture loss.
2. Immediately perform salt analysis by chloride analysis, pH by glass electrode and moisture by microwave.
3. Vacuum package the remaining cheese slabs individually, label, and store at 6°C.
4. Hold the cheese made on Monday until the Tuesday cheese has been cut and packaged.
5. Send three (3) slabs from each of the four salt treatments by Overnight Priority delivery to arrive at USDA ERRS the following (Thursday) morning, add coolant packs to keep the cheese cold. Keep the remaining cheese in storage at 6°C.
6. Perform proximate analysis on the cheese f:for salt, pH, fat, and moisture (by vacuum oveen) on day 7 after manufacture.
7. Prepare and ship samples for mineral analysis (Na).
8. After 12 weeks of storage at 6°C, ship tlthe other three (3) slabs of each cheese to USDDA ERRS as above.

RESULTS AND DISCUSSION

The first cheese manufacturing trial has been commpleted and the cheese has been shipped to USDA for analyssis. The composition of the cheeses is shown in Table 1.1.1.

NEXT STEPS

Additional cheese will be made as scheduled by UUSDA.

Table 1.1.1. Composition of the first replicate of cheese sent to USDA.

Block/Target Salt	Moisture	Fat	Salt	pH
Block 1 - 1.8%	37.34	31	1.64	5.07
Block 2 - 1.8%	37.56	31	1.6	5.09
Block 3 - 1.8%	37.86	31	1.44	5.03
Block 4 - 1.35%	38.01	31	1.27	5.01
Block 5 - 1.35%	38.36	31	1.25	4.99
Block 6 - 1.35%	38.31	30.5	1.2	4.97
Block 7 - 1.0%	39.20	30	1.09	5.19
Block 8 - 1.0%	39.64	30	1.12	5.19
Block 9 - 1.0%	38.70	30	1.09	5.20
Block 10 - 0.7%	39.78	30	0.84	5.11
Block 11 - 0.7%	40.09	30	0.87	5.11
Block 12 - 0.7%	39.91	30	0.84	5.11

FPreliminary investigation of block forming from 50:50 blend of comminuted aged and young cheese

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Funded by: Dairy Management Inc., January 2010 - December 2010

BACKGROUND

Theere is a need to quantify the sensory effect of salt level inn cheese without having confounding effects of low salt on aging. A potential way to do this is to blend aged cheesse (with normal salt content) with young cheese (with varyiingg salt content), press into a block and store for the miniimuum time for the cheese to knit together and then conduuct sensory analysis.

Before this can be done, it needs to be determined if commiinuted aged cheese (6 mo to 2 year old good aged cheddaar) can be mixed with comminuted young cheese (2 weeks old) that has no salt added or the normal salt level, then prressed into a block, vacuum packaged, stored cold (38 F) to restrict further aging, and whether a block of cheesse with reasonable cheese texture can be obtained, that can be used for sensory evaluation.

RESEARCH PLAN

Objeective 1.

Makee ttwo blocks of cheddar cheese with (1) no added salt, and ((2)) normal salt.

Objeective 2.

MMake reformed cheeses from comminuted young cheesse (14 day old) containing (1) no added salt and (2) normmall salt, with comminuted aged cheddar cheese (6 mo, 1 yr and 2 yr old).

MATERIALS AND METHODS

Objeective 1.

MManufacture Old Juniper Cheddar Cheese, and after milling but before salting, hoop and press one 22-lb block of cheese. Press for 3 hours only. The remainder of the cheese is salted at the normal level (curd to salt) and pressed. Remove one block after 3 hours.

Vacuum packaged and store at 38 F.

Repeat the process with White Pine Cheddar cheese.

Objective 2.

When the cheese is 15 days old, remove from storage. Also obtain a blocks of aged cheese that are 6 mo and 1 yr old.

Comminute:

- 16 lb of cheeses both young cheeses,
- 12 lb of 1-yr old cheese, and
- 4 lb of both 6-mo and 2-yr old cheese.
- Immediately store in sealed bags to prevent moisture loss.

Cut and package a 4-lb block of each of the aged cheeses, and store at 38 F as controls.

Retain samples of the cheeses for proximate analysis.

Return the remainder of the aged cheeses to 42 F storage.

Collect samples of the young cheeses (non-comminuted) for proximate analysis.

Re-package the remainder of the young cheeses, and store at 38 F as controls.

Blending Cheeses:

1. Young (no added salt)
2. Young (normal salt)

A. Aged 6 month

B. Aged 1 yr

C. Aged 2 yr.

Mix in 2 lb combinations as follows:

- 1-B
- 2-B
- Fill into square SS hoops and press for 3 hours.

Mix in 2 lb combinations as follows:

- 1-A
- 2-A
- 1-B
- 2-B
- 1-C
- 2-C
- Fill into round plastic hoops and press for 3 hours.

Fill 4 lb of cheese into round plastic hoops the following:

- 1
- 2
- A
- B
- C
- Press for 3 hours.

Mix in 2 lb combinations as follows

- 1-B
- 2-B
- Heat the cheese mixture to 120 F (enough to soften but not completely melt) then fill into square SS hoops and press for 3 hours.

Vacuum package all cheeses, label using the following codes (SS, PL or SSH, 1 or 2, A, B, or C) and store at 38 F.

After 2 wk, using the minimal material, determine cohesiveness by visual examination, TPA, hand compression, and chewing.

If satisfactory cohesion then send samples to DMI. This will enable a new workplan to be initiated that includes multiple salt levels and sensory analysis.

Repeat at 4 wk and 6 wk of age if necessary.

RESULTS AND DISCUSSION

Two blocks of cheese were manufactured, one containing salt, one without (Table 1.2.1). At 2 weeks of age these cheeses were comminuted along with cheese of 6, 12, and 18 months of age, and mixed as indicated in Table 1.2.2. The cheeses were pressed and stored for one month at 38°F, then sent to DMI for evaluation.

NEXT STEPS

Cheese will be sent for analysis as scheduled.

Table 1.2.1. Composition of cheese made with and without salt.

	Moisture	Fat	Salt	pH
No Salt Block 1	39.19	30	0.08	5.04
Salt Block 2	34.85	31	1.71	5.14

Table 1.2.2. Mixing ratio (pounds) of recombined and pressed cheeses.

Name	Hoop type	No salt 2 week old Block 1	Salted 2 week old Block 2	Salted, 6 mo. old Block A	Salted, 12 mo. old Block B	Salted 18 mo. old Block C	Total Wt. (lb)
1-B SS	Stainless Steel	2			2		4
2-B SS	Stainless Steel		2		2		4
1-A P	Plastic	2					4
2-A P	Plastic		2	2			4
1-B P	Plastic	2			2		4
2-B P	Plastic		2		2		4
1-C P	Plastic	2				2	4
2-C P	Plastic		2			2	4
1 P	Plastic	4					4
2 P	Plastic		4				4
A P	Plastic			4			4
B P	Plastic				4		4
C P	Plastic					4	4

Influence of 30% reduction of salt on Cheddar cheese flavor acceptability

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Funded by: Dairy Management Inc., May 2009 - August 2010

BACKGROUND

An increasing importance is being placed on sodium reduction in foods by the US nutrition and health community. Most cheeses have relatively high sodium contents because salt is an important component of cheese and is used for is ability to help prevent undesirable bacterial growth during storage of cheese and its contribution to desirable flavor and performance attributes. This project will evaluate whether a 30% reduction in salt content of Cheddar cheeses can be made without a detrimental effect on their flavor.

RESEARCH PLAN

Objecttive 1.

Determine the impact of salt level on the pH and moisture of Cheddar cheese.

Objecttive 2.

Determine if a 30% salt reduction in Cheddar cheese can be made without adversely .

MATERIALS AND METHODS

Objecttive 1.

Cheddar cheese stirred curd will be made from 1500 lb milk in a horizontal vat using the standard WDC Cheddar make procedures and a *Lactococcus lactis* starter culture that produces a cheese with 37% moisture, 52% FDB, 1.8% salt and pH 5.1. The curd will be divided into four 26.5-lb portions, transferred, and salt added to individual portionss to cover a range from 2 to .05% salt.

The curd will be packed into 20-lb Wilson hoops, pressed overnight at 15 psi, vacuum packaged, and stored at 42 °F. After 5 d, the cheese will be sampled for proximate analysis (moisture, fat, salt and pH) and the block be cut in to 10 pieces of approximately 2 lb each, vacuum packaged and stored at 42 °F.

The data from this objective will be used adjust cheese making parameters in Objective 2.

Objective 2.

Based on the proximate analysis data from Objective 1 cheeses, the necessary changes to the standard cheddar cheese make procedure will be determined so that all cheeses have a moisture content of 36.5% to 37.5% moisture, pH 5.05 to pH 5.25, and FDB 51% to 53%.

Individual vats of cheese for each salt level will be made. Five vats of cheddar cheese per replicate will be made from 550 lb milk each (over one week) using the make procedures determined above to yield two block of cheese weighing approximately 22 pounds each. The curd will be weighed and salt added that corresponds to a salt reduction of 0%, 25%, 33%, 50, and 61% as shown below.

% salt in curd	% reduction	% salt in moisture
1.80	0	4.86
1.35	25	3.65
1.2	33	3.24
0.9	50	2.43
0.7	61	1.89

The curd will be packed into two 20-lb Wilson hoops, pressed overnight at 15 psi, vacuum packaged, and stored at 42 °F. After 5 d, the cheese will be sampled (each block) for proximate analysis (moisture, fat, salt and pH) and sodium analysis by ICP spectroscopy, and one block will be cut in to 10 pieces of approximately 2 lb each, vacuum packaged and stored at 42 °F, the other block will also be stored.

After 3 and 6 months of storage the cheese will be tested for the following parameters:

1. pH.
2. Descriptive taste panel to quantify the following flavor descriptors in the cheese: sour, oxidized, bitter, salty, sweet, lactone/fatty acid, cooked, umami, nutty whey, buttery, fruity, rosy/floral, sulfur, and brothy.
3. A preference panel on cheese at room temperature, with recruiting limited to frequent consumers of cheese (people who consume cheese on at least 5 occasions per week) for flavor liking, texture liking, and overall liking.
4. A preference panel using the same panelists will evaluate the cheese when used to prepare cheese quesadillas that are served to consumers warm.
5. Texture profile analysis using 25% compression.

Experimental Plan Summary.

3	Replicates
20	Vats of cheese (5 preliminary, 15 study cheeses)
30	20-lb Blocks of cheese
3	Descriptive taste panels
3	Preference panels – cheese as table cheese
3	Preference panels – cheese in cheese quesadillas
5	Texture profile analysis with 25% compression

RESULTS AND DISCUSSION

Fifteen cheeses were made, three replicates for each of the requisite salt levels (Table 2.1.1). Consumer acceptance of the cheeses decreased with decreasing salt content (Figure 2.1.1).

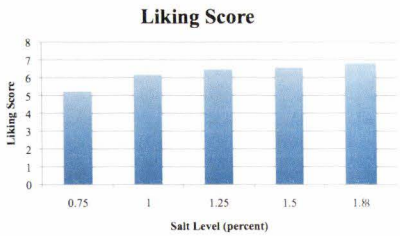

Figure 2.1.1. Average consumer liking scores of reduced salt cheeses.

CONCLUSIONS

During cheese making the ratio between added salt and retained salt varies as the salt concentration changes. Salt additions in the "normal" range (1.5-2.0%) must be increase to ensure that the proper amount of salt is retained in the curd. At these levels the percent salt loss is between 8-12%. At lower levels of salt in the cheese (0.9–1.2.%) there is less salt loss as less moisture is expelled from the curd. At 0.7% salt level there is no loss of salt due to the loss of moisture from the curd.

The level of salt added to the curd, also influences the final pH of the cheese. Low salt cheeses nearly always have a lower pH than high salt cheeses. As concentrations of salt increases in the curd, more syneresis takes place, and this expulsion of whey from the curd removes lactose, resulting in a decreased growth of the starter organisms. As the growth slows the pH drop is less significant. Consequently, the final pH of low salt cheese is lower than the final pH of a cheese with higher salt.

NEXT STEPS

Cheese will be analyzed as scheduled.

Table 2.1.1. Composition of reduced salt cheeses.

Salt Target	Rep	pH	% Fat	% Moisture	% Salt
0.75	Rep 1	4.99	34.50	36.55	0.73
0.75	Rep 2	4.99	35.00	37.00	0.72
0.75	Rep 3	5.00	35.00	36.44	0.77
1.00	Rep 1	5.00	34.50	36.47	1.64
1.00	Rep 2	5.29	31.50	37.26	0.92
1.00	Rep 3	5.00	34.00	36.24	1.04
1.25	Rep 1	5.10	30.00	37.46	1.28
1.25	Rep 2	5.02	30.00	38.43	1.28
1.25	Rep 3	5.09	30.00	37.43	1.28
1.50	Rep 1	5.04	30.00	38.02	1.32
1.50	Rep 2	5.01	34.50	36.68	1.78
1.50	Rep 3	5.04	30.00	37.52	1.47
1.80	Rep 1	5.28	33.00	34.60	1.90
1.80	Rep 2	5.16	32.50	35.59	1.84
1.80	Rep 3	5.33	31.50	35.44	1.98

Influence of 33% reduction of salt on Mozzarella cheese acceptability

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BACKGROUND

An increasing importance is being placed on sodium reduction in foods by the US nutrition and health community. Most cheeses have relatively high sodium contents because salt is an important component of cheese and is used for is ability to help prevent undesirable bacterial growth during storage of cheese and its contribution to desirable flavor and performance attributes. This project will evaluate the effect of reduction in salt content of Mozzarella cheese on its flavor and functionality.

RESEARCH PLAN

Objective 1.

Manufacture Mozzarella cheese with salt levels of 1.8%, 1.35%, 1.2%, 0.9% and 0.7%.

Objective 2.

Determine the effect of salt reduction in Mozzarella cheese on its flavor and functionality.

Objective 3.

Prepare report and write manuscript.

MATERIALS AND METHODS

Objective 1.

Mozzarella cheese (with fat content of 20% to 22%) will be made from 1500 lb milk using a horizontal vat using the standard WDC LMPS mozzarella cheese make procedure, on 2 separate occasions. The curd will be divided into 25-lb portions and then processed in the cooker-stretched as follows:

Target Salt	Dry Salting	% Salt in Cook Water	% Salt in Cool Water
1.80%	1.80%	4%	3.8%
1.35%	1.20%	4%	2.8%
1.20%	0.90%	4%	2.5%
0.90%	0.25%	4%	1.9%
0.70%	0.00%	4%	1.4%

The cheese will collected in approx. 6 lb portions as it exits the cooker/stretcher and then cooled in cold brine, then vacuum packaged, and stored at 38°F. After 5 d, the cheese will be sampled for proximate analysis (moisture, fat, salt and pH, and sodium content by ICP analysis, and returned to storage.

If the salt and composition levels are within target specifications then the cheese will be used for Objective 2. If not the cheese make procedure will be modified and a new batch of cheese made. Three replicates of cheese will be made for use in Objective 2.

Objective 2.

The mozzarella cheese from Objective 1 will be tested for functionality as follows:

- Meltability using the UW meltmeter on d 14, 28 and 56. [Changed to 21, 42, 63]
- Stretchability using the USU stretch test on d 14, 28 and 56.

The mozzarella cheese will be shredded and tested for flavor as follows:

- Descriptive taste panel of cheese at 2 wk to quantify the following flavor descriptors in the cheese: sour, oxidized, bitter, salty, sweet, lactone/fatty acid, cooked, umami, nutty whey,

- buttery, fruity, rosy/floral, sulfur, and brothy.

The mozzarella cheese will be tested for consumer acceptability as follows:

- Presented as cold cheese shreds at 3 wk using a preference panel, with recruiting limited to frequent consumers of cheese (people who consume cheese on at least 5 occasions per week) for flavor liking, texture liking, and overall liking.
- Presented as cheese on a hot pizza (60°C) at 3 wk using the same preference panel.

Experimental plan summary.

Objective 1.

Estimated 4 cheese makes to reach target salt levels.

Objective 2.

- 3 Replicates (vats of cheese)
- 5 Vats of cheese (5 treatments per vat
- 30 6-lb Blocks of cheese
- 3 Descriptive taste panels
- 3 Preference panels – cheese as shreds, and as pizza
- 9 Meltability (5 cheeses x 3 subsamples per test, 3 time points)
- 9 Stretchability (5 cheeses x 3 subsamples per test, 3 time points)

RESULTS AND DISCUSSION

The first replicate of cheeses has been made (Tabble 2.2.1).

NEXT STEPS

The second and third replicate cheeses have been scheduled. Analysis will be completed as scheduled.

Table 2.2.1. Composition of reduced sodium mozzarella cheese, trial 1.

NAME	% MOISTURE	% FAT	% SALT	pH
VAT 1 0.7%	48.10	17	0.75	55.3
VAT 2 0.9%	47.81		0.92	55.1
VAT 3 1.25%	47.36	18	1.17	55.3
VAT 4 1.35%	47.65		1.24	55.4
VAT 5 1.8%	45.21	18	1.77	55.9