2004 Annual Report

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Western Dairy Center
Annual Report
2004

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Annual Meeting
Utah State University
June 14, 2005

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Jeffrey Broadbent
Craig Oberg

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PI: Donald MacMahon

Pressure processing to improve milk freshness and refrigerated shelf-life
PI: J. Antonio Torres

Production of an extruded whey protein snack food
PI: Marie K. Walsh
Charles Carpenter
Western Dairy Center
Activities Summary
2004

The Western Dairy Center is a consortium of researchers devoted to improving the dairy industry in the United States by conducting research in all areas of dairy foods. The Center includes researchers from Utah State University, University of Idaho, Oregon State University, Brigham Young University, Washington State University and Weber State University. This report summarizes the research activities from January 1, 2004 through December 31, 2004.

The National Dairy Research Plan developed by Dairy Management Inc, industry and the 6 national Dairy Food Research Centers determines research priorities. The Western Dairy Center researchers have national recognition and expertise in the areas of dairy micro and molecular biology, cheese flavor development, cheese production, processing and functionality, fluid milk processing and utilization of dairy co-products.

The Center conducted two sessions of the 21st Annual Cheese Making Short Course in February 2004, at Utah State University with 12 attendees in each session. We limit the number of attendees to ensure a "hands on" learning experience. Our short course was able to use our new cheese making facilities, specifically two automated Scherping cheese vats. Our Scherping cheese vats have a 1500 lbm capacity and we also have a new finishing table with a 150 lbm capacity.

We conducted our 16th Biennial cheese Industry conference in Sun Valley Idaho in August 2004. There were over 35 attendees at this conference. This event included speakers from the dairy industry and academics who discussed the use of ultrafiltration with respect to economics, cheese processing and regulatory issues. Ultrafiltration of milk for use in dairy products will allow farmers who are currently a long distance from processing plants to sell more milk by increasing the number of processing plants they can economically ship to. We are soliciting topic areas of interest for the next meeting, in August 2006, so please contact the center for speaker and topic suggestions.

USU Licensed the patent “Textured whey Protein Product and Method” (patent number US 6,607,777, August 2003) to Grande Cheese Company (Lomire, WI) for commercialization of textured whey products. This will broaden the application of dairy proteins into markets currently dominated by soy protein by having dairy proteins as the major ingredient in meat analogs, meat extenders, and snack foods. This will increase milk sales for cheese production by making whey disposal more profitable.

In 2004, the number of new competitive grants awarded by Dairy Management was 2 and we had 6 continuing grants which resulted in $282,393 total research dollars. Project progress reports of all research projects active in 2004 are included in this report.
WESTERN DAIRY CENTER
OPERATIONAL ADVISORY COMMITTEE

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

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Publications and Presentations
2003 - 2004


Alred, A.L. 2004. Conjugated linoleic acid and omega fatty acids in milk and cheese from cows fed calcium salts of fish oil alone or in combination with soybean products. Dept. of Animal, Dairy & Veterinary Sciences


Noyes, B. L. 2003. Correlation between the USU stretch test and the pizza fork test.


Western Dairy Center
Project Report
Reporting Period January 1, 2003-December 31, 2004

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

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

Institution’s Project #:   03136

Project Completing Date:   June 30, 2005

Modifications to Project/Budget: None

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

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

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

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

To date, we have constructed plasmid-based gene complement systems that express the MR-2C epsA, epsD, epsG, and epsM genes. The epsG complement plasmid was transformed into strain MTC330 and we have been working to determine the effect of epsG expression on the sugar composition of MTC330 EPS. Unfortunately, our initial efforts to characterize EPS structure by NMR were confounded by contaminating sugar in the growth medium (M17). To overcome this limitation, we tested our strains for the ability to grow in a variety of chemically defined media that lack ingredients containing complex sugars (i.e., yeast or beef extracts). After considerable work, we identified a defined medium that supports the growth of our strains, and confirmed EPS purified from this medium is suitable for NMR studies. We are now using the medium to prepare sufficient EPS for NMR studies. In the coming months, we expect to complete our collection of eps mutants, and determine the effect of these genes on EPS production.

2. Significant Conclusions:
see progress, above

3. Anticipated Problems/Delays: None

Publications:

Theses: None

Published Abstract: None

Presentations:

Patent/Invention Disclosures: None

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

Visitors Hosted: None
Western Dairy Center
Project Report
Reporting Period: January 1, 2002—December 31, 2004

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

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

Institution’s Project #:  02132
Project Completion Date:  06/30/05

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

Modifications to Project/Budget:  None

Project Objectives:  (Include any revisions to objectives)
Objective 1: To determine the effect of oxidation-reduction potential (Eh) on the growth rate of selected starter and nonstarter lactic acid bacteria (NSLAB).
Objective 2: To determine if Eh can be used to preferentially control the growth of starter and NSLAB at the species or strain level.
Objective 3: To determine the correlation between manufacturing protocols, cheese Eh, and NSLAB populations in Cheddar-type cheese.

Project Summary:
All bacterial-ripened cheeses contain nonstarter lactic acid bacteria (NSLAB) that enter cheese through milk or processing equipment and grow to high numbers during ripening. NSLAB can have a significant effect on flavor development, but little is known about factors that influence the growth and composition of the nonstarter biota. As a result, the types and numbers of NSLAB in cheese, and their impact on flavor, is still largely a matter of chance.

This project will define influence of oxidation-reduction potential on growth of NSLAB and determine whether this property is a key factor in strain dominance.

1. Significant Progress against Objectives:
Research to date continues to focus on objectives 1 and 3. Redox experiments with different cheeses have worked well, and we have set up a fermentation system to study the relationship between growth of starter and NSLAB and redox. As reported previously, data from cheese trials performed under objective 3 support our hypothesis that Eh may be influenced by the cheese manufacturing process and by NSLAB populations. Colby cheese is a washed curd variety of Cheddar cheese, and has a higher moisture content and lower acidity than Cheddar because lactose and lactate are rinsed from the curd during washing. Previous workers have reported that the initial decrease in cheese Eh is due to oxidative fermentation of residual lactose by starter bacteria, so Colby cheese would be expected to show a higher initial Eh and more gradual Eh drop than milled or stirred curd Cheddar. These trends have been observed in our trials, but the Eh of Colby cheese ultimately reached values more negative
than those measured in milled or stirred curd Cheddar cheese. The latter observation was unexpected, but enumeration of NSLAB in each cheese revealed populations in Colby cheese were approximately two orders of magnitude higher than those of the milled or stirred curd Cheddar at 1 mo (105 versus 103 cfu/g, respectively). Starter numbers in all three cheeses were similar. Characterization of the NSLAB species in each cheese type was performed by API-50E Staph and by partial 16S rRNA sequence analysis, and showed both cheese types had NSLAB populations that were dominated by *Lactobacillus (Lb.) casei* and *Lb. curvatus*.

Research to study the relationship between NSLAB growth and redox initially examined the effect of starter and NSLAB isolates on redox. Results from that work have demonstrated that Eh goes up with lactose fermentation by some strains, but goes down with others. This strain-dependent phenomenon has been noted with strains of *Lactococcus lactis* starter bacteria as well as nonstarter and adjunct strains of *Lb. casei*. In the next series of experiments, we held pH constant at 5.1, temperature at 21°C, and determined the effect on NSLAB growth on redox. Next, we held redox constant (+150 or -50) and measured the growth rates of selected NSALB. Results showed growth was affected by redox, which indicated Eh is probably an important factor in strain dominance and success (objective 2).

2. Significant Conclusions: See progress, above

3. Anticipated Problems/Delays: None

Publications:

Theses: None

Published Abstract: None

Presentations:

Patent/Invention Disclosures: None

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

Visitors Lost: None

Invention Disclosures: (Title, Date) None
Patents: (Title, Date, #) None
Licensing Activities: None
Discoveries: See above
Western Dairy Center
Project Report
Reporting Period: January 1, 2002—June 30, 2002

Principal Investigators: Carl Brothersen
Co-Investigators:

Project Title: Optimization of high pressure liquid injection system for vitamin fortification of cheese

Institution’s Project #: 04148

Project Completion Date: June 30, 2005

Modifications to Project/Budget:
$1,375.00 transferred from Supply category to Salary category in order to accommodate accounting changes for cheese making. No change in overall budget.

Project Objectives: (Include any revisions to objectives)
Objective 1. Determine the effect of pressure, pulse duration, cheese age, and cheese temperature on injection penetration using a dye.
Objective 2. Determine the effect of injection frequency, cheese age and cheese temperature on injection retention using a dye.
Objective 3. Determine the retention and distribution of Vitamin D injected into cheese.

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

In a previous project we investigated the feasibility of fortifying cheese with vitamins. We found the flavor of the cheese was not adversely affected by addition of Vitamins D, B3 and folic acid, and the level of active vitamin remained high during aging of the cheese.

When vitamins are added to cheese milk, a large portion of the vitamin is lost in the whey. This reduces the efficiency of the process and renders the whey unsuitably for use in other products.

Researchers at the Western Dairy Center have developed technology to inject liquids into foods using high pressure. This technology utilizes narrow jets of liquid, which penetrate the product, rather than the traditional needle system. This eliminates cross contamination of samples by the needles. It also eliminates the whey contamination problem mentioned above.

The purpose of this proposal is to optimize this injection system for vitamin fortification of cheese. We will optimize pressure, pulse duration, and injection frequency in cheese at two temperature/age combinations: 1) room temperature when the cheese is removed from the press before the curd has begun to knit, and 2) in cheese that has been vacuum sealed and stored at 4°C for seven days and the cheese curd is sufficiently knit. We will then evaluate the cheese for even distribution of the vitamin. Cheese made from three manufacturing techniques will be examined, 1) matted and milled curd, 2) stirred curd...
Cheddar or Mozzarella, and 3) pasta filata Mozzarella.

Successful completion of this project will determine the injection parameters for the most efficient injection of matted curd, stirred curd and pasta filata cheese, with vitamins. It will determine the distribution of the vitamin injected into these three cheese manufacturing classes, and it will provide a cost estimate of the system.

1. Significant Progress against Objectives:
   Objective 1. Completed
   Objective 2. Completed
   Objective 3. In progress
   Objective 4. In progress

2. Significant Conclusions:

Objective 1. Cheese made by the following three different manufacturing techniques was obtained:
1) Mozzarella, pasta filata, brined for 12 hours, packaged in eight pound loaves.
2) Cheddar, stirred curd, dry salted, pressed in 20 pound blocks.
3) Cheddar, matted and milled curd, dry salted, pressed in 40-pound blocks.

The cheese blocks were randomly divided into two groups. Cheese from the first group was cut into blocks approximately 10cmX10cmX10cm when they were removed from the brine or press, and injected with solution containing red dye. The temperature of the cheese upon injection was 20°C. The stirred and matted curd cheese from the second group was vacuum packaged and stored at 4°C for 14 days. The cheese was then cut into 10cmX10cmX10cm blocks and injected with the dye solution. The pasta filata cheese in the second group was packaged and also stored at 4°C for 14 days. It was then cut into 10cmX10cmX10cm sized blocks and injected with the dye solution.

The smaller cheese blocks were randomly selected and injected at 1000, 1500, 2000, 2500, or 3000 psi. Each pressure treatment also received injection pulse time of 1, 2, 3, 4, and 5 seconds. Injections were spaced 1 cm apart. After injecting, the blocks were cut along the plane of injection, and depth of the injection measured. Examples of the injected blocks are shown in Figures 1-3.

Figure 1. Example of pasta filata cheese at one day of age and 20°C, injected with dye at 1,500 psi for 2 seconds
Figure 2. Example of stirred curd cheese at one day of age and 20°C, injected with dye at 3,000 psi for 5 seconds.

Figure 3. Example of matted curd cheese at one day of age and 20°C, injected with dye at 2,500 psi for 1 second.

Injection depth as a function of injection time, pressure, cheese type and cheese age/temperature is shown in Tables 4-9. Injection depth generally increased with increasing time and pressure for all cheese types, and age/temperature combinations. The data shown are averages of 45 injections for each data point. The variability between injections was large, due to the random nature of the curd in the block.

In the pasta filata cheese, there are no open channels to deflect the injectate, resulting in better penetration. The injectate had a tendency to travel with the protein fibers. Because of the circular motion of the agar in the cooker/stretcher, the fibers are layered in spirals, which appear as concentric circles in the sliced cheese (see Figure 11). Injection depth was greater in the older, colder cheese. This is due to a combination of the firmer texture of colder cheese and the lack of open channels to absorb the injectate.
Figure 4. Effect of injection pressure and time on injection depth in pasta filata cheese at one day of age and 20° C.

Figure 5. Effect of injection pressure and time on injection depth in pasta filata cheese at 14 days of age and 4° C.

The curd particles of cheese made by the stirred curd method are much smaller than those made by matting and milling. This results in more channels in which the injectate may flow, and reduces the penetration depth. It may however, allow a more even distribution of the injectate. Decreased temperature caused a decrease in the penetration because the open texture of the cheese was not reduced upon vacuum packaging, as it was with the milled curd.
Figure 6. Effect of injection pressure and time on injection depth in stirred curd cheese at one day of age and 20° C.

Figure 7. Effect of injection pressure and time on injection depth in stirred curd cheese at 14 days of age and 4° C.

In cheese made by matting and milling, the milled curd particles are relatively solid with large openings between adjacent particles. The injectate is deflected into these openings where it collects in pools. This decreases the depth of the injections. When the cheese is vacuum packaged, these openings are reduced in size and number, allowing better penetration of the injectate.
Objective 2.

Using the data and procedures from Objective 1, 10cm×10cm×10cm cheese blocks were injected to give the most efficient and uniform distribution of dye. The stirred and matted curd cheese at one day of age was the most difficult to inject, as the cheese had not knit, and was easily fractured by the injection process.

In order to evaluate the distribution of the injectate, randomly selected 10cm×10cm×10cm size blocks of each cheese type and age/temperature were injected on the top and bottom of each block. One block was then cut into 2 cm slices parallel with the direction of the injections, and inspected visually. A second block was sliced perpendicular to the direction of the injections and inspected. Figures 11-16 show examples of the slices of typical blocks. In each figure, the slices are arranged sequentially, as the were removed from the block, with the first slice being in the position row A, column 1; the second slice in row A, column 2; and so forth to the last slice.
Figure 10. Slices of a block of pasta filata cheese injected at one day of age and at 20°C, cut parallel to the direction of the injections.

Figure 11. Slices of a block of pasta filata cheese injected at one day of age and at 20°C, cut perpendicular to the direction of the injections.

Figure 12. Slices of a block of stirred curd cheese injected at one day of age and at 20°C, cut parallel to the direction of the injections.
Figure 13. Slices of a block of stirred curd cheese injected at one day of age and at 20°C, cut perpendicular to the direction of the injections.

Figure 14. Slices of a block of matted curd cheese injected at one day of age and at 20°C, cut parallel to the direction of the injections.
Figure 15. Slices of a block of matted curd cheese injected at one day of age and at 20°C, cut perpendicular to the direction of the injections.

Placing the injection points closer together than 1cm resulted in fragile cheese, which was easily broken when handled. The smaller sized blocks of cheese were injected with dye at one centimeter intervals, with alternating rows offset by 0.5 centimeters as shown in Figure 16. This configuration gave the best distribution and retention of the retentate while retaining the integrity of the block.

Figure 16. Most efficient injection pattern for all types of cheese.

3. Anticipated Problems/Delays:
We have experienced problems with mechanical breakdown of the injector, and therefore Objectives 3 and 4 have not been completed. We have received a six-month no-cost extension. A different injector has been modified to inject cheese and we plan to complete the project at the end of the extension.

Publications: None

Theses: None
Published Abstract: None

Presentations: None

Patent/Invention Disclosures: None

Technology Transfer Activities

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Visitors Hosted: None

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:
Western Dairy Center

Final Project Report
Reporting Period January 1, 2002 — June 30, 2004

Principal Investigators: Daren Cornforth, Utah State University
Co-Investigators: 

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

Institution's Project #: 01126

Project Completion Date: June 30, 2004


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

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

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

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

Objective 4: Determine the optimum use levels and economic viability of using dried WM as an antioxidant in processed meats (fresh pork sausage, Italian sausage, summer sausage).

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

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

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

Objective 1. Fresh Pork Sausage. Control (uncooked) pork sausage, with or without milk mineral (MM), had low rancidity, as measured by sensory evaluation and TBA values. TBA values for all uncooked sausages were less than 0.5 during 15 days storage at 2°C.

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

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

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

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

Objective 6. Comparison of MM to other antioxidants. 1.5% MM and 0.5% STP were both highly effective in prevention of rancidity in cooked ground pork stored for 15 days at 2°C. Sodium nitrite (156 ppm) was intermediate in prevention of rancidity, and Rosemary oil extract (0.2% of meat weight) or BHT (0.1% of meat fat content) were not effective antioxidants. Increasing the levels of Rosemary or BHT increased their effectiveness, but would also increase costs. Rosemary powder (0.4%) and BHT (0.1% of total meat weight) were effective antioxidants. However, use of BHT at 0.1% of meat weight would not be permitted by USDA regulations.
Comparison of Type 1 and Type 2 antioxidant effectiveness in cooked ground pork during refrigerated storage.
Preetha Jayasingh, Charles E. Carpenter, and D. P. Cornforth
(Presentation at the 2003 meeting of the Institute of Food Technologists, Chicago, IL)

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

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

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

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

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

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

2. Anticipated Problems/Delays: None

Publications:


Dissertation:

Published Abstracts:


Presentations:


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


Addendum


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

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

Invention Disclosures: (Title, Date)

Western Dairy Center

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

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

At currently accepted use levels, Type 2 antioxidants (Iron binding agents STP, MM, sodium nitrite) were more effective antioxidants in cooked ground pork than the type 1 antioxidants (BHT or Rosemary extract; electron donors).
Western Dairy Center
Project Report
Reporting Period August 1, 2004 - 2005

Principal Investigators: Conly Hansen, Utah State University
Co-Investigators: Seokhwan Hwang, Postech University; Donald J. McMahon, Utah State University

Project Title: Cultivation of Mushroom Mycelia Using Whey Permeate

Institution Project #: 03138

Project Completion Date: 12/31/04

Modifications to Project/Budget: None

Project Objectives: (Include any revisions to objectives)

Objective 1: Determine suitable bulk suspended growth conditions for mushroom mycelia grown on whey permeate including: concentration of permeate in the media to accelerate growth while avoiding inhibition, temperature, pH, mixing, and O2 requirements. [Hypothesis: there is an optimum level at which all variables (i.e. concentration of whey permeate, temperature, pH, mixing, and O2) must be maintained in order to promote maximum growth of the mycelia.]

Objective 2: Perform fermentations to determine if other cheese/whey/lactose byproducts can also be used to grow mycelia. [Hypothesis: whey byproduct streams other than whey permeate can be used as a growth medium for mycelia.]

Objective 3: Complete experiments to indicate nutrient supplementation requirements for whey permeate and delactosed whey permeate (mother liquor) when used as the media for growing mycelia. [Hypothesis: no additional nutrient supplementation is required.]

Objective 4: Develop a procedure for separating mycelia from spent media. [Hypothesis: centrifugation will effectively separate mycelia from the spent media.]

Objective 5: Determine residual chemical oxygen demand, solids, macronutrients N & P, and odor that remain in spent media after mushroom mycelia has been harvested to reveal how much the potential pollution problem of cheese byproduct is solved using this process. [Hypothesis: chemical oxygen demand (COD) of the spent media will be less than 100 mg/L and other discharge parameters will be
Project Summary: (Suitable for inclusion in Center documents released to the public)

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

Significant Progress against Objectives:

Objective 1
Determine suitable bulk suspended growth conditions for mushroom mycelia grown on whey permeate including: concentration of permeate in the media to accelerate growth while avoiding inhibition, temperature, pH, mixing, and O2 requirements.

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

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

Results and Discussion
The growth rate of the mushroom mycelium when grown on various commercial medium is shown in table 1. A comparison of the growth rate of the mushroom mycelia when
grown on whey permeate, whey, and the commercial media is shown in table 2. These tables show that the mycelia can be grown as quickly or more quickly using inexpensive whey permeate as they can be grown using expensive commercial media.

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

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

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

Table 2. Whey permeate and commercial media growth rate comparison

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

Conclusions
Whey permeate is a suitable growth medium for edible mushroom mycelium. In most cases the mycelia will grow even more quickly on whey permeate than on commercial media. With careful control of the growth parameters, it could be possible to achieve maximal growth rate and biomass production simultaneously. The parameters that yielded the fastest growth for the species *Lentinus edodes* were: temperature 23.6°C, pH 4.97, and substrate concentration 40g/l whey permeate.

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

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

**Results and Discussion**

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

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

**Maximum mycelial growth rate of *L. edodes* when grown on delactosed whey.** Low concentrations of delactosed whey allow vigorous growth of the mycelia whereas high concentrations exhibit no growth.
Conclusions

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

Objective 3

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

Results and Discussion.

The nature of this objective was such that if the original hypothesis was correct (i.e. no additional nutrient supplementation is required), then no experimentation would be necessary. Mycelia grown in 20% delactosed whey. These mycelia exhibit a viable colony that is constantly growing and expanding. The only source of nutrients for the mycelia is the delactosed whey substrate, yet they are finding adequate nutrition for growth. Confirm it. In other words, if we attempted to utilize whey products as a growth substrate for mushroom mycelia and those mycelia didn’t grow, we would have...
suspected that some vital nutrient was lacking in the growth media and subsequent experimentation would have been directed toward determining what nutrient/s were lacking. However, if our original attempts to cultivate the mushroom mycelia were successful, that would be an indication that no additional nutrients were needed for the cultivation process.

I Experiments conducted to meet objective one yielded growth rates that could exceed the growth rates obtained utilizing commercial media (see previous tables 1 and 2). Since our experiments were able to match or exceed the growth rates obtained using media engineered to contain all the necessary growth nutrients for the mycelia, we can safely conclude that no nutrient supplementation is necessary when utilizing whey permeate as a growth substrate.

I Experiments conducted to meet objective two utilized non-supplemented delactosed whey. In this media, the mycelia were able to obtain growth rates even greater than the rates obtained utilizing whey permeate. By the same logic, we may conclude that delactosed whey also requires no special nutrient supplementation.

Conclusion
No additional nutrient supplementation is required when using whey products as a growth substrate for the cultivation of mushroom mycelia.

Objective 4
I Develop a procedure for separating mycelia from spent media.

Result and Discussion
Mycelia were harvested at the stationary growth phase. Centrifugation was used to harvest the mycelia. After the thermal lysis treatment of the pellet (mycelia), protein-bound polysaccharides were obtained by precipitating the lysate with 95% ethanol.

The polysaccharide content as well as productivity of the extract is an important parameter to indicate efficiency in mycelial cultivation. A total of 1820 mg extract per liter was obtain from the submerged culture of *Ganoderma lucidum*.

Previous studies have shown that typical yield of polysaccharides varies from 0.6 to 1.1 g/g per liter used glucose medium. In our studies, the extract of polysaccharide were 11120Å°13 mg/L.
This high extract content have indicated that the process condition and harvesting condition were suitable for the application of whey permeate as an alternative substrate for mycelial production.

Objective 5
I Determine residual chemical oxygen demand, solids, macronutrients N & P, and odor that remain in spent media after mushroom mycelia has been harvested.
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The COD of the deproteinated whey was 54.1 g/L. This indicated an amount of potentially biodegradable substance in the deproteinated whey wastewater. There was also a high ratio of SCOD to COD (98%), which suggested that most of the organic materials in the waste were soluble. Lactose is the major organic component in whey permeate, which contributes to 83% of the COD.

**Result and Discussion**

The SCOD removal range from 80.7 to 93.1% within the design boundary, where the condition for maximum SCOD removal was pH 4.6 and 27.1°C. The model response at the estimated condition was 3639 ± 560 mg SCOD per liter, which was 93.1% SCOD reduction. In the case of response analysis for the mycelial yield, the quadratic model was selected to describe the response surface. The results showed that the condition for a maximum mycelium yield under optimal SCOD removal rate (0.35 mg mycelial weight per mg SCOD removed) was found at pH 4.2 and 28.5°C, based on response analysis. This was almost the same as the optimal condition for mycelia production (pH 4.2 and 28.3°C). This concluded that the optimal condition for SCOD removal could be used for the optimal mycelia production in *Ganoderma lucidum* cultivation with whey permeates.

**Anticipated Problems/Deays:** None

**Publications:** None

**Theses:** Cultivation of Mushroom Mycelia Using Whey Products as a Growth Substrate, by Boyd Inglet

**Publications:**


**Presentations:**

Boyd Inglet, Progress Report on Mushroom Mycelia Project. POSTECH University, Pohang, Korea. August, 2003

Boyd Inglet, Thesis defense [Title: Cultivation of Mushroom Mycelia Using Whey Products as a Growth Substate], Utah State University, July 2004

Song, M. Mushroom Mycelia Project. Presented at Utah State University, October, 2003.

**Patent/Invention Disclosures:** None
Western Dairy Center
Project Report
Reporting Period January 1, 2003 — December 31, 2004

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

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

Institution’s Project #: 89093

Project Completion Date: June 30, 2005


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

Objective 1: Determine the optimum conditions for measuring the functional properties of melted cheese using the USU Stretch test.

Objective 2: Correlate the melt and stretch parameters of cheese measured using the Stretch test with the fork stretch test used by the pizza industry.

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

The performance of cheese on a pizza depends on how readily the cheese melts and how well it can be stretched. Suppliers of cheese to the pizza industry, subjectively measure this performance using a fork test. A test has been developed at Utah State University that can be used to objectively measure cheese performance when heated. To determine if the “Stretch” test was a suitable replacement for the fork test, a comparison between the two tests was performed. The cheeses were tested in the range of 65°C to 85°C using the USU Stretch Test and then compared to evaluations of the same cheese by an industrial partner using the fork test. The USU stretch test provides a better characterization of a pizza cheese than can the pizza fork test. Where the pizza fork test is only able to record the distance that the cheese can stretch, the USU stretch test is capable of measuring a variety of parameters, which provides not only an indication of how far the cheese will stretch, but other important functional properties related to the cheese, such as elasticity, and the amount of cheese pulled into a strand as the cheese is being stretched.
**Significant Conclusions:**

An example of a typical stretch profile follows, indicating points where measurements of Melt Strength \( (F_M) \), Stretch Extension at \( F_M \) \( (SE_F) \), Stretch Load at 5 cm \( (SL_5) \), and Stretch Extension at 0.4 N \( (SE_{0.4}) \) are taken.

![Stretch Profile](image)

**Typical Stretch Profile of cheese using the USU Stretch Test.**

*Shredded versus block cheese.* Much of the pizza-type cheeses in the US is manufactured and sold in shredded form, so using plugs of cheese has limited application. When cheese was analyzed using plugs or after shredding, significant differences in test results were observed. Using shredded cheese resulted in lower \( F_M \) as well as lower loads during stretching \( (SL \text{ values}) \). This comes about because shredded cheese is loosely packed in the sample cup, resulting in more air being trapped in the melted cheese mass. Less resistance occurs as the probe ascends through the cheese mass, and less cheese is retained on the probe as it exits the reservoir of melted cheese.

**Sample size.** Sample size also influences test results. When a 30-g sample of shredded cheese was used, the probe tended to lift the entire mass of melted cheese from the sample cup resulting in erratic load measurements. When the sample size was increased to 50 g and a deeper sample cup used, this problem did not occur. An additional benefit was that larger load values were obtained using the 50-g sample. This resulted in increased repeatability and greater significant differences between cheeses.

**Probe size.** Probe size in relation to sample size is also important because as the probe size increases there is an increased likelihood that slippage of the cheese in the sample cup will occur as the probe ascends through molten cheese mass. This occurs usually during the first 10 cm of the test when the cheese fails to adhere sufficiently to the sides and bottom of the sample cup. When this happens, the resistive load exerted erratically changes resulting in false \( F_M \) values. Such slippage was reduced by roughing the inside walls of the sample cups but for sample cups with an inside diameter of 35 mm, a probe with outer prong dimensions of approximately 25 mm performed better than a probe that extended almost the full width of the sample cup.
Heating time. When using shredded cheese it was observed that a tempering time of 45 min was needed to reach the set temperature. After 30 min of heating in the water bath, the central portion of cheese was still 2 to 3°C below the set temperature, and at 65°C this results in some small cheese particles being unmelted. Although FM was the largest load value measured, it was the least repeatable parameter. When differences were observed between FM values for two or three replicates, the stretch curves tended to converge following as the probe exited the cheese mass. Differences in FM between replicates could have been caused by differences how well the shredded cheese was packed into the cup, the amount of air trapped in the melted cheese, or degree of adhesion of the cheese to the sample cup.

Temperature. The temperature at which the cheese was tested influenced the stretch profile. At 65°C, the resistive load exerted by the cheese as the probe was lifted upwards was greater and the cheese strands that formed as the probe exited the cheese mass were larger. As the test temperature was increased, there was less cheese left attached to the probe when the cheese strands broke, so the load at which SE was calculated needed to be decreased from 0.5 N at 65°C to 0.05 N at 85°C. This represents the load being exerted on the probe by the weight of cheese that had been pulled from the melted cheese mass.

It was expected that there would be a good correlation between the pizza fork test and the SE values because they are both measured as the distance at which strand breakage occurs. At lower temperatures most of the cheeses tested were capable of stretching the entire distance without breaking. At higher temperatures, some cheeses would break, but a significant amount of cheese would remain on the probe. In spite of these differences, relatively high correlations were still observed for each temperature.

Correlation using single parameters. After looking at various measurements of load and extension from the stretch profiles all the data at 65, 70, 75, 80, and 85°C, there were six parameters with the highest overall correlation to the pizza fork test. These were FM, the extension at which FM occurred (SEF), slope of the load after FM as measured between 10 and 15 cm, the average load during stretching between 10 and 15 cm (SL10-15), and 20 and 30 cm (SL20-30), and SE1. Greater correlation was observed at higher temperatures (80°C or 85°C) and the parameter with the highest correlation with the pizza fork test over the entire temperature range was SL10-15.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>FM</th>
<th>SL10-15</th>
<th>SL20-30</th>
<th>Slope from 10-15 cm</th>
<th>SL1</th>
<th>SL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C</td>
<td>0.538</td>
<td>0.521</td>
<td>0.344</td>
<td>0.429</td>
<td>0.000</td>
<td>0.083</td>
</tr>
<tr>
<td>70°C</td>
<td>0.532</td>
<td>0.410</td>
<td>0.499</td>
<td>0.207</td>
<td>0.711</td>
<td>0.715</td>
</tr>
<tr>
<td>75°C</td>
<td>0.371</td>
<td>0.351</td>
<td>0.417</td>
<td>0.168</td>
<td>0.300</td>
<td>0.288</td>
</tr>
<tr>
<td>80°C</td>
<td>0.377</td>
<td>0.735</td>
<td>0.745</td>
<td>0.463</td>
<td>0.590</td>
<td>0.701</td>
</tr>
<tr>
<td>85°C</td>
<td>0.497</td>
<td>0.606</td>
<td>0.483</td>
<td>0.640</td>
<td>0.600</td>
<td>0.130</td>
</tr>
</tbody>
</table>

This can be explained by considering the probe position relative to the melted cheese mass at this extension. The melted cheese mass is about 4 cm high, so at 10 cm the probe has lifted cheese strands to the top of the sample cup. At this point, strands from some of the low stretch cheeses begin to break while others continue to exert a relatively high load on the probe. The high degree of correlation with the pizza fork test
Western Dairy Center

is probably because the cheese in this region most closely resembles the cheese during the pizza fork test. At higher extension distances, the cheese strands have a tendency to harden because the rate at which they are lifted during the stretch test is slower than that occurring during the fork test.

The correlation of \( F_m \) with the pizza fork test is probably an indirect relationship as the load exerted on the probe as it passes through the cheese mass is related to both the viscosity and elasticity of the melted cheese. At lower temperatures (e.g. 65°C), the cheese remains fairly viscous which results in a larger \( F_m \) value compared to that at 85°C. However, at both temperatures the \( R^2 \) values were similar (0.54 and 0.50, respectively), even though at 85°C the melted cheese was much less viscous. This suggests that the intrinsic stretch properties of the cheese are an inherent function of the cheese itself and not dependent upon temperature. Thus, it is not necessary to conduct stretch characterization tests only at 65 to 70°C even though this is the typical temperature of the cheese on a pizza when it is consumed.

**Using multiple parameters.** Using the six parameters that had the most correlation over all temperatures, along with one other parameter from each temperature that had a high correlation at the selected temperature but not at any others, multiple linear regressions were then performed. As was observed with the individual correlations, there was better fit of the stretch test data with the pizza fork test when the cheese was tested at higher temperatures. Using two parameters, adjusted \( R^2 \) values > 0.8 were obtained at 80°C and 85°C. When four parameters were combined in the model, more of the variation was accounted for with adjusted \( R^2 \) values of 0.965 at 80°C and 0.889 at 85°C. A plot of the pizza fork test data (with 0.5 inch error bars added as this test measures stretch length in one inch increments) versus values predicted by the stretch test is shown below.

![Comparison of pizza fork test data for nine different cheeses with values predicted from the USU Stretch Test.](image)

The greatest correlation was found when the cheese tested by the USU stretch test was tempered at higher temperatures (80 and 85°C). At 80°C the highest individual correlation between the pizza fork test and the USU stretch test was seen when SL_{10:15}(R_2
2004 Annual Report

= 0.74), SL20-30 (R2 = 0.75), or SEF (R2 = 0.70) were used. Multiple linear regression studies show that at 80°C a combination of SEF and SL22 (R2 = 0.85, Adj. R2 = 0.80) or a combination of four parameters (FM, slope from 10 to 20 cm, SLO1, and SL22, R2 = 0.97, Adj. R2 = 0.93) provides an increased correlation to the pizza fork test. At 85°C, two values could be used in a multiple linear regression study, SEF and SE0.05, to provide a high correlation to the pizza fork test (R2 = 0.90, Adj. R2 = 0.85)

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

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Variables used in model</th>
<th>R²</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>65°C</td>
<td>FM, SL2-10, SE0.1</td>
<td>0.847</td>
<td>0.755</td>
</tr>
<tr>
<td></td>
<td>FM², SL2-2, SE0.5</td>
<td>0.886</td>
<td>0.818</td>
</tr>
<tr>
<td>70°C</td>
<td>SE0.7, SE0.1</td>
<td>0.732</td>
<td>0.642</td>
</tr>
<tr>
<td></td>
<td>SE0.7, SI0.50, SE0.3</td>
<td>0.767</td>
<td>0.628</td>
</tr>
<tr>
<td>75°C</td>
<td>SI20.30, SI0.1</td>
<td>0.793</td>
<td>0.724</td>
</tr>
<tr>
<td></td>
<td>SI20.10, SI21-30, SI0.50</td>
<td>0.883</td>
<td>0.813</td>
</tr>
<tr>
<td>80°C</td>
<td>SE0.7, SI22</td>
<td>0.853</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>FM, Slope from 10-20 cm, SE0.1, SI22</td>
<td>0.965</td>
<td>0.930</td>
</tr>
<tr>
<td>85°C</td>
<td>SE0.7, SE0.2</td>
<td>0.899</td>
<td>0.848</td>
</tr>
<tr>
<td></td>
<td>SI2-10, Slope from 5-15 cm, √SE0.10</td>
<td>0.944</td>
<td>0.889</td>
</tr>
</tbody>
</table>

An aging study was useful in demonstrating how the parameters of the USU stretch test, generally FM, SEF, and SLO1, could be used to characterize the functional properties of a pizza cheese. It was seen that not only is the USU stretch test much more objective than the pizza fork test, but the different parameters of the USU stretch test give a greater understanding of the how the pizza cheese stretches than does the single value of stretch supplied by the pizza fork test.
Changes in cheese stretch properties as the cheese ages as measure by the pizza fork test and the USU stretch test at 80°C.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Storage Time</th>
<th>L₀</th>
<th>SE₃</th>
<th>Slope from 10 to 15 cm</th>
<th>SE₁₀₁₅</th>
<th>SE₅₁</th>
<th>Fork Test Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(d)</td>
<td>(%)</td>
<td>(cm)</td>
<td>(N cm)</td>
<td>(N)</td>
<td>(cm)</td>
<td>(in)</td>
</tr>
<tr>
<td>BB</td>
<td>21</td>
<td>08</td>
<td>1.67</td>
<td>-0.0040</td>
<td>0.02</td>
<td>6.5</td>
<td>12</td>
</tr>
<tr>
<td>BB</td>
<td>40</td>
<td>05</td>
<td>0.83</td>
<td>-0.0000</td>
<td>0.01</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>BB</td>
<td>60</td>
<td>04</td>
<td>0.83</td>
<td>-0.0000</td>
<td>0.01</td>
<td>5.4</td>
<td>3</td>
</tr>
<tr>
<td>CC</td>
<td>21</td>
<td>15</td>
<td>2.25</td>
<td>-0.0120</td>
<td>0.07</td>
<td>10.2</td>
<td>12</td>
</tr>
<tr>
<td>CC</td>
<td>40</td>
<td>08</td>
<td>0.83</td>
<td>-0.0000</td>
<td>0.02</td>
<td>6.1</td>
<td>14</td>
</tr>
<tr>
<td>CC</td>
<td>60</td>
<td>07</td>
<td>0.83</td>
<td>-0.0000</td>
<td>0.00</td>
<td>5.7</td>
<td>6</td>
</tr>
<tr>
<td>DD</td>
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<td>08</td>
<td>1.67</td>
<td>-0.0020</td>
<td>0.04</td>
<td>7.0</td>
<td>16</td>
</tr>
<tr>
<td>DD</td>
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<td>08</td>
<td>2.50</td>
<td>-0.0020</td>
<td>0.04</td>
<td>7.0</td>
<td>18</td>
</tr>
<tr>
<td>DD</td>
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<td>06</td>
<td>1.67</td>
<td>-0.0020</td>
<td>0.04</td>
<td>6.7</td>
<td>7</td>
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<tr>
<td>EE</td>
<td>21</td>
<td>07</td>
<td>1.25</td>
<td>-0.0040</td>
<td>0.03</td>
<td>6.6</td>
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<tr>
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<td>05</td>
<td>0.83</td>
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<td>5.5</td>
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<tr>
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<td>0.83</td>
<td>-0.0000</td>
<td>0.01</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>FF</td>
<td>21</td>
<td>25</td>
<td>0.91</td>
<td>-0.0000</td>
<td>0.04</td>
<td>7.6</td>
<td>1</td>
</tr>
<tr>
<td>FF</td>
<td>40</td>
<td>22</td>
<td>0.96</td>
<td>-0.0020</td>
<td>0.09</td>
<td>9.9</td>
<td>0</td>
</tr>
<tr>
<td>GG</td>
<td>21</td>
<td>11</td>
<td>2.50</td>
<td>-0.0060</td>
<td>0.08</td>
<td>9.7</td>
<td>8</td>
</tr>
<tr>
<td>GG</td>
<td>40</td>
<td>07</td>
<td>2.50</td>
<td>-0.0020</td>
<td>0.03</td>
<td>5.8</td>
<td>3</td>
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<tr>
<td>GG</td>
<td>60</td>
<td>05</td>
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<td>-0.0000</td>
<td>0.01</td>
<td>4.0</td>
<td>-</td>
</tr>
</tbody>
</table>

Publications:

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

Published Abstract:

Presentations:

Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:
Visitors Hosted:

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:
Western Dairy Center

Project Report
Reporting Period January 1, 2004 — December 31, 2004

Principal Investigators: Donald McMahon, Jeffrey Broadbent, Craig Oberg

Co-Investigator:

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

Institution’s Project #:

Project Completion Date: December 31, 2006

Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

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

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


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

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

Significant Conclusions:

Extraction of cheese samples
Cheese samples were extracted in a citrate buffer as described by Kaiser et al. (1992) with some modifications. Cheese samples at 1 week, 2, 4 and 6 months were shredded, and 10% of each sample were homogenized with 40 ml of 500 mM sodium citrate solution (containing 1% sodium chloride) and 70 ml of deionized water at 40-50°C for 4 min at 260 rpm in a Seward Stomacher 400. The homogenate was then cooled to room temperature and made up to 200 ml with deionized water. An aliquot of this mixture (35 ml) was then centrifuged (Sorvall RC5C; 6000 rpm, 15 min, 4°C) and further dispensed into smaller volumes (1 ml, microcentrifuge tubes) and centrifuged at 13000 rpm for 5 min (Beckman Microfuge Lite). The supernatant (2 ml) was further concentrated (1.1X) using a 3 kDalton Centricon concentrator (Amicon) centrifuged for 2 h at 2500 rpm and 4°C (Sorvall RC5C). Both permeate and retentate were stored at -20°C until analyzed by RP-HPLC.
Reversed-phase HPLC of samples
Permeate and retentate samples were dissolved in 0.1% trifluoroacetic acid (TFA, 1:15) and centrifuged (Beckman Microfuge Lite; 13000 rpm, 5 min). RP-HPLC was performed using an automated Beckman System Gold (Autosampler 507, 168 Detector & 125 Solvent Module) fitted with an analytical Brownlee Aquapore C8 column (Applied Biosystems; RP 300 Å, 7 µm, 2 X 100 mm) and guard column (10 X 4.6 mm). A 25 µl aliquot of the sample was injected and eluted with a gradient of two solvent mixture: 0.1% TFA (Solvent A) and 0.085% acetonitrile (ACN, Solvent B). Solvent B was varied from 40 to 70% over 60 min with a final wash at 80%. The flow rate was maintained at 0.2 ml/min. The eluate was monitored at both 214 and 280 nm with the detector being interfaced with an IBM PC using System Gold software (version 8.10).

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

Reference

Figure 1. RP-HPLC analysis of 1-wk old cheese.
Figure 2. RP-HPLC analysis of cheese aged for 6 mo at 40°F.

Figure 3. RP-HPLC analysis of cheese aged for 6 mo at 55°F.
Figure 4. RP-HPLC analysis of permeate from 3 kDalton membrane filter of 1-wk old cheese.

Publications:

Theses:

Published Abstract:

Presentations:

Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:

Invention Disclosures: (Title, Date)

Patents: (Title, Date, #)

Licensing Activities:

Discoveries:
Western Dairy Center
Project Report
Reporting Period February 1, 2001 — June 30, 2004

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

Project Title: Rehydration and structure of reconstituted casein micelles.

Institution’s Project #: 01129

Project Completion Date: February 1, 2004

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

Project Objectives: (Include any revisions to objectives)
Objective 1: Characterization of the structure of casein micelles reconstituted from dry powders in comparison to the structure of native casein micelles in milk.
Objective 2: Investigate any differences in coagulation properties of milk containing reconstituted casein micelles.

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

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

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

Publications: None

Theses: None

Published Abstract:
microstructure of caseins in dried milk. 96th American Dairy Science Association
Meeting, J. Dairy Sci. 84(Supp. 1):381
Oommen, B. S., and D. J. McMahon. 2002. Effect of method and time of hydration on
structure of dried milk proteins. American Dairy Science Association Meeting, J.
Oommen, B. S., and D. J. McMahon. 2002. Coagulation properties of skin milk fortified
with various dried milk proteins. American Dairy Science Association Meeting, J.
Dairy Sci. 85(Supp. 1):380

Presentations:

Patent/Invention Disclosures:

Technology Transfer Activities
For information on licensing contact:

Visitors Hosted:
Western Dairy Center

Project Report
Reporting Period January 1, 2003 — December 31, 2004

Principal Investigators: J. Antonio Torres
Co-Investigators: Michael Qian, Dan Farkas1, Mina McDaniel2, Anna B. Marin, Vivek Savant3, Gonzalo Velazquez2

Project Title: Pressure processing to improve milk freshness and refrigerated shelf-life

Institution’s Project #: 03139

Project Completion Date: December 31, 2005


Modifications to Project/Budget:
We expect to require a no-cost extension to complete this project.

Project Objectives:
Objective 1: Evaluate the combination of mild heating and high pressure processing (HPP) to extend the shelf life of fresh milk.
Objective 2: Determine by sensory analysis “cooked” and “fresh” flavors in milk
Objective 3: Determine by chemistry analysis “cooked” and “fresh” flavors in milk
Objective 4: Conduct consumer evaluation of HPP-treated milk produced by a semi-continuous process.
Objective 5: Disseminate to peers and industry our findings and recommendations for the production of HPP-treated milk.

Project Summary:
The consolidation trend in the dairy industry will continue for the foreseeable future as processors seek to improve their competitive position in the market. This consolidation is leading to longer distribution chains and more and more U.S. companies need ways to extend shelf life to meet consumer expectations for freshness and safety. Considering this consolidation trend and the consumer demand for higher sensory quality, a longer shelf life, particularly of fresh milk, is critical to the future success of American dairy producers. This project will assess the combination of high pressure and moderate heating to meet these consumer and industry requirements. This report focuses on Objectives 1, 3 & 5.

1 No longer at OSJ
2 No longer in Food Science
3 Currently a Research Affiliate
Methods:
1. **Objective 1**: Operational parameters for the mild heating and high pressure processing (HPP) capable of extending the shelf life of fresh milk.

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

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

**Significant Conclusions:**
1. Microbial inactivation by HPP of raw milk at 1 and 3% fat level was shown to meet standard counts for pasteurized milk.
2. HPP treated raw milk at 1 and 3% fat level had lower counts than commercially pasteurized milk from the same processing plant.
3. HPP treated raw milk at 1 and 3% fat level had lower counts at day 45 than commercially pasteurized milk from the same processing plant at day 15 when both were stored at 5 °C.

2. **Objective 3**: Determination by chemistry analysis of “cooked” and “fresh” flavors in milk.

A headspace solid phase microextraction/gas chromatographic (HSSPME/GC) technique was developed for the quantitative analysis of the volatile compounds generated during the heat processing of milk and associated to the development of cooked, stale and sulfurous notes. The extraction temperature, time and sample weight were optimized using a randomized 23 central composite rotatable design with two central replicates and two replicates in each factorial point along with response surface methodology (RSM). Temperature had a highly significant effect (P = 0.007) on the total peak area, reaching maximum observed values at the maximum extraction temperature tested (35°C). Time of extraction showed to be the most significant (P < 0.001) factor affecting the sensitivity of the SPME technique. Even at low extraction temperature, increasing fiber exposure time to the headspace (up to 3 h for this experiment) improved the concentration of volatiles in the fiber. Sample size did not have a significant effect (P = 0.197) on the volatile extraction.

Highly significant (P < 0.001) correlation coefficient (R² > 0.944) calibration curves were obtained for twenty volatile compounds in milk using the standard addition technique and then used to quantify their concentration in raw, pasteurized and UHT milk samples with various fat contents. Concentrations of dimethyl disulfide, 2-hexanone, 2-heptanone,
2-nonanone, and \(-\)-undecanone, 2-methylpropanal, 3-methylbutanal, heptanal, and decanal were present at much higher concentrations in UHT milk as compared to raw and pasteurized samples. The concentration of volatiles in raw and pasteurized milk samples was not significantly different, except for dimethyl disulfide in raw and one of the pasteurized milk brands analyzed. Fat content had an effect on the concentration of volatiles in heat-processed milk, generally increasing with fat content.

A wide variety of sulfur compounds have been identified as responsible for the "cooked" off-flavor in heat-processed milk; however, their quantification in dairy matrices has not been reported due to their high reactivity and volatility. A headspace solid-phase microextraction coupled to a gas chromatographic technique using a pulsed-flame photometric detector (HS-SPE/GC-PFPD) for the quantitative analysis of sulfur compounds in milk was developed in this project. Calibration curves with highly significant (P ≤ 0.001) correlation coefficients (R² > 0.94) for seven sulfur-containing milk volatiles were obtained by the standard addition technique and then used to quantify their concentration in raw, pasteurized and ultra-high temperature (UHT) treated milk samples with various fat contents. All seven compounds were stable in the milk matrix and no artifact formation was observed.

When compared to raw and pasteurized samples, UHT milk contained significantly higher concentrations of hydrogen sulfide, carbon disulfide, dimethyl trisulfide, methanethiol and dimethyl sulfide with the two latter ones found at the highest levels. The concentration of dimethyl sulfone in 3% fat UHT milk was lower than in raw and pasteurized milk brand B. Raw unpasteurized milk samples had similar concentrations of volatile sulfur compounds except for carbon disulfide found at a higher level in pasteurized milk brand B. Finally, hydrogen sulfide, methanethiol, dimethyl trisulfide and dimethyl sulfide concentrations increased with fat content in UHT milk. This is the first time calibration curves of sulfur compounds are reported for a dairy matrix.

A 3×3 factorial experimental design will be used to test the effect of pressure, temperature, and time on the headspace volatile composition of milk using a temperature-controlled 2-L pilot plant HPP unit along with the volatiles quantification techniques described in previous paragraphs. The level values for each factor are:

<table>
<thead>
<tr>
<th>Factor</th>
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<tbody>
<tr>
<td>Pressure (kpsi)</td>
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</tr>
<tr>
<td>Temperature (°C)</td>
<td>4</td>
</tr>
<tr>
<td>Time (min)</td>
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</tr>
</tbody>
</table>

This design will provide complimentary supporting data for research data obtained in a previous experiment on the microbial and physicochemical stability of high pressure treated milk. It will include the pressure conditions found to be effective in extending milk storage time to 45 days.
•Significant Conclusions:
1. UHT milk contained higher concentrations of ketones, aldehydes, and sulfur compounds, when compared to raw and pasteurized milk. These chemicals are responsible for the off-flavor in heated milk.

2. Some sulfur compounds allowed for the differentiation of raw and pasteurized milk headspace composition, and even between different brands of pasteurized milk. This is an important achievement since commercial pasteurization is carefully controlled so off-flavors rarely occur, therefore highlighting the sensitivity of the techniques developed for the volatiles analysis. This will be particularly useful when analyzing HPP treated milk.

3. The techniques developed for the volatiles analysis of milk are fast and accurate enough so a large number of treatment combinations can be run to assess the effect of temperature-time-pressure on the milk volatiles, and give supporting data to shelf-life results from previous experiments. This will lead to a pressure-time-temperature treatment that extends milk storage time with minimal off-flavor formation.

Publications:


Theses:


Published Abstract:


Presentations: None

Patent/Invention Disclosures: None

Technology Transfer Activities None
For information on licensing contact:

Visitors Hosted:
Dr. Gonzalo Velazquez, Department of Food Science and Technology, U. A. M. Reynosa-Aztlán, Universidad Autónoma de Tamaulipas, Apdo. Postal 1015, Reynosa, Tamaulipas, 88700 Mexico

Invention Disclosures: (Title, Date) None

Patents: (Title, Date, #) None

Licensing Activities: None

Discoveries: None
Western Dairy Center

Final Report

Reporting Period November 1, 2000 — December 31, 2004

Principal Investigators: Marie K. Walsh
Charles Carpenter

Co-Investigators:

Project Title: Production of an extruded whey protein snack food

Institution’s Project #: 00119

Project Completion Date: December 31, 2004


Modifications to Project/Budget:

Project Objectives: (Include any revisions to objectives)

<table>
<thead>
<tr>
<th>Project Summary: (Suitable for inclusion in Center documents released to the public)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High protein snack foods are currently finding a large market as protein supplements, and we have described the use of thermoplastic extrusion to produce a high protein snack from whey protein (WPS). Our WPS is puffed during extrusion, although we have not yet achieved the expansion (puffing) desirable in this type of snack product. The purpose of this proposed research is to optimize the formulation and extruder configuration to allow extrusion into a puffed snack having acceptable texture. Variables being explored include concentration and type of WPC and type of starch.</td>
</tr>
</tbody>
</table>

1. Significant Progress against Objectives:

   a) Operational parameters to permit pilot scale production of an extruded whey protein snack food.
   b) Determine appropriate starch type (corn, modified corn, potato, modified potato, rice and bran), pH and use level which permits production of a puffed product.
   c) Product evaluation including sensory and stability analysis.

   The effect of two starch types (normal cornstarch and pregelatinized waxy starch), two protein types (regular and instantized WPC80), and three protein inclusion levels (16%, 32%, and 40%) on extrudate characteristics were studied. Each possible combination was extruded in triplicate, and values for the extrusion parameters of pressure, motor torque, TOM in the die and barrel, observed die and barrel temperatures, and product residence time were recorded. The physical parameters of expansion ratio, breaking force, density, color, and air cell size were examined. Extrudates were analyzed for the
chemical parameters of moisture content, WAI, WSI, total soluble protein, water-soluble protein, and water-soluble carbohydrate.

Three starch/protein combinations were chosen for sensory analysis (32% with corn starch, 32% and 40% with pregelatinized waxy). Extrudates were selected based on completeness of expansion and final protein level, with preference given to the two highest levels. As incomplete expansion was seen in the 40% extrudates with normal corn starch for both protein types, this combination was excluded. Protein type (regular or instantized) was found to have minimal effect on physical and chemical product characteristics, so extrudates containing only one protein type were included in sensory testing. Instantized WPC80 was chosen for ease of use. Fresh product was extruded and dried, then presented to an open consumer taste panel. The sensory characteristics of appearance, texture, flavor, aftertaste, and overall acceptability were rated using a hedonic rating scale (1, dislike extremely, to 9, like extremely). Judges were also asked to rank the three samples in order from best to worst.

Expansion ratio was influenced by starch type \( (p = 0.0003) \) and by protein to starch ratio \( (p < 0.0001) \), as were breaking force \( (p = 0.0069 \) and \( p < 0.0001 \), respectively), density \( (p < 0.0001, p < 0.0001) \), color \( (p = 0.0481, p < 0.0001) \), and air cell size \( (p = 0.0002, p < 0.0001) \).

In general, as the amount of protein in the extrudates increased, expansion ratio decreased. Expansion ratio for normal corn extrudates was significantly lower than that for pregelatinized waxy extrudates. The opposite trend was seen for breaking force, which tended to increase with protein content. Breaking force for normal corn extrudates was significantly higher than that for pregelatinized waxy extrudates. Similar trends to breaking force were seen for density.

Air cell size followed a similar trend to expansion ratio, though the difference between starch types was not as distinct. Only the 16% protein/pregelatinized waxy extrudates were seen to be significantly different from the others; in this case, the average air cell size was larger. Pregelatinized waxy extrudates consisted of fewer, larger cells, while normal corn extrudates exhibited more numerous, but significantly smaller cells.

There were no observed color differences between extrudates, with the exception of the 16% protein extrudates produced with pregelatinized waxy starch. These had significantly less color than any others.

As expected, all five physical parameters measured were significantly correlated with each other. Density was most positively correlated with breaking force and most negatively correlated with expansion ratio. Unexpanded, glassy sections were observed in 40% protein/normal corn extrudates, suggesting incomplete flash evaporation of water at the die. This results in an extrude with decreased volume, which translates to an increase in density. Extrudates with these hard glassy sections require more force to break; it was for this reason that this level of normal corn extrudate was excluded from the taste panel.

Color was negatively correlated with expansion ratio and air cell size; this association is most likely due to the effect of the starch type. The lightest colors were observed in the pregelatinized waxy extrudates (16% protein level), which were also the most highly expanded extrudates, and had the largest average cell size.
Chemical Parameters

Extrudate moisture content was influenced by protein type \((p = 0.0002)\), protein to starch ratio \((p < 0.0001)\), and the interaction between protein type and starch type \((p = 0.0092)\). Differences between moisture contents were negligible at the 16% and 32% protein levels. The only significant difference occurred with normal corn and instantized WPC80 extrudate at the 40% protein level, having a higher moisture content than any other extrudate. This extrudate also had a significantly higher density and breaking force than the others, consistent with the presence of unexpanded material. This suggests that the higher moisture content for this particular combination was due to incomplete expansion, and the presence of water that would have otherwise been lost to flash evaporation.

WAI and WSI were both influenced by starch type \((p = 0.0199\) and \(p = 0.0078\), respectively) and protein to starch ratio \((p = 0.0038\) and \(p < 0.0001)\). For WAI, opposite trends were observed between the starch types. For normal corn, water absorption decreased significantly as protein level increased. For pregelatinized waxy, water absorption increased with protein level, though not significantly. In all cases, normal corn extrudates absorbed significantly more water than pregelatinized waxy extrudates; as might be expected, WSI values for pregelatinized waxy extrudates were significantly higher than those of normal corn. For both starch types, WSI tended to increase as protein level decreased, though the trend was more significant for pregelatinized waxy extrudates.

Total soluble protein (given as the percentage of total protein that was resolublizable after extrusion) was influenced by protein to starch ratio \((p = 0.0004)\) and the interaction of starch type and protein type \((p = 0.0391)\). The preparation steps for total soluble protein involve exposing the ground extrudate to SDS to denature the proteins, and BME to cleave disulfide bonds in order to facilitate the denaturation. Theoretically, unless some other form of covalent bonding occurred among the protein molecules in the extruder, 100% of the protein present in the mix would be resolubilized by this method. This was not typically seen, though pregelatinized waxy extrudates at the 16% protein level averaged 90% protein resolublized. In general, ≥ 50% of the protein in the extrudates was resolublized.

The percentage of total soluble protein generally decreased as the protein level increased, and was higher for pregelatinized waxy extrudates. This could indicate starch/protein cross-bonding was more prevalent in normal corn products, due to the higher amylose content. Water-soluble protein (given as the percentage of total protein that was water soluble after extrusion) was influenced by protein to starch ratio \((p = 0.0306)\); however, no significant differences were found between means, and no noticeable trends were observed.

Water-soluble carbohydrate (given as the percentage of total carbohydrate that was water soluble after extrusion) was influenced by starch type \((p = 0.0090)\) and protein to starch ratio \((p = 0.0743)\). Differences within starch type were not significant.

Comparing total soluble protein with water-soluble carbohydrate suggests some degree of starch/protein cross-bonding. To examine this possibility, the results for water-soluble carbohydrate and total soluble protein were compared graphically. Because the effect of protein type was non-significant in both cases (water-soluble carbohydrate, \(p = 0.1593\); total soluble protein, \(p = 0.8326)\), values within each parameter were pooled and...
examined by protein content for the individual starches. Solubilities for the pregelatinized waxy extrudates changed independently of each other; while total soluble protein decreased as protein content increased, water-soluble carbohydrate remained relatively constant. For normal corn starch extrudates, however, values shifted consistently among protein levels.

**Sensory Testing**

Three extrudates (normal corn at a 32% protein level and pregelatinized waxy at a 32% and a 40% protein level) were evaluated by an open consumer taste panel. The rating values given to the sensory attributes texture (p < 0.0001) and aftertaste (p < 0.0001) varied significantly by sample. Differences in appearance ratings were non significant.

Flavor (p = 0.0277) and overall acceptability (p = 0.0005) varied by sample, but the ratings given to these two attributes depended on the sample position as well (flavor, p = 0.0415; overall, p = 0.0161). However, the interaction term of sample by position was not significant for these sensory attributes (p > 0.10), indicating that all three extrudates were affected equally. The sample in position 1 was given a lower rating than the sample in position 3 more often than the reverse. It could be that many judges experienced taste fatigue and became increasingly less critical in evaluating each subsequent sample, though the sample number was limited to three in an attempt to control for this situation.

Panelists were instructed to use a standard 1 to 9 hedonic scale to rate the sample attributes, with 1 being “Dislike Extremely” and 9 being “Like Extremely.” Mean values for samples fell primarily in the 5 to 6 range: “Neither Like nor Dislike” to “Like Slightly.” Overall acceptability scores ranged from 1-9 for the pregelatinized waxy, 32% protein sample, from 1-8 for the pregelatinized waxy, 40% sample, and from 2-9 for the normal corn, 32% sample.

2. Significant Conclusions:

This study was conducted to determine the effect of varying starch type, protein type, and protein concentration on the physical, chemical, and sensory characteristics of a second-generation extrusion expanded snack food. Two starch types, normal corn starch (25% amylose, 75% amyllopectin) and pregelatinized waxy starch (approximately 100% amyllopectin) were chosen, along with two WPC80 types (regular and instantized). Each combination of starch and protein was extruded at three protein concentrations: 16%, 32%, and 40%, for a total of 12 treatments. Each treatment was extruded in triplicate.

The extrudates were examined for physical characteristics (expansion ratio, breaking force, density, color, and air cell size) and chemical characteristics (moisture content, WAI, WSI, total soluble protein, water soluble protein, and water soluble carbohydrate). Based upon these examinations, three combinations were chosen for sensory analysis (normal corn starch at 32% protein, pregelatinized waxy starch at 32% protein, and pregelatinized waxy starch at 40% protein). The sensory attributes of appearance, texture, flavor, aftertaste, and overall acceptability were examined.
The results of the chemical and physical analyses show that starch type had the greatest impact on the characteristics of the extrudate. Specifically, normal corn extrudates exhibited less expansion and smaller air cell size, increased density, breaking force, and color development, and were less water soluble than the pregelatinized waxy extrudates. Though protein type was unimportant, the level of protein inclusion also influenced the characteristics of the extrudate. As protein levels increased, extrudates exhibited smaller expansion ratios, increased density and breaking force, and a decrease in water solubility.

In the case of the normal corn extrudates, the effect of the starch was likely modified by the presence of an amylose-protein complex. Upon comparing total soluble protein values with water soluble carbohydrate, a relationship between the two parameters was seen to exist for these extrudates. This relationship strongly suggests an amylose-protein complex that is covalent in nature.

Sensory results for the three tested extrudates indicated that an extruded snack food with decreased expansion ratio and increased breaking force (as was seen with higher protein extrudates) could still be acceptable to consumers, as mean hedonic scores were approximately 6, “Like Slightly.” Panelists rated the normal cornstarch with 32% protein extrudate higher than either of the less dense, more highly expanded pregelatinized waxy extrudates on texture, flavor, aftertaste, and overall acceptability. These results suggest that it is possible to incorporate WPC80 at a level above the previously recommended maximum of 20%.

3. Anticipated Problems/Delays:
Project is complete

Publications:
In preparation

Theses:

Presentations:


**Patent/Invention Disclosures:**
Technology covered in the following patent:

**Technology Transfer Activities** Licensed to Grande Custom Ingredients, Lomira WI

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

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

**Licensing Activities:**
Licensed to Grande Custom Ingredients, Lomira WI

**Discoveries:**