2005 Annual Report

Various Authors
Western Dairy Center
Annual Report
2005

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WESTERN DAIRY CENTER

ACTIVITIES SUMMARY

2005

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, Oregon State University, and Weber State University. This report summarizes the research activities from January 1, 2005 through December 31, 2005.

The National Dairy Research Plan developed by Dairy Management Inc, industry and the 5 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 19th Annual Cheese Making Short Course in February and March 2005, at Utah State University with 12 attendees in each session. One session was for industrial cheese makers while the other was tailored for Artisan cheese makers. 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.

In 2005 we had 3 new grants and 3 continuing DMI grants. Currently we have 4 new DMI grants starting in 2006. Project progress reports of all research projects active in 2005 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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Chen, Dong, Ming-Xiang Liang, Daryll DeWald, Bart Weimer, Michael D. Peel, Bruce Bugbee, Jacob Michaelson, Elizabeth Davis, Yajun Wu. 2006. Identification of drought response genes from two alfalfa cultivars using Medicago truncatula microarrays. (submitted) Plant Molecular Biology.

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


M.S. thesis titled, "Using Lactose and Lactose-Based Compounds for Controlling Erosion in Furrow Irrigation" has been written, successfully defended (3/23/2006), and published by Mr. Wes Hopwood. A final copy will be submitted to DMI.


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Food Biochem. 29:1-12.


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


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


Vázquez-Landaverde, P.A., Torres, J.A., Qian, M.C. 2006. Inhibition of off-flavor formation in milk subjected to high hydrostatic pressure combined with moderate heating. SUBMITTED.


1. Title of Project: Development of Lactose Hydrogels

2. Principal investigator: David Britt

3. Start Date: 1-1-05

4. End Date: 12-31-07

5. Executive Summary:

Since the project start date we have synthesized and characterized a library fatty-acid and fatty-amine based lactose-surfactants. The synthesis procedure was refined by determining the minimum reaction time (heating / cooling cycles) needed to achieve a stable gel with a defined gel transition temperature. The water holding capacity of the synthesized gels was also characterized as a function of reaction time. The influence of excess lactose (non-conjugated to fatty-acid or amine) on the gelation temperature was characterized using differential scanning calorimetry (DSC) to support visual observations of gelling. The DSC values were in excellent agreement with the visual method.


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

a. Introduction

b. Objective (s)

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

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

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

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

Objective 5: Reduce erosion and improve runoff water quality in laboratory scale soil beds.
Objective 6: Quantify lactose-surfactant bioactivity.

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

c. Method and Materials

d. Data or Results

Objective 1: For this period, we have restricted our focus to lactose-fatty amine conjugates. Surfactant amphiphilicity (hydrophobic / hydrophilic ratio) was systematically varied using fatty amines having alkyl-tails: C18, C16, C12. Conjugation to urea was also investigated as a negative control. Stable product was formed for all investigated alkyl-chain lengths.

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

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

![Graph showing % of available water retained vs surfactant: water (w/w) ratio.](image-url)
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Figure 1. Water holding capacity curves for tap water and deionized water. The ions in the tap water are suspected of causing the reduction in water holding capacity of gels.

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

<p>| Table 1. Average cumulative infiltration mannus through lactose treatments of suspended sediment solutions. Lactose treatments caused large infiltration improvements. n=3 |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative Infiltration (ml/min)</th>
<th>Standard Deviation</th>
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</thead>
<tbody>
<tr>
<td>Control: soil</td>
<td>0.42</td>
<td>0.03</td>
</tr>
<tr>
<td>Control: tap water</td>
<td>1.51</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Standard lactose solution:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil in 300 mg/L</td>
<td>0.50</td>
<td>0.05</td>
</tr>
<tr>
<td>Soil in 3,000 mg/L</td>
<td>0.59</td>
<td>0.01</td>
</tr>
<tr>
<td>Soil in 30,000 mg/L</td>
<td>0.68</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Pure lactose solution:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil in 3,000 mg/L</td>
<td>0.52</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Objective 6: The lactose-fatty amine conjugates (C16 alkyl tails) showed no intrinsic biocidal activity against E. coli or P. chlororaphis liquid cultures. Degradation of the product by bacteria was indirectly inferred by a sour smell and liberation of bubbles from soil columns treated with the product.

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

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

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

g. Conclusion and Next Step: We are encouraged by the reproducibility of our lactose-gels in terms of gel transition temperature, and we are working towards our original goals as well as developing systems with gel transition temperatures just above body temperature (37°C) for medically relevant applications such as controlled drug release matrices and wound healing scaffolds. We have been refining the gelation properties of our surfactants in order to determine the minimum amount of surfactant needed to completely gel and hold a given quantity of water. This is relevant for a wide variety of applications from soil treatment to cosmetics and medicine. We have discovered that excess lactose can significantly enhance the water holding capacity of a surfactant gel. Preliminary results are summarized in the graph below and ongoing experiments will investigate this over a greater range of concentrations and ratios:

![Graph showing water holding capacity vs. surfactant added](image)

Figure 2. Water holding capacity of lactose-fatty amine surfactant gels. A 150 mM solution of the surfactant (diamonds) was added in a volume indicated on the x-axis to 10 ml of tap water. The amount of water held by the resulting gel is indicated on the y-axis. The experiment was repeated with a 150 mM solution of the surfactant containing 100 mM excess lactose (squares). A dramatic increase in the water holding capacity is observed over the concentration range investigated. (J. Rasband, et al. unpublished)
Western Dairy Center

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

Yes [ ] No [ X ] Possible [ ]

8. Anticipated Delays or Problems: None

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts

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

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

M.S. thesis titled, "Using Lactose and Lactose-Based Compounds for Controlling Erosion in Furrow Irrigation" has been written, successfully defended (3/23/2006), and published by Mr. Wes Hopwood. A final copy will be submitted to DMI.

Abby Taylor: Senior design report

A Paper has been accepted for publication (galley proof sent previously):

Dhruv, H., M. Draper, D. Britt, Role of lactose in modifying gel transition temperature and morphology of self-assembled hydrogels, Chemistry of Materials, Accepted for publication, 2005.
1. Title of Project: **Analysis of Capsule Production in *Streptococcus thermophilus* by Comparative Genomics**

2. Principal investigator: **Jeffery R. Broadbent**

3. Start Date: 1-1-03

4. End Date: 6/30/06

5. Executive Summary:

Capsule-producing *Streptococcus thermophilus* can significantly improve cheese functionality. However, most exopolysaccharide (EPS)-producing strains of *S. thermophilus* in industry are ropy which means the EPS is released into the growth medium instead of being attached to the cell surface (which results in production of a cell capsule). Identification of genes involved in attachment of EPS to the cell surface will facilitate the identification or development of new strains of capsule-producing *S. thermophilus* suited for cheese applications.


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

a. Introduction

Our group demonstrated that encapsulated, but not ropy, exopolysaccharide-producing (EPS) *S. thermophilus* strains can be used to significantly increase cheese yield and improve functionality without deleteriously affecting cheese quality, whey viscosity, or UF concentration time. We 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 are working to create isogenic derivatives of these strains in order to investigate the role of these genes in cell capsule attachment.

Nucleotide sequence and structural organization of the 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.

b. Objective(s)
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.

c. Method and Materials

**Identification of *cps* genes.** We have sequenced large *cps*-related loci in MR-2C, MTC 330, and MTC 360 by traditional and inverse PCR techniques (Broadbent et al., 1998). After the structure and organization of *cps* gene cluster was determined, the degree of similarity between individual *cps* homologs in each strain was analyzed using GCG sequence analysis software.

**Comparative genomics of *cps* genes.** To identify genes that may encode enzymes involved in exopolysaccharide synthesis, we performed BLASTx protein homology searches against the nucleotide sequence data using software available at the National Institutes of Health Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The BLASTx tool translates a nucleotide sequence in all six reading frames and compares the putative translation products to all non-redundant GenBank translations and several protein databases. Database proteins that share homology with each query protein (i.e., putative translation products from the sequence) are arranged in an output file by descending alignment scores. The statistical significance of a given alignment is represented by an E (expected) value that reflects the probability that the alignment is due simply to chance. The more significant the alignment (i.e., the more likely a query protein is a true homolog to a known protein), the lower the E value. By this approach, we identified a number of previously uncharacterized *cps* genes in *S. thermophilus* MR-2C that appear to encode enzymes involved in regulation, assembly, and membrane translocation of exopolysaccharide. A comparative analyses of these genes between MR-2C, MTC 330, and MTC 360 implicated *wzg*, *wzd*, and *cpsM* as candidate genes with possible roles for capsule attachment.

Physiological role for *cps* genes in capsule attachment. Since comparative sequence data suggested *wzg* and *cpsM* genes in ropy strains were derived from the MR-2C ortholog (and not vice-versa), we performed complementation experiments to determine whether introduction of MR-2C genes (with promoter sequences) would lead to conversion of MTC360 or MTC330 from ropy to capsular phenotype. Individual DNA fragments containing MR-2C *wzg*, *wzd*, and *cpsM* genes were constructed by PCR using oligonucleotide primers with restriction endonuclease linkers and cloned into pTRK687p*wzg*. Cells of MTC360 and MTC330 were separately transformed by electroporation with the recombinant *wzg*, *wzd*, and *cpsM* vectors, and transformants containing each plasmid were isolated by incubation at 37°C on M-17 agar that contained erythromycin. The presence of recombinant plasmid in cells was confirmed by agarose gel electrophoresis, then cells were examined for the presence of capsular polysaccharide by the Duguid stain method.

**Comparative genomics of *pbp* genes.** Basic mechanisms for EPS biosynthesis in streptococci and other Gram-positive bacteria have a great deal of similarity to that for cell wall synthesis. Both entail intracellular assembly of sugar repeat units on an undecaprenyl lipid carrier, and translocation of the lipid-linked intermediate across the cell membrane is
catalyzed by a "flippase". Peptidoglycan biosynthesis also involves later polymerization of the glycan chains and chain cross-linking by interpeptide bridge formation. The former reaction is catalyzed by glycosyltransferase enzymes whereas the latter is performed by transpeptidases. Both of these activities are resident on the extracellular domains of bifunctional (class A) penicillin-binding proteins (PBPs). Because of the similarities between EPS and peptidoglycan synthesis, and because capsule attachment in streptococci probably involves a covalent link between EPS linkage and the cell wall, we speculated that one or more PBPs may provide the glycosyltransferase activity involved in capsule attachment. To pursue this hypothesis, we screened the complete genome sequences for 3 different S. thermophilus strains for pbp genes whose products were predicted to include a glycosyltransferase domain, then used that information to design PCR primers for the amplification of orthologous sequences from our capsular (MR-2C and MR-1C) and ropy (MTC 330 and MTC 360) EPS-producing strains.

d. Data or Results

To ensure that the entire cps gene cluster of each strain has been identified, we first extended DNA sequence data from the genomic regions flanking known portions of the cps gene clusters of MR-2C, MTC360 and MTC330. BLASTx protein homology searches against the nucleotide sequence data strongly indicate that we successfully identified all of the genes associated with exopolysaccharide synthesis in each of these strains.

Comparative genomic analysis of MR-2C, MTC360, MTC330, and Sf6 cps sequences identified three conserved mutations in ropy strains that were not present in the capsule-producing strain MR-2C; one in the wzg (formerly called wzg) gene, another in wzd (formerly called cpsC) and the third in cpsM. The wzg and wzd genes are reportedly involved in regulation of polysaccharide synthesis and polymerization of the repeat unit, respectively, while the function of the cpsM product 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).

To investigate the relationship between these genetic polymorphisms and exopolysaccharide type and composition, we constructed an expression vector, pTRK687_wzg, that replaces a high-level constitutive promoter with the S. thermophilus wzg promoter. The MR-2C wzg wzd, and cpsM genes were then individually cloned into pTRK687_wzg, and separately transformed into MTC 330 and MTC 360. Visual examination of wzg wzd, and cpsM transformants from each host for capsule production by the Duguid stain procedure showed none had acquired a CPS+ phenotype. As was noted in our previous report, none of our complementation experiments resulted in production of a cell capsule, these results may not be valid because parallel studies with the CPS+ strain MR-1C have recently suggested that expression of insert DNA from cloned genes by the pTRK687_wzg promoter is too weak (or is adversely regulated) to be detected by phenotypic assays. As a result, we have been working to develop a new expression vector that uses a moderately (and constitutively) expressed promoter so that we can re-evaluate the effect of MR-2C wzd, wzg, and cpsM genes in MTC 330 and MTC 360.
elongation factors, and included copies of the *E. coli lac* Operator both upstream and downstream of the promoter (as it appears naturally in the *E. coli lac* operon). This fragment was introduced into pTRK687, then we tried to insert *S. thermophilus eps* genes into the new construct. Unfortunately, all of the recombinant plasmids we recovered contained truncated (deleted) insert genes, suggesting the promoter is still too strong and the amount of *lac* repressor in cells too low to control expression in *E. coli*. Plans to recover from the current situation are to clone and express the *E. coli lac* repressor protein gene from another vector (this has been done in other situations for the same purpose) and in this way shut off expression of the genes in *E. coli*. In any event, this effort is ongoing despite completion of DMI funded work.

Screening genome sequences for *S. thermophilus* strains LMG18311, CNRZ1066, and LMD-9 identified genes for 3 different PBPs, named 1A, 1B, and 2A, that were predicted to include a glycosyltransferase domain. PCR primers for the region surrounding this domain were designed for each gene, and have been used to to isolate and sequence the corresponding fragments from genes for all three PBPs in our capsular and ropy isolates. DNA sequence analysis of these fragments showed GTF domains for PBP 1B and 2A were highly conserved in all four strains, and found no relationship between the protein sequence and the presence or absence of a cell capsule. In contrast, the gene for PBP 1A showed one nucleotide polymorphisms that would result in an amino acid substitution. This mutation results in a change from Asp to Asn in a highly conserved region (YDPY) of the glycosyltransferase domain. This mutation was found in MR-1C only. Asp has a negatively charged R group, while Asn has a polar, uncharged R group, so this mutation may have an effect if it is involved in glycosyltransferase activity. Sequence comparison against PBP genes from other bacteria showed YDPY was present in *Enterococcus fecalis*, *E. hirae*, *S. pyogenes*, and *S. mitis*, while the YNPY block seen in MR-1C is present in *Listeria monocytogenes*.

e. Discussion

Efforts to evaluate the contribution of specific genes to capsule attachment in *S. thermophilus* have been stymied by the need to construct a suitable expression vector that can be shuttled through an *E. coli* host background. The need for such a vector is particularly acute in light of the recent work by Morona et al. (2006, PNAS) who showed mutations in *cpsC* (*wzd*) affected capsule attachment to the cell wall of *Str. pneumoniae*. As was noted above and in previous project reports, our comparative genomic analysis of MR-2C, MTC360, MTC330, and Sf66 *cps* sequences identified *wzd* as one of three candidate genes involved in capsule attachment. As a consequence of the Marona et al. report, we have focused all our current efforts toward investigating the role of *wzd* in capsule attachment to the cell wall of *S. thermophilus*, although we are still interested in pursuing the mutation we discovered in the MR-1C PBP 1A gene.

f. Conclusions

Our work and the work of others (Morona et al., 2006) has pinpointed *wzd* and *pbp1A* as the most promising candidate genes for capsule attachment in *S. thermophilus*. Though DMI
funding for this work has been exhausted, we will continue our work to define the contribution of each gene to capsule formation.

7. Intellectual Property (if applicable)

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

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>Possible</th>
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<td>[X]</td>
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8. Anticipated Delays or Problems

Not applicable.

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts

Western Dairy Center

PROJECT PROGRESS REPORT
of
Western Dairy Center

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

2. Principal investigator: Donald J. McMahon

3. Start Date: 1/1/05

4. End Date: 12/31/07

5. Executive Summary:


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

a. Introduction

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

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

Hypothesis 2: Cheese flavor characteristics in a low fat cheddar cheese can be optimized by using adjunct cultures that eliminate bitterness and that increase production of flavor compounds known to be important in cheddar cheese flavor.

b. Objective (s)

Objective 1. Determine the best combinations of low fat cheese make procedure strategies for making a low fat cheddar cheese.

Objective 2. Determine the best combinations of low fat cheese make procedure strategies for making a low fat cheddar cheese.

Objective 3. Combine the results of Objectives 1 & 2 above to develop a low fat cheese make procedure suitable for use by commercial cheese companies.

c. Method and Materials

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

Cheese body, texture and flavor will evaluated by our cheese research team at 7 d and 28 d of storage at 5°C, and by using texture profile analysis at 28 d of age. A sample of each cheese will also be maintained in the aging room for possible future testing.

The following strategies will be initially tested individually using a milled curd make procedure and using a stirred curd procedure that includes washing the curd with cold water:
- Elevated pasteurization temperature of 82°C for 15 secs.
- Low-pressure homogenization of milk at 800 psi with 120 psi second stage.
- Pre-acidification of milk to pH 6.3 using vinegar
- Addition of hydrogenated lecithin at the rate of 0.2%

Each strategy will be tested in duplicate on separate days using 600 lb of milk split between two vats to make the milled curd and washed curd cheeses. A nonbitter Lactococcus starter culture will be used during one replicate, and the Lactococcus starter culture in conjunction with the CNRZ32 adjunct will be used in the second replicate. It is not expected that this would influence the body or the texture of the cheese during 30 d of storage. These experiments will help to establish a flavor baseline for further study in Objective 2.

Objectives 2 and 3 not funded.

d. Data or Results

After developing a stirred and washed curd procedure that would yield cheese in the moisture range 50 to 55%, the project was concluded with a 2x2 factorial experiment with 3 replicates made with a standardized make procedure comparing milk pasteurized at either 163 F or 185 F, and renneted at either pH 6.65 or pH 6.30. Homogenization of the milk was not shown to provide any improvement in cheese properties at this low level of fat.

Composition
Using a high heat treatment during pasteurization (185°F compared to 165°F) resulting in a cheese with about 3% more moisture. Consequently, salt contents were slightly higher as less whey was expelled during pressing. Acidifying the milk to pH 6.3 prior to renneting had a slight influence on moisture content of the cheese, with the cheese made from pre-acidified milk having slightly higher moisture content. This may also be a function of the changes in make procedure that was necessary rather than the pre-acidification itself.
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<table>
<thead>
<tr>
<th>Cheese Composition</th>
<th>pH 6.7, 165F</th>
<th>Milk Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Moisture</td>
<td>52.5</td>
<td>57</td>
</tr>
<tr>
<td>% Fat</td>
<td>4.75</td>
<td>4.25</td>
</tr>
<tr>
<td>% FDB</td>
<td>10.0</td>
<td>9.9</td>
</tr>
<tr>
<td>%MFFS</td>
<td>55.1</td>
<td>59.5</td>
</tr>
<tr>
<td>%Salt</td>
<td>1.88</td>
<td>2.03</td>
</tr>
<tr>
<td>pH</td>
<td>5.21</td>
<td>5.21</td>
</tr>
</tbody>
</table>

Melting
No major differences in meltability were observed except for one set of cheeses that had a much higher moisture content than the other cheeses.

![Melting Graph](image)

Texture
Some differences in texture were observed, but data analysis is not completed yet. Our observations were that preacidifying the milk produced a cheese that appeared to be less rubbery but was slightly more sticky.

Flavor
Descriptive sensory flavor analysis was performed at North Carolina State University on two of the replicates when the cheese was 2 to 3 months old. Duplicate replications of four low fat cheeses were received. Each cheese replicate was evaluated in duplicate by 10 highly trained panelists (each panelist evaluated each cheese 4 times) using the Cheddar cheese sensory language developed by Drake et al. (2001). Data were analyzed by analysis of variance with Fisher’s least significant difference as a post hoc test.
Sensory profiles of low fat Cheddar cheeses

<table>
<thead>
<tr>
<th>Cheese</th>
<th>Pasteurization Temperature</th>
<th>Renneting pH</th>
<th>Flavor Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>165°F</td>
<td>6.7</td>
<td>Cooked 3.1a</td>
</tr>
<tr>
<td>B</td>
<td>185°F</td>
<td>6.7</td>
<td>Whey 3.3a</td>
</tr>
<tr>
<td>C</td>
<td>165°F</td>
<td>6.3</td>
<td>Diacetyl 1.2a</td>
</tr>
<tr>
<td>D</td>
<td>185°F</td>
<td>6.3</td>
<td>Milkfat ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sulfur 1.1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Brothy 1.3a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sweet 1.8a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sour 3.1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Salty 4.2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Umami 1.7a</td>
</tr>
</tbody>
</table>

Means in row followed by different letters are different ($p<0.05$).

ND - not detected

Attributes not listed (fruity, free fatty acid, catty, nutty, bitter) were not detected in cheeses.

Descriptive sensory flavor analysis was performed on the cheese.

Compared to full fat cheese made with the same cultures, the low fat cheeses had more intense cooked flavor, whey flavor, diacetyl flavor and sulfur flavors. They had less milkfat (lactone) flavor. The cheeses made from milk heated to 185°F were observed to have a slight rosey flavor, which in this analysis is incorporated as part of the brothy flavor (as well as beefy, veggie/mushroom, and other flavors) as so was not differentiated from the other cheeses on total brothy flavor.

e. Discussion

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

f. Conclusion and Next Step.
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The make procedures developed during this study will serve as a starting point for the DMI Low Fat Cheese Flavor Strategic Platform project that is scheduled to commence in July 2006.

7. Intellectual Property (if applicable)

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

Yes [ ]

No [ X ]

Possible [ ]

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

8. Anticipated Delays or Problems: None, other than funding of Phase 2 and 3.

9. Manuscripts/Abstracts Submitted for Publication: None

10. Published Articles/Abstracts: None
1. Title of Project: Molecular basis of cheese melting in relation to proteolysis

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

3. Start Date: 1-1-04

4. End Date: 12-31-06

5. Executive Summary:

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


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

   h. Introduction

   i. Objective (s)

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

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


   j. Method and Materials

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

**Reversed-phase HPLC of samples**

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

**Cheesemaking**

Using the hypothesis that different coagulants, their levels and different ripening temperatures will produce varying proteolytic patterns and consequent melting properties, cheese was made using chymosin and *C. parasitica* rennet. A split-split plot design was used with the block treatment being the starter and adjunct cultures, split into 3 coagulant levels (chymosin, *C. parasitica* rennet and a combination of them), further split into 2 ripening temperatures (40 and 55°F). Sampling was carried out at 1 week, 1, 2, 3, 4, 5 and 6 months. The cheese samples were being analyzed for proteolytic patterns using RP-HPLC and CE, soluble nitrogen at pH 4.6 and 12% TCA, melt properties using a UW Meltemeter, and TPA with a TPA Analyzer.

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

**Cheese Opacity**

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

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

see previous report for chromatographs

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

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

see previous report for chromatographs

Table 1. Areas of peaks assumed to be intact αs1- and β-casein and αs1f(24-199) (αs1-1) at 1 week, 2, 4 & 6 months determined by RP-HPLC.

<table>
<thead>
<tr>
<th>Cheese</th>
<th>1 wk</th>
<th>2 mo</th>
<th>4 mo</th>
<th>6 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>αs1</td>
<td>375.8</td>
<td>196.9</td>
<td>52.8</td>
<td>58.0</td>
</tr>
<tr>
<td>A*</td>
<td>450.8</td>
<td>126.7</td>
<td>4.5</td>
<td>10.3</td>
</tr>
<tr>
<td>αs1-I**</td>
<td>888.5</td>
<td>296.1</td>
<td>77.7</td>
<td>82.9</td>
</tr>
<tr>
<td>β</td>
<td>531.7</td>
<td>306.8</td>
<td>23.6</td>
<td>27.6</td>
</tr>
<tr>
<td>B*</td>
<td>541.1</td>
<td>86.2</td>
<td>6.5</td>
<td>13.3</td>
</tr>
</tbody>
</table>
Cheeses made using different cultures combinations: *Lactococcus lactis* starter culture with *Lac-* *Lactococcus lactis* and/or a *Lactobacillus helveticus* adjunct cultures, are being aged. Two storage temperatures, 6°C and 13°C are being used so as to give two different rates of proteolysis and aging.

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

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

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

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

Figure 10. Cheese opacity (measured as L* values) as cheese was heated from 10°C to 90°C for cheeses made using chymosin (left) and Sure Curd (right).
Comparing the peptide profiles in Figs. 5, 6 and 7 there is an obvious difference in the initial hydrolysis of the caseins even within the first week after manufacture, and during the first month of storage. Elevated levels of the coagulant do change the pattern of hydrophobic proteins/peptides present.

At 6 months, all cheese samples had very little intact αs1- and β- caseins. Further, in the case of cheese made with elevated levels of chymosin (Figs 8 and 9), at 1 week, the hydrolysis of αs1- casein into αs1l- casein appears to be fairly rapid due to the excess enzyme (chymosin) available to act on the caseins (primarily αs1- casein).

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

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

m. Conclusion and Next Step

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

7. Intellectual Property (if applicable)

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

Yes [ ] No [x] Possible [ ]

8. Anticipated Delays or Problems.

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

9. Manuscripts/Abstracts Submitted for Publication


10. Published Articles/Abstracts None
Western Dairy Center

PROJECT PROGRESS REPORT
of
Western Dairy Center

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

2. Principal investigators: J. Antonio Torres, PI
   Michael Qian and Gonzalo Velazquez, co-PI

3. Start Date: 1-1-04

4. End Date: 6/30/06

5. Executive Summary:

   High pressure processing (HPP) at low temperature inactivates pathogenic and spoilage microorganisms without altering sensory and nutrition food quality. The objective of this project was to evaluate the effect of HPP combined with moderate heating on the flavor quality and stability of fresh milk. The goal was to identify treatments to produce extended-shelf-life (ESL) refrigerated milk with a shelf life exceeding 30 d.

   Based on microbial inactivation, enzyme activity, viscosity and color studies on whole (3.25%) and 1% reduced fat milk, we identified three processes at 586 MPa (1 and 3 min at 60°C and 3 min at 55°C) producing milk with a shelf-life exceeding 45 days under refrigeration (5°C). Flavor analysis studies showed that these treatments do not affect the volatile composition of milk yielding similar values to those measured for thermally pasteurized milk. These results open a totally new field of dairy chemistry research since it was demonstrated that high pressure combined with heat has processing effects that differ to a large extent from heat-only treatments in the formation of off-flavor volatiles in milk. At the same temperature and time, high pressure inhibited the formation of many off-flavor compounds. The flavor quality of high pressure/moderate temperature processed milk needs to be evaluated by sensory methods as its aroma profile is dramatically different from that of conventionally treated milk. Further inhibition of cooked aroma compounds was achieved by natural and synthetic antioxidants generating valuable information on developing extended shelf-life (ESL) refrigerated dairy beverages with the superior flavor required to increase milk consumption. BHA and epicatechin showed a 95% of inhibition for straight chain aldehydes, keeping concentration values of volatile off-flavor compounds close to those of raw milk. Based on aroma profile determinations, our conclusion is that consumers should not be able to distinguish conventionally pasteurized (15-day shelf-life) from pressure-treated milk (shelf-life exceeding 45 d). Therefore, ESL milk produced by combined heat-pressure treatments has a high probability of being accepted by consumers. Furthermore, kinetic analysis of off-flavor compounds formation strongly suggest the possibility of producing by combined pressure/heat treatments shelf-stable milk with an aroma profile superior to that achieved by UHT technology.

n. Introduction

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 dairy producers. This project will assess the combination of high pressure and moderate heating to meet these consumer and industry requirements.

b. Objective(s)

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.

NOTE: Objectives 2 and 4 were not financially feasible due to the large delay between submission of project budget and reception of research funds at Oregon State University. All other objectives were met or exceeded.

c. Methods and Materials

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

A two-step search was used to find a combination of mild heating and high pressure processing (HPP) extending the shelf life of fresh milk. In the first step, six combinations of pressure (586 MPa), time (1, 3 and 5 min) and temperature (40 and 55 °C) were used to evaluate the treatment effect on mesophiles, pseudomonas, psychrotrophs and coliforms counts in 1% and 3.25% fat content milk stored at 5 °C for up to 45 days. Microbial counts immediately after pressure treatments showed that all combinations were able to inactivate the microorganisms analyzed at the same level as thermally pasteurized milk. When compared to the commercially pasteurized milk from the same processing plant, microbial loads remained lower for all treatments during refrigerated storage up to 45 days. An HPP treatment at 586 MPa for 5 min at 55°C yielded the largest reduction in microbial counts for mesophiles, psychrotrophs, pseudomonas and coliforms in milk samples with 1 and 3.25%
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fat. A much shorter pressurization time (1 min) yielded microbial counts at day 45 that were lower than those observed for samples of pasteurized milk obtained from the same commercial processing plant at day 15 but detectable.

For the second step, we evaluated three possible processes based on microbial deactivation, enzyme activity, viscosity and color of whole and 1% fresh milk observed in step 1. 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>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure, MPa</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>Time, min</td>
<td>586</td>
<td>586</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Two M.S. thesis published in Spanish (G.Tovar-Hernandez and H. Peña) have been completed reporting the details for this search, confirmation of microbial stability during the desired 45-day shelf life and other quality factors. Technical presentations were done at the 2005 Annual Meeting of the Institute of Food Technologists and two manuscripts are currently in their final preparation stage.

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

A headspace solid phase microextraction/gas chromatographic (HS-SPME/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).

d. Results

Objective 1:

1. Microbial inactivation by HPP of raw milk at 1 and 3.25 % fat level was shown to meet standard counts for pasteurized milk
2. HPP treated raw milk at 1 and 3.25 % fat level had lower counts than commercially pasteurized milk from the same processing plant.
3. HPP treated raw milk at 1 and 3.25 % 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.
Objective 3:

Development of improved analytical techniques:

The optimization of the solid phase microextraction (SPME) showed that temperature had a highly significant effect ($P = 0.007$), reaching maximum values at the highest extraction temperature estimated to be free of introducing measurement artifacts (35°C). Extraction time was an even more significant factor ($P < 0.001$) and even at low extraction temperature, increasing fiber exposure time improved the extraction of volatiles in the SPME fiber. Sample size did not have a significant effect ($P = 0.197$) on the volatile extraction.

Highly significant ($P < 0.001$) correlation coefficient ($R^2 = 0.984$) 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 2-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 matrixes has not been reported by other researchers due to their high reactivity and volatility. The headspace SPME technique coupled to a gas chromatograph with a pulsed-flame photometric detector (HS-SPME/GC-PFPD) used in our work for the quantitative analysis of sulfur compounds in milk is an important contribution to dairy research. Calibration curves with highly significant ($P \leq 0.001$) correlation coefficients ($R^2 = 0.94$) 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 sulfoxide with the two latter ones found at the highest levels. The concentration of dimethyl sulfone in 3.25% fat UHT milk was lower than in raw and pasteurized milk brand B. Raw and pasteurized 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 sulfoxide concentrations increased with fat content in UHT milk. This is the first time calibration curves of sulfur compounds are reported for a dairy matrix.
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Evaluation of volatile concentrations in pressure treated milk: A 2 x 3 x 3 factorial experimental design was 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>
<th>levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (kpsi)</td>
<td>70</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25, 60</td>
</tr>
<tr>
<td>Time (min)</td>
<td>1, 3, 5</td>
</tr>
</tbody>
</table>

This design included the treatment conditions found to be effective in controlling the natural microflora in milk to extend refrigerated milk storage time to 45 days (85 kpsi, 60°C, 1 and 3 min). The results were analyzed using Analysis of Variance (ANOVA) for factorial designs and Principal Component Analysis (PCA) of 28 different flavor volatile compounds.

Temperature-time effects on the volatile composition of milk differed from those by pressure-temperature-time combinations. Temperature-time treatments promoted the formation of hydrogen sulfide, methanethiol, nonanal, 2-nonanone and carbonyl sulfide, listed in order of higher to lower concentration. Pressure-temperature-time treatments promoted the formation of hydrogen sulfide, heptanal, nonanal, carbon disulfide and 2-nonanone, listed in the same order.

In heat-only treatments, temperature was the most significant factor affecting the concentration of volatiles, although time became more important as temperature was increased. When using heat and pressure combinations, temperature was the most significant factor affecting the concentration of volatiles. Pressure level became more important when the temperature was increased while time was the least significant factor, being slightly important only for the highest pressure and temperature combination used (90 kpsi and 60°C).

Changes in the concentration of volatiles after treatment combinations of pressure, temperature and time (see Table below) were used to obtain kinetic parameters (rate constant and activation energy), to achieve a better understanding of the formation mechanisms of volatile compounds in milk subjected to high pressure and moderate temperature treatments. The concentration of 27 volatile off-flavor compounds of different chemical classes (aldehyde, ketone and sulfur compounds) was determined experimentally. Experimental data for the formation of off-flavor compounds were fitted with zero-order, first-order, and second-order methods. Rate constants were then obtained by plotting concentration versus time. The activation energies for the formation of volatile compounds at different pressures were calculated using the Arrhenius equation.
Temperature, pressure and time showed a strong interaction affecting the formation of some of the compounds ($P < 0.05$), being larger when the levels of the factors were at their highest values. The formation of hexanal, heptanal, octanal, nonanal, and decanal, followed first-order kinetics, with rate constants increasing when the pressure and temperature were raised. Activation energies for these five aldehydes decreased when the pressure was higher, suggesting that increasing pressure has a catalyst effect on their formation reactions in milk. 2-Methylpropanal, 2,3-butanedione, and hydrogen sulfide followed zero-order kinetics for their formation. Their rate constants increased when the temperature was raised, but the effect of raising the pressure level was not very clear. The activation energies for 2-methylpropanal and 2,3-butanedione showed a trend to increase when the pressure applied was higher, whereas the activation energy for hydrogen sulfide remained stable through the pressure range studied. Dimethyl trisulfide concentration remained stable under pressure until 75°C, where it clearly showed a zero-order kinetic behavior with rate constants increasing with the pressure level. The concentrations of any other off-flavor compounds studied, including the very important methanethiol, remained stable when under pressure. In the case of methyl ketones (2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 2-decanone, and 2-undecanone), only a slight increase in the average concentration was noticed when the temperature of treatment was higher, maybe due to the time required to load and unload the samples in the preheated pressure vessel. In conclusion, the pressure levels used in this study inhibited the formation of methyl ketones and most sulfur compounds. Hydrogen sulfide formation was not affected by the pressure applied. Only straight chain aldehydes formation was promoted by high hydrostatic pressure, therefore some experiments about their inhibition by the addition of antioxidants were needed.

The data obtained in this work can be used to construct models for the design of high pressure processes that keep the fresh flavor of milk at a maximum, which is an important quality for the consumer, while accomplishing the desired safety and shelf life standards. Along with this, sensory studies are needed to assess the impact of aldehydes formation under pressure on the final flavor perception of milk.

Considering that a few aroma compounds were not inhibited by pressure, we added to this project a study on the inhibition of off-flavor aroma compounds in high pressure-moderate temperature treated milk by the addition of antioxidants. This experiment was used to study the development of undesirable flavor compounds generated during heated pressurization. A 3 X 2 X 5 experimental design for time and antioxidant type was run in triplicate to evaluate the use of one synthetic and four natural antioxidants. Pressure and temperature conditions were kept constant at 95 kpsi and 75°C respectively, which were the conditions that previously demonstrated to have the highest formation of off-flavor compounds.
volatiles in milk under high hydrostatic pressure. Reduced oxygen samples were also treated in the same way.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>3</td>
</tr>
<tr>
<td>Antioxidant concentration</td>
<td>Low level</td>
</tr>
<tr>
<td>Antioxidant</td>
<td>BHA</td>
</tr>
</tbody>
</table>

Off-flavor compounds including aldehydes, ketones and volatile sulfur compounds were quantified using headspace solid-phase microextraction and gas chromatography. BHA and epicatechin showed up to a 95% of inhibition for straight chain aldehydes, keeping concentration values of volatile off-flavor compounds close to those of raw milk. Ascorbic acid, b-carotene and L-cystine showed less aldehyde inhibition. However, L-cystine was the only compound capable of inhibiting hydrogen sulfide and methanethiol formation. In general, off-flavor inhibition was proportional to the concentration of antioxidants. The reduction of oxygen in milk showed only a slight reduction of off-flavor compounds. Results suggested that off-flavor formation under high pressure is free radical related, and addition of antioxidants can inhibit their formation upon pressure processing. Off-flavor analysis indicated that with the combination of high pressure, high temperature and antioxidants, it is possible to achieve milk with microbial stability and a minimum formation of off-flavor compounds. In addition, due to its anticarcinogenic properties, epicatechin can be taken as a functional ingredient in milk and not as an additive, opening an option for the marketing of shelf-stable fortified milk with superior flavor.

e. Conclusions and Next Steps

1. An improved technique allowed more accurate analysis of UHT milk showing 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 sensitivity has been 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.

4. Pressure-temperature treatments below the combination 60°C-90 kpsi did not affect significantly the volatile composition of milk and yielded values similar to those measured for commercial conventionally pasteurized milk. This leads to the conclusion that the previously reported treatments (85 kpsi and 60° for 1 and 3 min), and yielding milk with shelf-life exceeding 45 days under refrigeration, can be predicted that consumers will not be able to distinguish it from commercial conventionally pasteurized milk. Therefore, ESL refrigerated milk produced by combined heat-pressure treatments have a high probability of being accepted by consumers.

5. Kinetic data demonstrated for the first time that off-flavor formation in milk under high pressure and mild temperature is different from that of milk subjected to heat treatments only, therefore opening a new field of study for reactions under high hydrostatic pressure.

6. Data obtained from the inhibition experiment above mentioned, along with the kinetic information, will help elucidate the pathway of formation for these off-flavor compounds. This will generate valuable information on improving the flavor of extended shelf-life (ESL) refrigerated milk and provide insights into an HPP process for shelf-stable milk.

7. The above findings suggest the possibility that a continuation project could address developing shelf-stable milk with a flavor profile superior to that of current products on the market.

7. Intellectual Property (if applicable)

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

Yes [ ]
No [ ]
Possible [x ]

8. Anticipated Delays or Problems

9. Manuscripts/Abstracts Submitted for Publication

10. Published Articles/Abstracts
Western Dairy Center

PUBLICATIONS:


to Journal of Agricultural and Food Chemistry.


PRESENTATIONS


PROJECT PROGRESS REPORT
of
Western Dairy Center

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

2. Principal investigator: **Marie Walsh**

3. Start Date: 1-1-05

4. End Date: 12-31-06

5. Executive Summary:

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

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

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

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

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


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

o. Introduction

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


p. Objective (s)

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

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

q. Method and Materials

**Project overview.**

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

**Whey protein extrusion**

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

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

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

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

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

Extrudate Analysis.

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

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

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

\[
\text{Force (Pa)} = \left(9.7865 \text{ N/kg} \right) \left(\text{breaking strength (kg)}\right) \times 1000 \times \frac{\pi \left(\text{extrudate diameter (mm)/2}\right)}{2}
\]
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The ratio of cross sectional area of each extrudate (CS) to the area of the die exit was used for expansion ratio calculation. Ten values for obtained for randomly selected extrudate samples.

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

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

where \( \text{d}_{ave} \) is the average of the initial and final diameters of a given extrudate segment taken at point of shear. Length, and weight measurements of each extrudate sample used for breaking strength determination where used for density calculation.

Chemical tests. Moisture Determination. Four 20 second samples randomly collected during extrusion and weighed for product flow determination were used for moisture determination. The first and third samples were immediately dried overnight for at least 16 hours at 70 °C in a drying oven while the second and third samples were them dried 24 hours later under same conditions. Pans were allowed to cool and were weighed. Moisture content was calculated as a percent of the weight difference before and after drying.

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

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

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

a. Vitacel Orange fiber OF400 (J. Rettenmaier USA LP, Schoolcraft, MI)
b. Vitacel Apple fiber AF 401 (J. Rettenmaier USA LP, Schoolcraft, MI)
c. Vitacel Oat fiber HF 600 (J. Rettenmaier USA LP, Schoolcraft, MI)
d. Vitacel Oat fiber HF 401 (J. Rettenmaier USA LP, Schoolcraft, MI)
e. Vitacel Wheat Fiber WF 600 (J. Rettenmaier USA LP, Schoolcraft, MI)
f. Vitacel Powdered Cellulose L601 FCC (J. Rettenmaier USA LP Schoolcraft, MI)
g. Oatvantage Oat fiber (Nuture Advanced Oat Technologies, Devon, PA)
Three fiber sources were not successfully extruded: Oat fiber X, Litesse, and Fibersol-2 fiber sources. Unsuccessful extrusion was determined by seizing of extruder before extrusion parameters could be obtained and or inability to consistently form an extrudate. Apparent radial expansion was best seen in the extrudates extruded from Vitacel Oat fiber HF600, Vitacel Apple fiber AF401, Cargill Corn fiber Maizewize 60, Vitacel Powdered Cellulose L601 FCC, and Vitacel Wheat fiber HF600. For this experiment, samples with similar %TDF content were desired. Vitacel Apple fiber AF401 and Cargill Corn fiber Maizewize 60 only contained 60% TDF. Vitacel Powdered Cellulose L601 FCC was considered 100% TDF, and Vitacel Oat fiber HF600 and Vitacel Wheat fiber WF600 had 96 and 97% TDF, respectively. Cargill Corn fiber Maizewize 60 was in the prototype stages and sufficient quantities of fiber sample could not be
based on the observations stated above, Vitacel Powdered Cellulose L601 FCC, Vitacel Oat fiber HF600 and Vitacel Wheat Fiber WF600 were then selected to complete objective 2.

**Extrudate Physical Parameters**

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

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

Expansion ratio is usually attributed to the degree of starch gelatinization. As stated previously, dietary fibers bindis water more tightly than starch (Harper, 1981; Gomez and
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Aguilera, 1984; Bhattacharya and Hanna, 1987; Moraru and Kokini, 2003). Therefore, as more water is structurally bound by fiber, less water may become available for starch gelatinization and moisture flash-off as the extrudate leaves the die (Onwulata et al., 1998; Lue et al., 1991). This would result in decreased extrudate expansion because less steam would be available to act as a leavening agent (Camire, 1991). Decreased expansion may be attributed to insufficient amount of starch in the extrudate and not just the presence of dietary fiber.

Extrudate density (Fig. 3) and breaking force (Fig. 4) where both strongly influenced by fiber type, fiber level and the fiber type/fiber level interactions with \( p < 0.0001 \) for all parameters. The same trends were observed with both density and breaking strength. Each fiber type at 48% TDF was significantly different than the other fiber levels. Each fiber type had an increase in extrudate density and breaking force with an increase in the amount of fiber in the extrudate. Thus, as breaking strength and density increased, extrudate expansion ratio and air cell size decreased. Powdered cellulose had the lowest density and breaking strength. Wheat usually had the highest density and breaking strength at all fiber levels. There was no difference between samples with added fiber and the control for breaking force at 18% TDF. Powdered cellulose was not significantly different from the control at 18% TDF for density. Despite no differences between fiber types with air cell size at 36 and 48% TDF, and expansion ratio at 48% fiber levels, there were statistical differences between fiber types with density and breaking strength at 36 and 48% TDF levels. It is therefore possible other parameters are affecting the expansion ratio and air cell formation in the extrudates. The water absorption ability of the fiber types and extrudate moisture content may affect the air cell formation and expansion ratio of the extrudates.

Extrudate Chemical Parameters

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

Generally, moisture content (Fig. 5) and WAI (Fig. 6) increased with increasing fiber level of the extrudate. With moisture content, there was no difference between fiber types at each fiber level. The 48% TDF level was statistically different from the other fiber levels. Oat fiber at 18% TDF level was the only fiber type to not be statistically different from the control.
The significant increase of moisture content at 48% TDF level can be attributed to increase fiber leading to more water being bound during extrusion (Moraru and Kokir, 2003; Onwulata et al., 1998). Thus, as more water is structurally bound by fiber, less water may become available for moisture flash-off as the extrudate leaves the die (Onwulata et al., 1998; Lue et al., 1991), allowing for extrudates with higher moisture content.

The WAI is the amount of water an extrudate can absorb. WAI is usually dependent on the starch present in the extrudates. It was found that WAI generally exhibited an increase with an increase in fiber. There were no differences between fiber types at 18% TDF. However, at the 36% and 48% TDF levels, powdered cellulose was statistically different from the other fiber types. Powdered cellulose had no differences in WAI at all fiber levels. Both oat and wheat fibers were not statistically different from each other at all fiber levels. Oat and wheat fibers absorbed the most water, thus having the highest WAI. Powdered cellulose at 48% TDF was significantly different from the control and oat and wheat fibers.

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

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

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

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

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

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

Fig. 4. Breaking force of extrudates (_, Powdered Cellulose; _, Wheat; _, Oat). Control (0% TDF), not shown, has a mean of 16.68 and letter sharing of ‘a’. Points are means of all three extrusion blocks. Means sharing letter are not different at p 0.05.
Fig. 5. Moisture content of extrudates (—, Powdered Cellulose; —, Wheat; —, Oat). Control (0% TDF), not shown, has a mean of 9.7325 and letter sharing of ‘a’. Points are means of all three extrusion blocks. Means sharing letter are not different at p < 0.05.

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

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

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

s. Discussion

We have successfully evaluated 12 different fiber types for the production of an extruded-expanded product and selected three fibers to complete objective 2. The extrudates have been analyzed for physical and chemical properties and the data analyzed by ANOVA. The level of fiber had a greater impact than the type of fiber added on extrudate characteristics. Generally, as the amount of fiber increased, moisture content increased leading to decreased expansion ratio, WSI, and air cell size and an increase in total soluble protein, density, WAI, and breaking strength. It is unclear whether the increased amount of fiber affected chemical and physical parameters more so than the decrease in starch. We believe that dietary fiber can be incorporated into an extruded snack product. Extrudates with 18% TDF were comparable to extrudates with 0% TDF (control). Extrudates with 48% were significantly different than the control (0% TDF). It is concluded that 48% fiber addition under the aforementioned extrusion conditions is too high for product requirements of low extrudate density and high expansion ratio. Extrudates containing TDF as high as 36% may be acceptable for some products, such as chips, crackers, and other snack foods.
We have also evaluated 4 different fiber types for the production of a fibrous-textured extruded product and have narrowed the list to 3 different fibers (Vitacel Oat fiber HF 600, Vitacel Wheat Fiber WF 600, and Vitacel Powdered Cellulose L601 FCC and all from J.Rettenmaier USA LP, Schoolcraft, MI) to continue with objective 1. Each fiber was blended with WPC 80, fiber and starch to yield compositions of 50.4 % protein with fiber levels of 0%, 7%, 15%, and 22% (w/w) and extruded in triplicate producing 30 TWP samples. Each of these TWP samples is now being analyzed for physical and chemical properties and data will be analyzed by ANOVA.

Conclusion and Next Step

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

References


Western Dairy Center


7. Intellectual Property (if applicable)

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

Yes [ x ]  No [ ]  Possible [ ]

A patent application was filed to the US patent office on August 19, 2003 entitled

Textured whey protein product and method (Walsh and Carpenter authors) which covers the research described in this report.

8. Anticipated Delays or Problems

none

9. Manuscripts/Abstracts Submitted for Publication

62
none

10. Published Articles/Abstracts

None