National ADSA Dairy Foods Oral: Dairy Foods Oral Student Competition

127 The effect of sodium gluconate on pH, lactose, lactic and, and water soluble Ca changes during Cheddar cheese ripening. C. Phadungath*[1] and L. E. Metzger[2], [1]*Midwest Dairy Foods Research Center, University of Minnesota*, *St Paul,* [2]*Midwest Dairy Foods Research Center, South Dakota State University*, *Brookings*.

Sodium gluconate increases the solubility of calcium late in model solutions by forming soluble sodium-lactate-gluconate complexes. However, its effect on the main components responsible for calcium lactate crystals in Cheddar cheese, which are lactic acid and water soluble calcium, has not been reported. The objective of this study was to determine the effect of sodium gluconate on pH, lactose, lactic acid, and water soluble Ca changes during Cheddar cheese ripening. Six Cheddar cheeses with 2 salting levels (2 and 2.5%) and 3 sodium gluconate levels (0, 0.5 and 1%) were manufactured in triplicate. Composition and chemical analysis was performed at 1 week of ripening, and at 1 week, 3, 6, 9, and 12 mo of ripening. Cheeses were analyzed for pH, lactose and lactic acid, and water soluble calcium (WSC). Compositional analyses at 1 week indicated that sodium gluconate addition had a significant effect on cheese pH, moisture, Na, lactose, and lactic acid. Cheddar cheeses from both 2% and 2.5% salt levels with higher concentration of sodium gluconate exhibited higher pH than the control cheeses throughout the ripening time, which corresponded to the concentration of lactic acid in the cheeses. HPLC results from Cheddar cheeses from both 2% and 2.5% salt levels indicated that cheeses with higher concentration of sodium gluconate addition had higher concentration of lactose, but lower concentration of lactic acid when compared with the control cheeses throughout the ripening time. WSC results indicated that Cheddar cheeses from both 2% and 2.5% salt levels with higher concentration of sodium gluconate addition had lower WSC concentration when compared with the control cheeses throughout the ripening time. From the results, we concluded that sodium gluconate could have an effect on starter culture activity and could also act as buffering agent, which would cause a higher cheese pH. A higher cheese pH resulted in less soluble of calcium in the cheese serum; thus, resulting in less calcium and lactate ions in the cheese serum.

Key Words: calcium lactate crystal, water soluble calcium, sodium gluconate

128 The impact of starter culture and annatto on the flavor and functionality of whey protein concentrate. R. E. Campbell*, R. E. Miracle, and M. A. Drake, *North Carolina State University*, *Raleigh*.

The flavor of whey protein can carry through into ingredient applications and negatively influence consumer acceptance. Understanding sources of flavors in whey protein is crucial to minimize flavor. The objective of this study was to evaluate the impact of annatto color and starter culture on flavor of whey protein concentrate (WPC). Cheddar cheese whey with and without annatto (15mL/454kg with 3% norbixin content) was manufactured using a mesophilic lactic starter culture or by addition of lactic acid and rennet (rennet-set). Pasteurized fat-separated whey was then ultrafiltered and spray dried into WPC62. The experiment was replicated 4 times. Flavor of liquid wheys was evaluated by sensory and instrumental volatile analyses, and sensory and instrumental analyses, color analyses (Hunter Lab and norbixin extraction) and functionality (solubility and heat stability) were performed on WPC. Both main effects (annatto, starter) and interactions were investigated. No differences in sensory properties or functionality were observed among WPC ($P > 0.05$). Lipid oxidation compounds were higher ($P < 0.05$) in WPC manufactured from whey with starter culture compared with WPC from rennet-set whey. WPC with annatto had higher concentrations of p-xylene, diacetyl, pentanal, and decanal ($P < 0.05$) compared with WPC without annatto. Interactions ($P < 0.05$) were observed between starter and annatto for hexanal, suggesting that annatto may have an antioxidant effect when present in whey made with starter culture.

Key Words: whey, antioxidant, flavor

129 Exopolysaccharides modify the functional properties of whey protein concentrate. G. Deep* and A. Hassan, *Midwest Dairy Foods Research Center, Dairy Science Department*, *South Dakota State University, Brookings*.

The objective was to study the effect of exopolysaccharides (EPS) on the functional properties of whey protein concentrate (WPC). Exopolysaccharides producing cultures are used to improve the physical properties of reduced fat cheeses. It was hypothesized that EPS released in the whey would modify the functional properties of WPC due to their interaction with whey proteins. An EPS-producing culture of *Lactococcus lactis* ssp. *cremoris* (JFR), an EPS-producing culture of *Streptococcus thermophilus* (DP) and an EPS-nonproducing commercial cheese culture (DVS 850) were used in this study. Cultures were grown overnight in reconstituted WPC (10% w/w) which was then added, directly or after overnight cooling, at 2% to rehydrated dry whey (6% w/w). This gave a level of EPS similar to that found in whey from cheese made with EPS-producing cultures. Whey was then pasteurized at 75°C for 35 s and ultrafiltered 5 times. Ultrafiltered whey (retentate) was spray dried at inlet and outlet air temperatures of 200°C and 90°C, respectively. The protein level in WPC was about 32%. Results showed that EPS decreased ($P < 0.05$) the minimum gelling concentration and protein denaturation, and increased emulsifying capacity and gel hardness of WPC. Exopolysaccharides from DP culture showed more pronounced changes in functionality of WPC than did EPS from the JFR culture. Cooling of the fermented medium containing EPS, before its addition to whey, increased ($P < 0.05$) gel hardness and emulsifying capacity of WPC. This is possibly due to effect of cooling on interactions within EPS molecules and between EPS and proteins. This study demonstrated that application of EPS-producing cultures in cheese making modified the functional properties of WPC. Further studies will be directed toward understanding the effect of EPS structure and their interaction with whey protein on WPC functionality.

Key Words: exopolysaccharides, whey protein concentrate, functional properties

130 Evaluation of the effects of cheese milk fat content on the lipid composition and flavor of liquid whey and whey protein concentrate. A. E. Croissant*[1], L. Dean[2], and M. A. Drake[1], [1]*North Carolina State University*, *Raleigh,* [2]*USDA-ARS, North Carolina State University*, *Raleigh.*

Lipid oxidation is generally considered the most important degradation process to food products, and whey products are no exception. Ongoing research has established that lipid oxidation is initiated during the cheese make process. A relationship between lipids and phospholipids and flavor and flavor stability of WPC80 has not been established. The objective of this study was to evaluate the effect of 3 milk fat levels in cheese milk on the lipid composition of fluid whey and WPC80 and subsequent effects on flavor and flavor stability. WPC80 was manufac-

tured from uncolored Cheddar whey in triplicate. For each replication, pasteurized whole, low fat, and skim milk (3.25, 1, and 0.25% milk fat, respectively) were acquired from a single batch of milk. Spray dried WPC80 was produced from each milk on successive days and order was balanced between replications. WPC80 were evaluated by sensory and instrumental analyses as well as proximate analyses. Lipid concentrations in WPC80 from whole, low fat, and skim milk were 5.03, 3.90, and 3.45%, respectively. Whole milk WPC80 was higher in cardboard flavor ($P < 0.05$) compared with low fat and skim WPC80. WPC80 from whole milk also had higher relative abundances of oxidation reaction products including heptanal, hexanal, octanal, nonanal, and 1-octen-3-one ($P < 0.05$) compared with low fat and skim WPC. Results indicate that cheese milk lipid concentration does impact the lipid composition of WPC as well as the flavor and volatile compounds associated with lipid oxidation. Currently, low and reduced-fat cheese whey streams are combined with full-fat cheese whey streams in WPC manufacturing. Reduced-fat cheese production is situated to increase greatly given the growing consumer and governmental interest in fat reduction. The examination of the impact of reduced-fat cheese production on the flavor and lipid composition of whey protein concentrates has the potential to influence the creation of a value-added whey protein product.

Key Words: WPC, lipids, low fat

131 Growth and production of volatile compounds by *Lactobacillus casei* in Cheddar cheese extract under cheddar cheese ripening condition. H. Cai*[1], M. Budinich[1], W. Tan[1], E. Miracle[2], J. Broadbent[3], M. A. Drake[2], and J. Steele[1], [1]*University of Wisconsin*, *Madison*, [2]*North Carolina State University*, *Raleigh*, [3]*Utah State University*, *Logan*.

Lactobacillus casei are commonly used as adjunct cultures for the control of off-flavors, as well as the intensification and acceleration of beneficial flavors in bacterial-ripened cheeses. In this study, 22 *Lb. casei* strains were screened in a model system for attributes likely to influence their potential to serve as adjunct cultures for the manufacture of Cheddar cheese. The model system used was 4-mo-old Cheddar cheese extract supplemented with citrate (4mCCE–cit) and the experiment was conducted under Cheddar cheese ripening condition (pH5.1, 3.1% NaCl, and 8°C). The attributes screened for were the ability to dominate the NSLAB microbiota, produce volatile flavor compounds, and hydrolyze bitter peptides. None of the *Lb. casei* strains examined could degrade the model bitter peptide β-CN(f193–209) under the condition examined. Six strains (A2-309, CRF28, L9, M36, UW1 and UW4) exhibited growth parameters in the model system, relatively short lag phase, rapid growth rates, and high final cell densities, likely to be associated with the ability to dominate the NSLAB microbiota of Cheddar cheese. Significant increases in 2,3-butanedione accumulation was observed with 7 of the *Lb. casei* strains examined; 2,3-butanedione is strongly associated with the beneficial buttery note in young Cheddar cheese. Significant decreases in phenylethanal concentrations were observed for 9 strains and significant increases in phenylethanol concentrations were also observed for 9 strains. The use of culture adjuncts that reduce the level of phenylethanal would likely result in Cheddar cheese with a reduced rosy note. The results of this study provided a starting point for the rational selection of culture adjuncts to control cheese flavor development in Cheddar cheese.

Key Words: *Lactobacillus casei*, volatile, Cheddar cheese ripening

132 Interaction between casein micelles and serum protein/κ–casein complexes during renneting of heat–treated skim milk. P. Kethireddipalli*, A. R. Hill, and D. G. Dalgleish, *University of Guelph*, *Guelph, ON, Canada*.

Heat-induced complexes of whey protein (WP) and κ-casein (κ-CN) can impair rennet clotting of native casein micelles; coagulation can be mostly restored by dialyzing the complex–laden serum against unheated milk (to be published). The present study investigated the mechanism of interaction between casein micelles and WP/κ-CN complexes during renneting of heat-treated milk. Native casein micelles were separated from unheated skim milk and re–suspended in the serum of heated (90°C, 10 min) milk, with and without dialysis of the serum against unheated milk. Using size exclusion chromatography, it was found that in both milk systems, the WP/κ-CN complexes progressively bind to the casein micelles as renneting proceeded. At about 85% κ-CN cleavage (caseinomacropeptide was quantified), the protein complex peak area decreased by 30 ± 5% and at 90% cleavage (prior to onset of clotting), peak area reduction was 50 ± 4%. In the absence of micelles, renneting of protein complexes in isolation showed no significant self aggregation, even when 90% κ-CN had been proteolyzed. Enzyme treatment of the dialyzed serum, however, resulted in significant aggregation of complexes (20 ± 2% at about 90% κ-CN cleavage), but to a much lesser extent than when casein micelles were present. Similar trends were noted when casein micelles from milk heated at native pH 6.7 (some surface-bound WP/κ-CN complexes), pH 7.1 (nearly complex-free micelle surface) or pH 6.3 (complex-saturated surface) were suspended in the serum of heated milk. No matter what the micelle surface was, all micelles were capable of binding more serum protein complexes in the course of renneting. These results are strong evidence that impaired rennet clotting of native casein micelles suspended in the serum of heated milk is due to the binding of WP/κ–CN complexes to the micelle surface prior to the onset of micelle aggregation, thereby sterically impairing the aggregation process. For reasons possibly related to ionic equilibria, heat-induced protein complexes in serum dialyzed against unheated milk are less detrimental to micelle fusion.

Key Words: whey protein/κ-casein complexes, heated milk, rennet

133 Starter cultures and cattle feed manipulation enhance conjugated linoleic acid levels in Cheddar cheese. M. S. Mohan*, S. Anand, K. F. Kalscheur, and A. N. Hassan, *Midwest Dairy Foods Research Center, South Dakota State University*, *Brookings*.

Conjugated linoleic acid (CLA) is a fatty acid that provides several health benefits to humans. In our previous research, a CLA-producing starter culture of Lactococcus lactis (CI4b) increased the CLA content in Cheddar cheese. Addition of fish oil to cattle diets has also been reported to increase the CLA content in milk. Thus, it was hypothesized that the use of the CLA-positive starter (CI4b) along with high CLA milk (obtained through dietary manipulation in cattle) would enhance the CLA content in Cheddar cheese. A diet containing fish oil (0.75% of dry matter) was fed to 32 dairy cows grouped in a pen for 15 d. This increased the CLA cis-9, trans-11 (CLA1) and CLA trans-10, cis-12 (CLA2) content from 0.8 and 0.13 g/100 g of fatty acids in the normal milk to 1.22 and 0.27 g/100g fatty acids in the treatment milk, respectively. A 2 × 2 factorial treatment design was used to test the effect of culture (DVS vs. CI4b) and type of milk (normal vs. treatment milk) on CLA content in Cheddar Cheese. A commercial cheese starter (DVS) was selected as the CLA nonproducing culture. Chemical composition (moisture, salt, fat, protein) of cheese was not affected by the type of culture used ($P > 0.05$). The textural properties (hardness, gumminess, chewiness) showed an interaction between milk and culture ($P < 0.05$) in the 1 mo old cheese. The 1 mo old cheese made from normal milk with DVS culture, normal milk with CI4b culture, treatment milk with

DVS culture and treatment milk with CI4b culture contained 0.34, 0.39, 1.03, 1.10 (±0.09) of CLA1 concentration and 0.06, 0.10, 0.18, 0.21 (±0.13) of CLA2 concentration (g/100 g of fatty acid), respectively. The treatment milk resulted in increased ($P < 0.01$) CLA1 and CLA2, while the CI4b culture increased only CLA2 levels ($P = 0.03$) in cheese. The results indicated that the combination of a CLA producing starter culture and dietary manipulated milk could enhance levels of CLA (CLA1 + CLA2) in Cheddar cheese by up to 3.3 times.

Key Words: conjugated linoleic acid, fish oil, cheddar cheese

134 Transcriptional stress responses to hydrogen peroxide in *Bifidobacterium longum*. T. S. Oberg*[1], J. L. Steele[2], S. C. Ingham[2], and J. R. Broadbent[1], [1]*Utah State University*, *Logan,* [2]*University of Wisconsin*, *Madison*.

Commercial application of bifidobacteria probiotics in function foods has increased dramatically in recent years. Due to the anaerobic nature of bifidobacteria, however, oxidative stress can significantly diminish viability of bifidobacteria during food production and storage. To better understand mechanisms for oxidative stress resistance in these cells, we examined the transcriptional stress responses to hydrogen peroxide (H_2O_2) of *Bifidobacterium longum* NCC2705, a strain that exhibits oxidative stress resistance, and *B. longum* D2957, a strain that does not. Bacteria were grown in MP5 medium to late logarithmic phase under anaerobic conditions with pH control, then suspended in MP5 with 0.625 mM H_2O_2 and incubated 5 or 20 min (NCC2705) or for 5 or 60 min (D2957). mRNA was isolated from each sample, converted to cDNA, and hybridized to a custom made Affymetrix DNA array. Three biological replicates of each treatment were performed. The microarray data was preprocessed using the RMA-MS method, filtered to only include genes with high signal intensity and a low coefficient of variation, then tested for differential expression using the limma/eBayes method (P value <0.05). Results showed *B. longum* NCC2705 had 316 genes that were differentially expressed after the 5-min treatment and 131 differentially expressed genes after the 20-min treatment. In contrast, D2957 had only 24 and 116 differentially expressed genes after the 5-and 60-min treatments, respectively. Both strains showed upregulation of genes associated with an oxidative stress response, including thioredoxin reductase, peroxiredoxin, ferridoxin and glutaredoxin. However, NCC2705 showed more extensive upregulation of genes involved in transcription regulation, a greater number of downregulated genes involved in sugar transport and metabolism, and several other differences. These observations provide a platform for future functional genomics research to determine the molecular basis for differences that are observed in oxidative stress resistance among strains of *B. longum*.

Key Words: probiotics, bifidobacteria, oxidative stress

135 Positive influence of milk on the expression of some stress-induced genes in *Bifidobacterium longum*. W. Dominguez* and D. J. O'Sullivan, *University of Minnesota*.

The objective of this study is to define the genes encompassing the yogurt fermentation stress regulon in *B. longum* DJO10A. A genome-wide microarray analysis was conducted on total RNA from samples taken at different time points during a yogurt fermentation with *B. longum*DJO10A. A control yogurt fermentation revealed that cross-hybridization of the yogurt starter culture transcripts with the microarray was essentially negligible under these fermentation conditions. During the fermentation, a set of genes located in 5 clusters, some of which have previously been associated with stress protection, were significantly expressed. These consisted of the *dnaK* and ibpA gene clusters, *groL*, a predicted gene BLD_1532, and genes encoding the F_0F_1-type ATP synthase. These 5 gene clusters were defined as the bifidobacteria stress regulon for yogurt fermentation and 4 genes were selected for further analysis using real-time PCR. Primers and TaqMan probes were designed to monitor the expression of these genes when the culture was exposed to milk and media supplemented with glucose or lactose and when the pH was decreased to 4.5. As the pH dropped in the media, low upregulation (<2 times) was observed in all the tested genes, except for *ibpA*, which was 8 times higher at the final pH (4.5) compared with the initial pH (6.4). However, when the cells sensed the pH drop in milk, they responded by upregulating *ibpA* and BLD_1532 by 30 and 5 times respectively. The expression of *ibpA* is induced by acid stress and milk components, other than lactose. A low pH in milk upregulates *ibpA* at a higher level than the presence of acid by itself and BLD_1532 expression is upregulated at low pH only when milk components are present. This suggests that the higher expression of these stress-induced genes in milk may afford a protective effect on bifidobacteria from acid or other stresses.

Key Words: bifidobacteria, probiotics, microarray

136 Impact of color of low fat Cheddar cheese on consumer preference. R. Wadhwani*, D. J. McMahon, and C. Maughan, *Utah State University*, *Logan*.

To observe if color impacts low fat cheese consumer preference, 9 batches of low fat Cheddar cheeses were manufactured at USU with 3 different levels of annatto (0, 7.34, and 22 g/100 kg) and TiO_2 (0, 7.67, and 40 g/100 kg) in a 3 × 3 completely randomized block design and aged 60 d before consumer liking assessment. The IRB approved consumer panel was conducted with 120 panelists recruited from the vicinity through newspaper advertisements, emails, and flyers. Panelists evaluation and responses were recorded anonymously in computers using SIMS 2000 program. Population studied were 18 to 35 years of age with >60% were frequent cheese consumers. Among 9 combinations, cheese with no color, intermediate and maximum level of annatto and TiO_2 combination were rated significantly higher for overall liking than other combinations ($P < 0.05$) on a 9-point hedonic scale of degree of liking. Interestingly, maximum level of TiO_2 when added singly, was rated higher (6.27) along with the combination of both TiO_2 and annatto, however, annatto when added singly, was rated significantly lower (4.82). Regarding the flavor and appearance liking, exactly same responses were observed and both intermediate and maximum levels of both colors in combination and maximum level of TiO_2 were rated significantly higher than other levels tested. However, texture rating was slightly different than previous attributes. For texture liking, consumers did not prefer the maximum level of TiO_2 and rated significantly lower (5.52) than intermediate and maximum levels. Panelists were also asked to evaluate these samples for the sharpness level on a 4-point scale where 1 = mild and 4 = extra sharp. The panelists rated maximum level of TiO_2 as significantly milder (1.48) and maximum level of annatto as significantly sharper (2.76) than other levels of color ($P < 0.05$). All panelists unanimously responded that these samples contained 24–26% fat when asked to assume the fat level wherein reality they all contained 6% fat. This study clearly indicated a significant impact of color on overall liking of Cheddar cheese and consumers' responses were driven by the appearance of cheese.

Key Words: color, Cheddar, liking