Dairy Foods: Microbiology and Probiotics

496 Use of high pressure processing to control *Listeria monocytogenes* in packaged Queso Fresco. P. Tomasula*[1], L. Leggett[1], R. Kwoczak[1], D. Van Hekken[1], M. Tunick[1], J. Renye[1], M. Toht[1], S. Mukhopadhyay[2], A. Porto-Fett[3], and J. Luchansky[3], *[1]USDA/ARS/ERRC/Dairy and Functional Foods Research Unit, Wyndmoor, PA, [2]USDA/ARS/ERRC/Residue Chemistry and Predictive Microbiology Research Unit, Wyndmoor, PA, [3]USDA/ARS/ERRC/Food Safety Interventions Research Unit, Wyndmoor, PA.*

Queso Fresco (QF), a fresh, Hispanic-style cheese, is manufactured using pasteurized milk; however, its high pH (>6) and moisture content (>50%) coupled with post-pasteurization labor intensive practices may lead to contamination with Listeria monocytogenes (LM). The objective of this study was to evaluate the effectiveness of high pressure processing (HPP) as an intervention applied to QF after packaging to control LM. QF was manufactured from pasteurized milk using a commercial-make procedure. In preliminary experiments, about 8 kg of the dry, finely milled and salted curd was packed into a mold and held at 4°C overnight before the mold was removed. QF was then cut into slices with dimensions of about 12.7 cm x 7.6 cm x 1 cm. QF slices were surface inoculated on both sides with 50 µL of a 5-strain LM cocktail (ca. 5.0 $\log_{10}$cfu/g), individually double vacuum-packaged and then cooled to 4°C. The slices were then warmed to either 22 or 40°C and treated using HPP at pressures of 200, 400, and 600 MPa for holding times of 0, 5, 10 or 20 min. Only HPP treatment at 600 MPa, at both temperatures and all holding times, reduced LM to below the detection level of ≤0.91 $\log_{10}$cfu/g. Processing at 40°C resulted in visible textural changes and significant "wheying-off" of the cheese and was not investigated further. In subsequent experiments, QF slices were treated at 22°C and 600 MPa at holding times of 0, 3, 10 and 20 min and then stored at 4 and 10°C for 7 d. For the 3 min holding time, LM populations increased to 2.65 ± 0.92 and 4.50 ± 0.50 $\log_{10}$cfu/g, when QF was stored at 4 and 10°C, respectively. For the 10 min holding time, LM populations in QF remained below the detection level when stored at 4°C but increased to 2.00 ± 0.00 $\log_{10}$cfu/g when stored at 10°C. For the 20 min holding time, LM populations were not evident in QF when stored at 4°C but approached the detection level at 10°C. These results show that HPP was most effective when conducted at 600 MPa for 20 min, but HPP pressures >600 MPa, pulsed HPP, or addition of antimicrobials, may be necessary for ultimately controlling this pathogen.

Key words: *Listeria monocytogenes*, high-pressure processing, cheese

497 High-pressure processing of lowfat Cheddar cheese. M. Ozturk*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], K. Houck[2], M. E. Johnson[2], and J. A. Lucey[1], *[1]University of Wisconsin, Madison, [2]Wisconsin Center for Dairy Research, Madison.*

A major problem with lowfat cheese is the difficulty in attaining a strong flavor that is typical of full fat versions. Some studies have suggested that the use of high hydrostatic pressure (HHP) can accelerate cheese ripening by increasing starter lysis. Our objective was to investigate the use of HHP on lowfat Cheddar cheese with the goal of improving or accelerating flavor and texture development. To study the impact of pressure and holding time on the rheological, physical, chemical and microbial characteristics of lowfat Cheddar cheese, we used a central composite rotatable design with response surface methodology. A 2–level factorial experimental design was chosen to study the effects of the independent variables (pressure and holding time) with 2 star points (α = 1.414) and 2 replicates of the center point. Pressures varied from 50 to 400 MPa and holding times ranged from 2.5 to 19.5 min. We performed the design in 2 blocks (i.e., replicated the design), and validated its predictions with another trial. HHP was applied one week after cheese production, and analyses were performed at 2 wk, and 1, 3, and 6 mo. With an increase in pressure, cheeses had higher pH and residual lactose levels, and lower starter and non-starter bacteria counts. Compared with untreated cheeses, all HHP-treated cheeses had lower acid/base buffering areas due to solubilization of residual insoluble calcium phosphate. Cheeses treated with higher pressures were softer and had more homogeneous texture. Pressure had a larger impact on cheese properties than holding time. Several conditions exhibited buttery flavor notes in cheeses that were perceptible within a few weeks of HHP treatment. High pressure treated cheeses also had "sweet" notes due to their high pH. We did not observe any significant difference in proteolysis rates. This study indicates that holding times of around 5 min and pressures of ≥300 MPa could potentially be used to improve excessive firm textured cheese or reduce unwanted microbial activity.

Key words: high-pressure processing, lowfat cheese

498 The effect of UV light treatment and processing method on the microbial reduction of pasteurized whole milk. J. Tharani*, A. Laubscher, A. M. Lammert, and R. Jimenez-Flores, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

UV (UV) irradiation does not involve heat to kill microorganism. Therefore, it is of interest to the dairy food industry as a potential low cost non-thermal method of preservation. The objective of this study was to determine the impact of UV treatment immediately before or after pasteurization on microbial reduction in whole milk. A total of four controls and four test variables were produced. The controls were single or double pasteurized milk with or without the UV equipment. The test variables were UV light followed by pasteurization or pasteurization followed by UV at either 138 J/L or 920 J/L. Raw whole milk was standardized to 3.5% fat and each variable thermally processed at 166°F for 16 seconds and homogenized at 500/2000 psi. A Sure Pure Photo-Purification unit was used for UV treatment. Microbial testing was completed every 3 d until 21 d post processing. All variables were replicated 5 times. The results show that the reduction in microbial load was from 91,000 cfu/ml to 20 cfu/ml, 16 cfu/ml, 13 cfu/ml and 19 cfu/ml for the controls of single pasteurized with UV, single pasteurized without UV, double pasteurized with UV and double pasteurized without UV respectively. Alternatively, the reduction in microbial load was from 91,000 cfu/ml to <10 cfu/ml, 13 cfu/ml, <10 cfu/ml and 14 cfu/ml for UV treatment at 920 J/L followed by pasteurization, UV treatment at 138 J/L followed by pasteurization, pasteurization followed by UV treatment at 920 J/L and pasteurization followed by UV treatment at 138 J/L respectively. At the end of three weeks, the microbial load increased for all controls and test variables except for UV treatment at 920 J/L followed by pasteurization where the microbial population was still below 10 cfu/ml. After 3 d, differences were found in cardboard, fruity, and light oxidized flavors. Results indicate that when raw whole milk is treated with UV light at 920 J/L followed by pasteurization, there is a reduction in microbial load and the low microbial load is maintained throughout 21 d.

Key words: fluid milk, UV light

499 Tina wooden vat biofilms used in Sicilian PDO Ragusano cheese provide a new cluster of *Streptococcus thermophilus* strains. V. Florence[1,2], C. Delorme[3], C. Pediliggieri[4], M.-N. Madec[1,2], V. Chuat[1,2], S. Parayre[1,2], S. Carpino[4], P. Campo[4], P. Renault[3], S. Lortal*[1,2], and G. Licitra[4], *[1]INRA, UMR1253, STLO, Rennes, France, [2]Agrocampus Ouest, UMR1253, STLO, Rennes, France, [3]INRA, Micalis, Jouy en Josas, [4]CoRFiLaC, Ragusa, Sicily, Italy.*

Ragusano cheese (PDO) is a brine-salted pasta filata cheese from Sicily. Raw milk is directly placed in the traditional wooden vat (tina) for cheese making. The biofilm present on this tina was shown to be a safe and very efficient lactic acid bacteria delivering system. Depending on the farm, the biofilm exhibited 2 to 10 co-dominant species, thus representing a valuable source of biodiversity. In this work, *S. thermophilus* was shown, by RT-PCR-TTGE, to be the more active species in 3 different tinas over a 2 years period. To explore the biodiversity within *S. thermophilus*, 25 clones per tina and per year were isolated from M17 plates and analyzed by Pulsed field gel electrophoresis (PFGE) and multi locus sequence typing (MLST). Many different PFGE profiles were obtained highlighting the strain biodiversity within each tina. Some of the profiles were highly related suggesting clones from the same initial strain derived by minor variations; however, each tina contained at least 4 co-dominant strains of *S. thermophilus*. From the cheese at d 1, 22 clones were also isolated and analyzed by PFGE. Most of the profiles were similar to the one of the biofilm, confirming the crucial role of the tina in inoculating *S. thermophilus* for the acidification step. A MLST analysis was applied on 50 strains of *S. thermophilus* isolated from the biofilm using the 6 following genes glck, ddlA, tkt, proA, ptsi, serB. Seven completely new sequence types were found. When compared with 170 other strains of *S. thermophilus* coming from all over the world analyzed by MLST in the same conditions, strains isolated from Tina whatever the year and the farms formed a completely separated group, a new cluster. Special phenotypic traits of these new strains of *S. thermophilus* are under investigation.

Key words: cheese, *Streptococcus thermophilus* biodiversity, biofilm

500 Molecular identification and characterization of *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* by FTIR and its utilization for Cheddar cheese production. H. U. Rehman*[1], M. Nasir[1], S. U. Rehman[2], M. A. Jabbar[1], and M. A. Ali[1], *[1]University of Veterinary & Animal Sciences, Lahore, Punjab, Pakistan, [2]University of Agriculture Faisalabad, Faisalabad, Punjab, Pakistan.*

Cheese starter cultures are expensive and precarious in subcontinent countries like Pakistan. Hence, it is imperative to isolate and characterize indigenous cultures for sustainable and low priced cheese production in the country. The present study was designed to isolate, identify and characterize the *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*, from milk samples. The cultures were isolated through agar media, from milk samples collected from different locations of Faisalabad, Pakistan and then identified on morphological basis by Gram staining, sugar fermentation and catalase tests. Phenotypic characterization of commercial and local made cultures was done by FT-IR spectroscopy; a rapid and novel phenotypic finger printing method, that creates holistic biochemical profile of microorganisms by developing spectra in a specific range. The preservation of isolated bacterial cultures was done at 4°C, which decreased significantly ($P \leq 0.05$) with time but the viability was non-significant compared with commercial culture. Isolated and commercial culture were used at 1% to produce Cheddar cheese for physico-chemical and sensory evaluation. The texture of test cheeses produced with local culture was less elastic compared with control cheese sample produced with commercial culture. The color, flavor and overall acceptability scores of cheese samples with different cultures were not significantly different ($P \geq 0.05$). It is concluded that cheese prepared from indigenous isolated culture was comparable with that of commercial culture cheese and therefore, further studies should be carried out for commercial production of various cheese cultures.

Key words: Cheddar cheese, starter culture, FTIR

501 Transcriptional and physiological responses of *Bifidobacterium animalis* ssp. *lactis* strains to hydrogen peroxide stress. T. S. Oberg*[1], R. E. Ward[1], J. L. Steele[2], and J. R. Broadbent[1], *[1]Utah State University, Logan, [2]University of Wisconsin, Madison.*

Consumer interest in probiotic foods containing bifidobacteria is increasing, but industry efforts to secure high cell viability in foods is undermined by the sensitivity of these anaerobes to oxidative stress during food production or storage. To address this limitation, we investigated transcriptional responses of 2 fully sequenced *Bifidobacterium animalis* ssp. *lactis* strains, BL04 and DSM10140, to hydrogen peroxide (H_2O_2) exposure. Although the genome sequences for these strains are virtually identical, they display different levels of intrinsic and inducible H_2O_2 resistance. For transcriptomics, late log phase cells were exposed to a sub-lethal H_2O_2 concentration for 5 or 60 min, then mRNA was isolated, converted to cDNA, and hybridized to an Affymetrix microarray. Data analysis by the limma/eBayes method found significant ($P < 0.05$) changes in 158 genes in BL04 after 5 min, and 30 differentially expressed genes after 60 min. Surprisingly, no significant changes in gene expression were detected in DSM10140 at either time. Examination of genomic data for each strain suggested differences in H_2O_2 stress resistance might be related to membrane lipid composition, due to genetic mutations in genes for long chain fatty acid-coA ligase. To address this hypothesis, membrane fatty acids were isolated and analyzed by GC-MS. Results confirmed the 2 strains had significantly different lipid profiles. In particular, the BL04 membrane contained higher percentages of C14:0 and C16:0, and lower percentages of C16:1n7 and C18:1n9. These differences could affect membrane fluidity and, potentially, transduction of stress signals, either of which could explain the observed contrasts in H_2O_2 stress resistance.

Key words: *Bifidobacterium*, stress response, hydrogen peroxide

502 Fresh cheese containing higher inoculation of *L. acidophilus* and its effect on the functionality and metabolism of probiotic culture. A. Cruz, J. Faria*, W. Castro, R. Cadena, and H. Bolini, *University of Campinas (UNICAMP).*

This work aimed to evaluate the effect of supplementation with increasing *L. acidophilus* counts on the physicochemical parameters and functionality of fresh Minas cheese. Probiotic fresh cheese (Minas cheese) was supplemented with *L. acidophilus* La-14 (0, 0.4 or 0.8 g/L milk) being submitted to physico-chemical analysis (pH, proteolysis and organic acids levels) and microbiological analysis (probiotic and starter viable count) for 21 d refrigerated storage. In addition, conventional and probiotic commercial cheese supplemented with *Bifidobacterium animalis* Bi-07 were submitted to the same analysis. It was observed an effect of the storage time on the physical-chemical parameters and the microbiological counts ($P < 0.05$). Probiotic cheeses inoculated with increasing concentrations of *L. acidophilus* and the

commercial probiotic cheese presented lower pH values, greater proteolysis level and organic acids production along the refrigerated storage. *L. acidophilus* ranged from 9.42 to 9.11 log cfu/g, maintaining the functionality of the product throughout the shelf life. *B. animalis* counts ranged from 8.36 and 8.91 log cfu/g, while *Lactococcus lactis* count ranged from 8.93 to 7.49 log cfu/g. Additional consumer test should be performed to evaluate the changes due the supplementation of higher *L acidophilus* counts during the fresh cheese processing.

Key words: probiotic cheese, *L. acidophilus*, functionality

503 Microbiological and physico-chemical properties of probiotic whey beverages processed with different whey concentrations. W. Castro, A. Cruz, J. Faria*, M. Bisinotto, and R. Celeghini, *University of Campinas (UNICAMP).*

The use of whey in probiotic beverage formulations can be an option to address environmental challenges faced by the dairy industry. This work aimed to evaluate the microbiological and physico-chemical properties of probiotic whey beverages processed with increased whey concentrations. Whey probiotic beverages were manufactured with 0, 20, 35, 50, 65 and 80% (w/w) liquid sweet whey (pH = 6.48, Minas fresh cheese whey) concentrations and were subjected to physico-chemical analysis (pH, proteolysis, color values) and microbiological count (*Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactobacillus acidophilus*). The whey concentration had an effect on all parameters analyzed. The pH values ranged from 4.07 to 4.14 ($P > 0.05$), while proteolysis was inversely proportional to the whey concentration. The color values (Hunter Lab Color, Minolta) were inversely proportional to the whey concentration, without difference between samples except for 65 and 80% whey ($P > 0.05$). *S. thermophilus* counts ranged from 8.37 to 9.05 log cfu/mL ($P > 0.05$), while *L. bulgaricus* count ranged from 8.01 to 8.31 log cfu/mL ($P < 0.05$). Regardless of the whey concentration, all products presented high values for *Lactobacillus acidophilus* count (minimum value of 8.1 log cfu/mL; $P > 0.05$), suggesting that the whey beverage was an adequate food matrix to be supplemented with probiotic bacteria. The findings indicate that increased whey concentrations had and effect on the physico-chemical parameters of whey probiotic beverages. Additional consumer testing is needed to evaluate their acceptance.

Key words: whey probiotic beverage, functionality, stability