Dairy Foods: Microbiology

T70 Fluid milk quality survey. C. Boeneke*, J. Vargas, and K. Aryana, *Louisiana State University Agricultural Center, Baton Rouge.*

Whole and 2% milks were received from 17 dairy processing plants located in the west, midwest, and southern regions of the United States in duplicates. All milks were shipped overnight in Styrofoam coolers filled with ice to maintain the temperature of the samples. The samples were pasteurized at the processing plants by high-temperature short-time pasteurization. The first set of milk samples was evaluated for standard plate count, coliforms, and psychrotrophic counts using a standard method as well as a rapid method, heat-resistant spore-forming psychrotrophs, aerobic spores, HR testing (HR-1, HR-2 and HR-3), fat percentage, protein percentage, somatic cell count and a sensory evaluation immediately upon arrival. Milks were evaluated for flavor using the Collegiate Dairy Products Evaluation Score Sheet. The duplicate set was evaluated for standard plate count, coliform count and a sensory evaluation at the end of 2 weeks storage time at 7°C. Three replications were conducted. Five percent of the 2% milk samples presented psychrotrophic counts (3 samples in the first replication of the study, 2 samples in the third replication) with a mean values of approximately 2 cfu/ml. For heat resistant spore forming psychrotrophs, 10% of the samples showed the highest counts of approximately 1 cfu/ml, mainly in the second replication of the study. Ninety percent of the samples showed zero counts. Sensory evaluation scores ranged from 1 to 10 out of 10 possible points. The most common flavor criticism found by the panelists was a cooked off-flavor as well as rancid and oxidized criticisms.

Key words: fluid milk, shelf life, flavor

T71 Seasonal variation of psychrotrophic bacteria isolated from raw milk in South Korea. H. A. Lee*, J. H. Myung, Y. H. Park, and Y. K. Shin, *Institute of Dairy Food Research, Seoul Dairy Cooperative, Ansan, Kyunggi, South Korea, 425-838.*

The aim of this study was to examine the distribution and diversity of psychrotrophic bacteria from raw milk sampled from 9 provinces in South Korea by seasons. Raw milk was collected from farms in the central, eastern, western, southern, northern, northern east, southern east Kyunggi, Ansan and Incheon province at 4 different seasons. The samples were diluted and plated on sterile plate count agar and incubated at 7°C for 10 d. Then, 20 colonies per province every season were randomly selected and subcultured at least 3 times at 30°C for 48 h. For bacterial sequencing, 16s rDNA region was amplified using RT-PCR, then the products were sequenced using the 3130 Genetic analyzer. As a result, psychrotrophic counts were higher in winter than in other seasons as 5.43 log cfu/mL ($P < 0.05$). Among 9 provinces, the population in raw milk sampled from Incheon province was in significantly greater numbers and from the western Kyunggi province was in significantly lower numbers than from any other provinces, 5.38 log cfu/mL and 3.56 log cfu/mL, respectively ($P < 0.05$). Among 720 bacterial isolates, the predominant class was Gammaproteobacteria (84.01%) and genus was *Acinetobacter* (36.99%), especially *Acinetobacter johnsonii* (17.24%).

Key words: psychrotrophic bacteria, raw milk, seasonal

T72 Influence of multilayer packaging on pasteurized milk quality. M. da Silva Pinto, A. F. Carvalho*, J. Y. Suda, A. C. P. Silveira, and A. C. dos Santos Pires, *Food Science Department, Federal University of Viçosa, Viçosa, MG, Brazil.*

The type of packaging used for pasteurized milk can substantially affect their quality characteristics by directly controlling the amount of oxygen and light which comes into contact with food or for providing perfect isolation to avoid post-processing contamination by microorganisms. In this study, we tested 3 different high density polyethylene (HDPE) multilayer films and one HDPE monolayer film with the objective to evaluate the effects of the 4 different packaging types on milk quality: (1) multilayer with high barrier to light, high oxygen barrier, high tensile strength; (2) multilayer with high oxygen barrier; (3) multilayer with high barrier packing light, high tensile strength and (4) monolayer. The milk stored in each type of packaging was analyzed as the microbiological (mesophilic and psychrotrophic bacteria), physicochemical (pH, titratable acidity, dry matter content, protein content, fat content, lactose content, density and cryoscopy) and nutritional (fat oxidation and vitamin degradation) characteristics besides sensory acceptance of pasteurized milk stored at 5°C for 21 d. Three replications were analyzed using the general linear models procedure of SAS (SAS Institute Inc., Cary, NC) version 9.1, licensed by University Federal of Viçosa, 2008. The milk quality was in accordance with the Brazilian legislation. The various film properties provided by the different packaging systems demonstrated similar milk characteristics ($P > 0.05$) during the 21 d of storage at 5°C, although there were differences between the barrier packaging. All packaging materials evaluated can provide sufficient protection to the quality of pasteurized milk that can achieve a shelf life of 21 d/ 5°C, considering the conditions of the quality of the raw material and the integrity of the entire cold chain.

Key words: packaging, shelf-life, milk quality

T73 Microbiological quality of UHT dairy products analyzed by rapid, reference, and ATP bioluminescence methods. A. F. Cunha[1], A. D. Lage[1], M. M. P. Araújo[1], C. F. Abreu[2], A. R. Tassinari[2], M. R. Souza[1], C. F. A. M. Penna[1], L. M. Fonseca[1], M. O. Leite[1], and M. M. O. P. Cerqueira*[1], *[1]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]3M do Brazil, Sumaré, São Paulo, Brazil.*

Besides traditional methods used for microbiological analysis of UHT dairy products, rapid methodologies based on ATP bioluminescence have been developed to detect contamination of these products in a shorter time. The use of these new approaches for monitoring the quality of UHT products such as Brazilian flavored milk and milk cream was compared with the traditional microbiological methods. Sixty-six samples of UHT dairy products, from different sources, incubated at 48, 72, and 168 h, were analyzed for the counts of mesophilic and psychrotrophic aerobic microorganisms using plate count agar (PCA) and brain heart infusion (BHI) agar and also PetrifilmAC plates. The data were compared with results of the ATP bioluminescence technique performed by the 3MMicrobial Luminescence System expressed in RLU and submitted to the MacNemar test at 95% of confidence. At all incubation times, the majority of UHT dairy samples showed low counts of mesophilic and psychrotrophic microorganisms and values lower than 150 RLU by ATP bioluminescence. Only one sample showed mesophilic count above the limit established by the Brazilian legislation - 100 cfu/mL - in PCA (2.6×10^2 cfu/mL) and PetrifilmAC (1.1×10^2 cfu/mL) at 168 h. These high counts were also detected by the ATP bioluminescence (416 RLU) and were mathematically equal.

It can be concluded that the ATP bioluminescence results were similar to those obtained by traditional methods. For the dairy industry, the ATP bioluminescence using the MLS system may become an important tool for rapid monitoring of microbiological quality of UHT dairy products, releasing the products in a shorter time than that established by the Brazilian legislation.

Key words: UHT dairy products, ATP bioluminescence, quality

T74 Phylogenic analysis and characterization of bacterial sporeformer isolates obtained from raw milk, pasteurized milk, and dairy farm environments. R. A. Ivy*, M. L. Ranieri, N. H. Martin, H. C. den Bakker, B. M. Xavier, M. Wiedmann, and K. J. Boor, *Cornell University, Ithaca, NY.*

The presence of psychrotolerant bacterial sporeformers represents a major challenge to extending the shelflife of pasteurized dairy products. The objective of this study was to identify prominent phylogenetic groups of psychrotolerant dairy-associated sporeformers from a collection sporeformer isolates from dairy systems (i.e., raw and pasteurized milk and dairy farm environments). Partial *rpoB* sequences of 1425 sporeformer isolates had been obtained previously, and partial 16S rDNA sequencing was used to assign species identifications to unique *rpoB* allelic types (ATs). A maximum likelihood phylogenetic tree was constructed for 288 unique *rpoB* ATs. Major *rpoB* clades consisted of Bacillaceae (i.e., *Bacillus* spp., *Lysinobacillus* spp., and *Viridibacillus* spp.) and *Paenibacillus* spp. Most Bacillaceae isolates grouped into clades with known contaminants of raw milk or with species associated with high altitudes, soil, and organic material. Major *Paenibacillus* clades were identified as *P. ordorifer*, *P. amylolyticus*, and *P. graminis*, and many uncharacterized *Paenibacillus* species were identified. Among the 8 major Bacillaceae clades, only 2 included isolates that grew to >4 log (cfu/ml) in skim milk broth (SMB) at 6°C, whereas 6 out of 8 major *Paenibacillus* clades contained isolates that grew to >4 log(cfu/ml) in SMB at 6°C. Though all *Paenibacillus* clades tested positive for β-galactosidase activity at 32°C and most Bacillaceae clades tested negative, *Bacillus licheniformis* (13% of Bacillaceae isolates) showed variable β-galactosidase activity. β-galactosidase activity alone, therefore, could not reliably distinguish between Bacillaceae and *Paenibacillus*. Therefore, consensus sequences of predominant *Paenibacillus rpoB* ATs indentified in this study will be used to design a DNA-based system to rapidly and specifically detect these psychrotolerant sporeformers in fluid milk and dairy ingredients (e.g., milk powders).

Key words: sporeformers, dairy microbiology, *Paenibacillus*

T75 Spores in dairy products: Characterization and destruction by pulsed light. A. Laubscher* and R. Jimenez-Flores, *California Polytechnic State University, Dairy Products Technology Center, San Luis Obispo.*

The USDA has reported an increase in the consumption of nonfat dry milk and skim milk powders due to the opening of international markets for these products. However, the main limitation to international sales is the spore counts in dairy powders. The objective of this study was to examine the characteristics of the spores in California milk powders, and to evaluate the potential quality improvement using pulsed light covering all wavelengths. We designed our experiments around 4 liquid matrices in which to contain the spores: sterile nanopure water, sterilized whey, non-fat UHT milk, and 2% UHT milk. In addition we tested dry matrices in 6 commercial skim milk and buttermilk powders. The bacteria used to inoculate these medias include ATCC reference strains (*Geobacillus* spp. and *Paenibacillus* spp.) and a collection of aerobic spore-forming bacteria isolated from California milk powders. These strains were selected because they were considered to be highly heat-resistant after exposure to excessive heat treatments. UV treatments with the Xenon pulsed lamp consisted of 4 different levels (1 burst, 2 bursts, 3 s, and 20 s) and included a post 4°C incubation and HTST pasteurization. Protein profiles were obtained for each strain used, and compared before and after treatments. With pure water we observed a seven logarithmic reduction in spores with two bursts of pulsed UV light. However, cloudy solutions were much less permissive of the lethal action of light. These matrices had the same seven logarithmic reductions but only after 3 seconds. Powders were treated with variable success, and much detriment to the flavor and chemical structure. However, while the same reduction of spore viability was achieved, the powder buttermilk was significantly more resistant to off flavor development.

Key words: spores, pulsed light, milk quality

T76 The effect of different sweeteners on growth and survival of *Lactobacillus rhamnosus* GR-1 in milk. S. Hekmat*[1,2] and G. Reid[2], *[1]Brescia University College, London, Ontario, Canada, [2]Canadian Research and Development Center for Probiotics, London, Ontario, Canada.*

There has been an increasing demand by consumers for low-calorie and low-sugar products with functional properties. *Lactobacillus rhamnosus* GR-1 is a probiotic agent with therapeutic properties. The objective of this study was to monitor growth and survival of *L. rhamnosus* GR-1 in milk sweetened with various sweetening agents during storage period. Six formulations of milk (1% fat) with 7% xylitol (X), 0.04% stevia (ST), 5% sucrose (S), 0.04% stevia-inulin-chromium mixture (SIC), 1.25% sucralose (SU) and one with no sweetening agents (C) were prepared. The mixtures were autoclaved for 15 min, cooled to 37°C and inoculated with 1% of starter culture. The samples were then incubated anaerobically at 37°C overnight. Selective MRS agar containing 0.015 g/L fusidic acid was used to enumerate *L. rhamnosus* GR-1 after 1, 14, and 28 d of storage at 4°C. All sweetening agents supported the growth and survival of *L. rhamnosus* GR-1; however, it showed a higher survival rate ($P < 0.05$) in the ST and SIC treatments. After 1 d of storage, the total colony counts of treatments ST and SIC for *L. rhamnosus* GR-1 were 7.4×10^9 and 1.1×10^9 cfu/mL, respectively. The total colony counts for all treatments decreased slightly after 28 d of storage. The results indicate that *L. rhamnosus* GR-1 can remain viable in presence of various sweetening agents during the storage period and there is potential for incorporating these sweetening agents in other probiotic dairy products.

Key words: probiotics, sweeteners, milk

T77 Detection and transfer of the glutamate decarboxylase gene in *Streptococcus thermophilus*. G. Somkuti*, J. Renye, and D. Steinberg, *Eastern Regional Research Center/USDA, Wyndmoor, PA.*

γ-Aminobutyric acid (GABA) is generated from glutamate by the action of glutamic acid decarboxylase (GAD) and characterized by hypotensive, diuretic and tranquilizing effects in humans and animals. The production of GABA by lactic acid starter bacteria would enhance the functionality of fermented dairy foods including cheeses and yogurt. The survey of 42 strains of the yogurt starter culture *Streptococcus thermophilus* by PCR techniques indicated the presence of

a glutamate decarboxylase gene (*gad*) in 15 strains. DNA sequencing data indicated that in the genome of GAD+ *S. thermophilus* strains the *gad* as a rule is flanked by a transposase gene (5′) and a HD-superfamily hydrolase gene (3′). Specific primers were designed to amplify a 1.75-kb genomic fragment in *S. thermophilus* to include *gad* and its putative promoter region. The resulting PCR product was inserted into the 5.48-kb pMEU5a shuttle vector which was used to transform *Escherichia coli* DH5α. Subsequently, the recombinant plasmid pMEU5a-1/*gad* (7.24 kb) was transferred by electroporation into the GAD-negative strain *S. thermophilus* ST128. The ST128 transformants carrying the plasmid-encoded *gad* produced fully functional GAD enzyme as evidenced by the conversion of glutamate to GABA at a rate similar to strains with *gad* located on the chromosome. The results demonstrated the potential to equip non-GABA producing strains *S. thermophilus* and possibly other lactic acid bacteria with the capacity to produce GAD to improve culture performance in the development of functional foods.

Key words: γ-aminobutyric acid, *Streptococcus thermophilus*

T78 Development of a real-time PCR assay for rapid detection of spoilage *Paenibacillus* spp. in fluid milk. M. L. Ranieri*, W. R. Mitchell, R. A. Ivy, N. Martin, M. Wiedmann, and K. J. Boor, *Cornell University, Ithaca, NY.*

Psychrotolerant sporeforming bacteria, specifically *Paenibacillus* spp., represent the current biological limit to the extension of fluid milk shelf-life. *Paenibacillus* have been found in farm environments, raw milk, processing plant environments, and in pasteurized fluid milk across the United States. While typically present at low levels in raw milk, *Paenibacillus* spores can survive conventional pasteurization temperatures, germinate, and grow to numbers capable of negatively impacting the sensory characteristics of refrigerated milk products. A real-time PCR assay was designed to detect *Paenibacillus* spp. in fluid milk and discriminate between *Paenibacillus* and other closely related sporeforming bacteria. Partial 16S rDNA sequences, representing a total of over 1400 *Paenibacillus* and *Bacillus* spp. isolated from dairy farms, milk processing plants, and fluid milk products, were compared with identify appropriate primer and probe regions specific to *Paenibacillus* spp. To confirm specificity, genomic DNA from 16 *Paenibacillus* and 17 *Bacillus* isolates were tested with the assay; these isolates represented the 9 most frequently isolated *Paenibacillus* and *Bacillus* allelic types, plus additional allelic types to include genetic diversity. All 16 *Paenibacillus* isolates were detected with a mean cycle threshold (Ct) of 19.14 (±0.54). While 14/17 *Bacillus* isolates showed no signal (Ct > 40), 3 *Bacillus* isolates showed very weak positive signal (Ct = 38.66 [±0.80]). When total genomic DNA was also extracted from milk samples inoculated with *Paenibacillus*, we found a detection limit of 1,000 *Paenibacillus* cfu/mL. We also showed that this assay could be used to screen colonies collected from standard plate count agar, using crude colony lysates. Despite the additional incubation time necessary to develop colonies on plate media, this method eliminates the time and cost associated with genomic DNA preparations. Overall, this assay represents a specific and rapid tool to identify *Paenibacillus* spp. and therefore provides a method for determination of sources or milk containing spoilage bacteria.

Key words: fluid milk, spoilage, *Paenibacillus*

T79 Genetic analysis of a novel plasmid encoded durancin locus in *Enterococcus durans* 41D. L. Du[1], G. Somkuti*[2], and J. Renye[2], [1]*Nanjing University of Finance and Economics, Nanjing, China,* [2]*Eastern Regional Research Center/USDA, Wyndmoor, PA.*

Enterococcus durans is commonly found in the intestinal tract in humans and animals and several strains are known to produce bacteriocins. Durancin GL, a novel bacteriocin of *Enterococcus durans* 41D with antilisterial activity was isolated from artisanal cheese samples and its genetic determinants were characterized. The bacteriocin operon included the structural (durA) and immunity (durB) genes among the 9 putative ORFs identified on pDGL1, an 8.3-kb plasmid which was fully sequenced. The deduced DurA protein was a 71-amino acid peptide with 74% identity to the class II bacteriocin BacA of *E. faecalis*. DurA had a potential signal peptidase cleavage site indicating the involvement of a sec-dependent transport system in yielding a 7.97 kDa mature peptide. The mature DurA peptide had the typical structure of subclass IIa bacteriocins, including a conserved YYGNG motif and a hydrophobic C-terminal region. The immunity gene (durB) encoded a 94-amino acid peptide with 85% identity to the BacB immunity gene. The minimum requirement for durancin GL production was defined by translationally fusing a 547 bp fragment containing the durAB genes with the *Streptococcus thermophilus* chromosomal promoter P2201 by PCR, and subcloning in vector pMEU5a. Isolates of the yogurt starter culture *S. thermophilus* electrotransformed with the recombinant plasmid pMEU5a-1 secreted durancin GL into the medium and inhibited the growth of *Listeria*. The results showed the possibility of transferring the durancin GL gene into food-grade lactic acid bacteria to serve as the source of a natural bioprotective agent for *Listeria* control.

Key words: durancin, *Listeria*, *Streptococcus thermophilus*

T80 Development of a qPCR method for monitoring strain dynamics during yogurt manufacture. D. Miller*, E. G. Dudley, and R. F. Roberts, *The Pennsylvania State University, University Park.*

Starter cultures used in the manufacture of yogurt consist of multiple strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST). Existing tools for monitoring ST and LB levels during yogurt manufacture rely on conventional plating methods, which require 48–72 h of incubation and do not allow for quantification of individual strains. The objectives of the present work were to i) design and evaluate primers for quantification of individual strains in a commercial starter culture by quantitative polymerase chain reaction (qPCR), ii) to identify an external control for DNA isolation and qPCR, iii) to prepare standard curves with individual starter culture strains and combinations of starter culture strains spiked in bulk starter base, and iv) to evaluate strain balance in bulk starters prepared at 3 different temperatures. Strain-specific primers were designed for 2 ST strains (ST DGCC7796 and ST DGCC7710), one LB strain (DGCC 4078) and one *Lactobacillus delbrueckii* ssp. *lactis* strain (DGCC4550). Primers for the individual ST strains were designed to target unique DNA sequences in clustered regularly interspersed palindromic repeats (CRISPR, GenBank Accession no. EF434468 and EF434469). LB primers targeted a CRISPR locus based on sesquence information provided by the culture supplier. Primers for LL were designed to target mannitol-specific IIbC component of the PTS system (GenBank Accession no. AF496224.1). *Lactobacillus farciminis* ATCC 29644^T (LF) was selected as an external control for the DNA extraction procedure and qPCR because it has a similar peptidoglycan structure as the LB and LL strains. Following evaluation of primer specificity, standard curves relating cell number to cycle threshold (Ct) were prepared for each strain individually and for each strain in 3-strain combinations when spiked bulk starter base, and no significant

differences in the slopes were observed ($\alpha = 0.05$). Strain balance data was collected for bulk starter cultures prepared at 37, 41, and 43°C to demonstrate the potential application of this method for evaluation of the influence of fermentation conditions on strain balance.

Key words: yogurt, qPCR, LB and ST

T81 Binding and efficacy of a natural biopreservative (nisin) in different food matrices. R. Niewohner*, S. Anand, and R. Nauth, *South Dakota State University, Brookings.*

Nisin is a natural biopreservative produced by lactic acid bacteria. We explored its ability to bind in different food matrices and to protect them from bacterial spoilage in ranch sauce, vegetable soup, and hot dogs with a well plate assay. Skim milk was used as a control for these studies. Challenge studies were also conducted by spiking specific spoilage bacteria such as *Lactobacillus plantarum* in ranch sauce and hot dogs, and *Bacillus cereusi* in vegetable soup. The hypothesis of this experiment was that the more recovery of nisin over time of the sample matrix, the fewer bacteria will be able to grow, since the "free nisin" is not bound and available to act against the bacteria. *Lactococcus lactis ssp. cremoris* was used as a test culture in the entire study. All experiments were replicated thrice. In the first part of the experiment, a nisin standard was established using 10, 100, 1,000, and 10,000 IU Recovery studies were conducted at 0 and 24 h intervals in each of the food matrices, including milk, by spiking 1000 IU of nisin. The Dunnett multiple comparisons showed that skim milk had the highest recovery. Ranch sauce showed a slight decrease with about 900 IU recovered, however, in vegetable soup only 20 IU could be recovered. Hot dogs showed a slight decrease, but it was not significantly different from the milk. After 24 h, the ranch sauce and the hot dog proved to not be significantly different than the milk. However, the vegetable soup showed a decreased recovery over time with a final estimate of 5 IU of nisin. In the ranch sauce challenge study, with nisin addition at 1,000 IU, a decrease in *L. plantarum* counts were observed up to 24 d of the storage studies from an initial count of 10^9 cfu/g of the sample. The hot dogs with 1,000 IU of nisin showed a decrease in the number of *L. plantarum* up to 10 d of the storage studies. On the other hand, *Bacillus cereus* (10^4 cfu/g) in vegetable soup challenge test did not show any reduction by nisin at 1,000 IU. The study thus demonstrated variations in the binding of nisin to different food matrices with variable protective effect.

Key words: recovery of nisin, preservative, ranch sauce, hot dog, and vegetable soup

T82 Resistance of membrane biofilms to cleaning and sanitation treatments. D. Singh* and S. K. Anand, *Midwest Dairy Foods Research Center, Dairy Science Department, South Dakota State University, Brookings.*

Previous studies conducted in our lab have established the formation of bacterial biofilms on reverse osmosis (RO) whey concentration membranes. In the present study we evaluated the effectiveness of different chemicals of a cleaning in place (CIP) protocol against the constitutive microflora of biofilms formed on whey RO membranes. Different bacterial isolates that were a part of biofilm consortia of 2 to 12 mo old membranes included *Bacillus* sp., *Escherichia coli*, *Klebsiella oxytoca, Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Aeromonas* sp., *Corynebacterium* sp., and *Pseudomonas* sp. The CIP protocol tested against the planktonic and 12-h-old biofilms of above microflora included 5 treatment steps based on; alkali, surfactant, acid, enzyme, a second surfactant, and a weekly sanitizer application. The results confirmed the resistance of isolates in both planktonic and embedded states against most of the 5 treatment steps. The only effective step was acid based treatment, which resulted in 5–8 logs reduction in case of planktonic cells, and 2–5 logs reduction against embedded cells in 12 h old biofilms formed under lab conditions. The sanitizer treatment step also showed similar results and caused a reduction of 6–8 logs against planktonic cells. On the other hand, it was much less effective against the embedded cells in biofilms, and resulted in a reduction of only 1 to 3 log counts. *Bacillus* sp. showed highest resistance in planktonic cell, as well as, embedded cell state. Differences were noticed in the resistance pattern of biofilm isolates as the membranes aged. In general, isolates from the older membranes showed higher resistance to CIP chemicals and sanitizer treatment. Data were statistically analyzed using the SAS program. On the basis of these results, it can be concluded that the biofilms formed on the RO whey concentration membranes were resistant to the CIP protocol being used.

Key words: planktonic, biofilm, embedded

T83 Effect of low sonication intensities on the growth of *Streptococcus salivarius* ssp. *thermophilus* ST-M5 subjected to different temperatures. M. Moncada* and K. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

Whether low sonication conditions stimulate the growth of bacterial cultures is not clearly understood. The objective was to study the influence of low sonication intensities on the growth characteristics of *Streptococcus salivarius* ssp. *thermophilus* at different temperatures. Freshly thawed culture was suspended in 0.1% peptone water and 18 mL of sample was sonicated using horn (diameter 13 mm) set at a maximum acoustic power output of 750 W, frequency 24 kHz. The treatments were 4 sonication intensities of 8.07, 14.68, 19.83 and 23.55 Watts/cm^2 randomized over 3 different temperatures (4, 22 and 40°C) of inoculated peptone water before sonication. The energy input (1500 J) was kept constant in all treatments. Control samples did not receive any sonication treatment. Growth of treatment and control samples was determined hourly during 12 h of incubation at 37°C. Data were analyzed using proc mixed model of statistical analysis system (SAS). At all 3 temperatures growth of *Streptococcus thermophilus* ST-M5 subjected to low sonication intensities were significantly ($P < 0.05$) better than control. At 4 and 40°C all low sonication intensities showed better growth than control. At 22°C 8.07, 14.68 and 19.83 Watts/cm^2 showed higher ($P < 0.05$) viable counts than control and 23.55 Watts/cm^2. Some low sonication intensities improved growth of *Streptococcus salivarius* ssp. *thermophilus* ST-M5.

Key words: mild sonication, probiotic

T84 Low sonication intensity influences on the protease activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 at different temperatures. M. Moncada* and K. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

Low sonication intensity is an acoustic energy which involves the conversion of an electrical signal into a physical vibration. It uses the sound for modifying the permeability of the cell plasma membrane. *Lactobacillus delbrueckii* ssp. *bulgaricus* is a widely used bacterium used for the production of some fermented dairy products. The objective was to study the influence of low sonication intensities on protease activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 at different

temperatures. Freshly thawed culture was suspended in 0.1% peptone water and 18 mL of sample was sonicated using horn (diameter 13 mm) set at a maximum acoustic power output of 750 W, frequency 24 kHz. The treatments were 4 sonication intensities of 8.07, 14.68, 19.83 and 23.55 Watts/cm^2 randomized at 3 different temperatures (4, 22 and 40°C) of inoculated peptone water before sonication. The energy input (1500 Joules) was kept constant in all treatments. Control samples did not receive any sonication treatment. Protease activity was determined at 0, 12 and 24 h of incubation spectrophotometrically at 340 nm. Differences of least squares means were used to determine significant differences at $P < 0.05$ for main effect (low sonication intensity) and interaction effect (low sonication intensity * time * temperature). At 4°C the sonication intensities of 14.68, 23.55 and 19.83 W/cm^2 significantly ($P < 0.05$) increased protease activity compared with the control. At 22°C the sonication intensities of 23.55 and 19.83 W/cm^2 had protease activities significantly ($P < 0.05$) higher than the control. The optical density (OD) values for control bacterium and culture sonicated at 19.83 and 23.55 W/cm^2 at 12 h were 0.38, 0.46 and 0.59 absorbance units respectively. The OD at 24 h for control bacterium and bacterium sonicated at 19.83 and 23.55 W/cm^2 were 0.68, 0.92 and 0.96 absorbance units. At 40°C all treatments exhibited significantly ($P < 0.05$) higher protease activity than control. Protease activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 was improved by some low sonication intensities.

Key words: mild sonication, probiotic

T85 Influence of low sonication intensities at different temperatures on the bile tolerance of *Streptococcus salivarius* spp. *thermophilus* ST-M5. M. Moncada* and K. Aryana, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

Low sonication intensity condition is a non-destructive technique that uses sound waves to cause cavitation in aqueous solutions and may improve the permeability of membrane, speed up the transfer of substrates and promote cellular growth and propagation. Bile tolerance is an important probiotic characteristic. *Streptococcus salivarius* spp. *thermophilus* is widely used in the fermentation of dairy products. The objective was to determine the effect of various low sonication intensities at different temperatures on bile tolerance of *Streptococcus salivarius* spp. *thermophilus*. Freshly thawed culture was suspended in 0.1% peptone water and 18 mL of sample was sonicated using horn (diameter 13 mm) set at a maximum acoustic power output of 750 W, frequency 24 kHz. The treatments were 4 sonication intensities of 8.07, 14.68, 19.83 and 23.55 W/cm^2 randomized over 3 different temperatures (4, 22 and 40°C) of inoculated peptone water before sonication. The energy input (1500 J) was kept constant in all treatments. Control samples did not receive any sonication treatment. Bile tolerance of samples were determined hourly for 12 h of incubation. Data were analyzed using the using proc mixed model of statistical analysis system (SAS). At 22°C culture sonicated at 19.83 W/cm^2 and at 4°C culture sonicated at 8.07 and 14.68 W/cm^2 showed similar bile tolerance compared with control. At 40°C bacterium subjected to intensity of 14.68 W/cm^2 showed significantly ($P < 0.05$) higher bile tolerance at hours 6 to 12 compared with control.

Key words: mild sonication, probiotic

T86 Screening of mild pulsed electric field parameters for enhancing acid tolerance of *Streptococcus salivarius* spp. *thermophilus* ST-M5. N. Najim and K. Aryana*, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

Aim of the present study was to screen mild PEF conditions for increasing acid tolerance. Freshly thawed frozen concentrated culture of *Streptococcus salivarius* spp. *thermophilus* ST-M5 was suspended in 0.1% (wt/vol) sterile peptone water and treated in pilot plant PEF system. A range of mild PEF treatments included positive and negative square unipolar pulse widths of 2, 3, and 4 µs, pulse periods of 0.3 and 0.6 s, voltages of 1 and 3 kV/cm at 2 temperatures, 21.6°C and 40.5°C. The control was run through the PEF system without receiving any pulsed electric field condition. The flow rates of both the control and PEF treated samples were kept constant at 60 mL/min. Samples were individually inoculated in acidified M17 broth at pH 2.0. Samples were plated in duplicates and incubated aerobically at 37°C for 48 h. The acid tolerance was determined at 0, 30, 60, 90 and 120 min of incubation. Three replications were conducted for each experimental condition. The acid tolerances using the 3 positive square unipolar pulses of 2, 3 and 4 µs for both pulse periods of 0.3 and 0.6 s and voltage of 1 kV/cm at both 21.6°C and 40.5°C PEF treatment temperatures were significantly the highest compared with both the control and negative square unipolar pulses at the same PEF conditions. The acid tolerance with positive square unipolar pulse width of 3 µs for both pulse periods of 0.3 and 0.6 s using voltage of 1kV/cm at 40.5°C PEF treatment temperature was significantly higher than both pulses of 2 and 4 µs. Furthermore, the same PEF conditions at 40.5°C PEF temperature showed significantly higher acid tolerance than pulses of 2, 3 and 4 µs at 21.6°C. The acid tolerances using positive square unipolar pulse widths of 2, 3 and 4 µs for both pulse periods of 0.3 and 0.6 s with voltage of 1 kV/cm were significantly higher than both the control and those subjected to voltage of 3 kV/cm at the same PEF conditions. Positive square unipolar pulses significantly improved acid tolerance compared with the negative square unipolar and square bipolar pulses.

Key words: mild pulsed electric fields, probiotic

T87 Mild pulsed electric field conditions identified for improving growth, protease activity and acid tolerance of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K. N. Najim and K. Aryana*, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

The objective was to determine the effect of mild pulsed electric field (PEF) conditions on acid tolerance, growth and protease activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K. Freshly thawed frozen concentrated cultures were individually inoculated in 0.1% (wt/vol) sterile peptone water and treated in pilot plant PEF system. The treatment was positive square unipolar pulse width of 3 µs, pulse period of 0.5 s, voltage of 1 kV/cm, delay time of 20 µs and flow rate of 60 mL/min at 40.5°C PEF treatment temperature. The control was passed through the PEF system at the same flow rate (60 mL/min) without receiving any pulsed electric field condition. The acid tolerance was determined every 30 min for 120 min of incubation in acidified MRS broth at pH 2. Growth of the cultures was determined hourly for 32 h of incubation of *Lactobacillus acidophilus* LA-K and for hourly for 25 h of incubation of *Lactobacillus delbrueckii* ssp. *bulgaricus*LB-12. Protease activity was determined at 0, 12, 24, 36 and 48 h of incubation. Three replications were conducted. The viability of the control *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 was lost after 30 min of incubation in acidified MRS broth (pH 2), whereas, the bacterium subjected to mild PEF treatment was acid tolerant until the end of 120 min of incubation.

Mild PEF treated cultures of both *Lactobacillus acidophilus* LA-K and *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 reached the logarithmic growth phase an hour earlier than the control. Mild PEF treatment significantly ($P < 0.0001$) enhanced the protease activity of both *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K compared with the control. Mild PEF conditions studied significantly improved acid tolerance, growth and protease activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 and *Lactobacillus acidophilus* LA-K.

Key words: mild pulsed electric fields, probiotic

T88 Impact of mild pulsed electric field conditions on improving bile tolerance, protease activity and growth of *Streptococcus salivarius* ssp. *thermophilus* ST-M5. N. Najim and K. Aryana*, *School of Animal Sciences, Louisiana State University Agricultural Center, Baton Rouge.*

Certain mild pulsed electric field (PEF) conditions show promise in enhancing probiotic characteristics and need to be further explored. In an earlier study on screening mild PEF conditions for enhanced acid tolerance, a condition was identified that enhanced acid tolerance. Aim of the present study was to evaluate the identified mild PEF conditions' influence on the bile tolerance, growth and protease activity of this probiotic bacterium. Freshly thawed frozen concentrated culture of *Streptococcus salivarius* ssp. *thermophilus* ST-M5 was suspended in 0.1% (wt/v) sterile peptone water and treated in pilot plant PEF system. Treatment was positive square unipolar pulse width of 3 µs, pulse period of 0.5 s, voltage of 1 kV/cm, delay time of 20 µs and flow rate of 60 mL/min at 40.5°C PEF treatment temperature. Control was passed through the PEF system at the same flow rate (60 mL/min) without receiving any pulsed electric field condition. Growth and bile tolerance of the control and PEF treated samples were determined hourly throughout 20 and 16 h of incubation respectively. Protease activity was determined at 0, 12, 24, 36 and 48 h of incubation at 37°C. Three replications were conducted. The mild PEF conditions had non significant influence on the bile tolerance of *Streptococcus salivarius* ssp. *thermophilus* ST-M5 throughout the entire incubation time period of 16 h. The control and mild PEF treated samples had the same counts of 10.97 (+/− 0.25) log cfu/ml at 0 h of growth. Mild PEF treated samples reached the logarithmic growth phase an hour earlier than the control. Mild PEF treated samples had significantly higher counts compared with the control for all the time points over the logarithmic phase but had nonsignificant ($P > 0.05$) difference for all the time points over both the stationary and the decline phases of the growth curve. *Streptococcus salivarius* ssp. *thermophilus* ST-M5 subjected to mild PEF throughout the incubation time points of 12, 24 and 36 h had significantly enhanced proteolytic activity compared with the control.

Key words: milk pulsed electric fields, probiotic

T89 Resistance of *E. coli* and *L. rhamnosus* to acid stress is affected by the presence of pepsin-treated caseinomacropeptide. G. Robitaille, C. Lapointe, D. Leclerc, and M. Britten*, *Food Research and Development Centre, Agriculture and Agri-Food Canada, St Hyacinthe, Quebec, Canada.*

Caseinomacropeptide (CMP) is a 7 kDa phosphoglycopolypeptide released from κ-casein during milk digestion and during chymosin-induced renneting of caseins. The objective of the study was to analyze the effect of pepsin-treated CMP from cow and goat milk on the resistance of *E. coli* and *L. rhamnosus* during acid stress. Bacterial cells in exponential growth phase were suspended in acidified phosphate buffered saline with or without pepsin-treated CMP. Viability was determined during a 90 min incubation period. Pepsin-treated CMP exhibited bactericidal activity at pH 3.5 when added in a dose dependent manner to *E. coli*, reducing survival by more than 90% within 15 min at 0.25 mg/mL. At pH >4.5 the bactericidal activity was lost indicating that pepsin-treated CMP was efficient at low pH only. The effectiveness of pepsin-treated CMP at pH 3.5 was not affected by the presence of glycoconjugates linked to CMP or by the bovine or caprine origin of milk. In contrast, *L. rhamnosus*, a probiotic, was more resistant to acid stress when pepsin-treated bovine or caprine CMP was added to the media. The viability reached 50% after 60 min incubation at pH 3 compared with 5% survival without additive. Neither the glycosylation extent nor the sequence variations between CMP from bovine and caprine milk affected the protective activity of hydrolysed CMP toward *L. rhamnosus*. This suggests that encrypted bioactive peptides released by the pepsin treatment of CMP was antibacterial toward *E. coli*, and improved acid stress resistance of *L. rhamnosus* in an acidic media that mimicked gastric environment.

Key words: caseinomacropeptide, antibacterial, acid stress

T90 Effect of microencapsulation on survival of *Lactobacillus acidophilus* La5 during simulated gastrointestinal conditions of stirred yoghurt after refrigerated storage. M. C. E. Ribeiro, K. S. Chaves, C. G. M. S.C. Tenório, F. N. Souza, C. R. F. Grosso, and M. L. Gigante*, *State University of Campinas, Campinas, SP/Brazil.*

Microencapsulation is an effective method for maintaining high viability of probiotic bacteria, as it protects probiotics both during food processing and storage as well as in gastric conditions. The aim of this study was to evaluate the viability of *Lactobacillus acidophilus* La5 in both free and microencapsulated forms in stirred yogurt during simulated gastrointestinal transit. Probiotic bacteria were microencapsulated using pectin and whey protein, associating ionotropic gelation and complex coacervation. Three batches of stirred yogurt were made with *L. acidophilus* incorporated into the product in different states: free (1% v/v), moist microcapsules (10% w/v) and rehydrated freeze-dried microcapsules (13% w/v). After 35 d of storage the survival of *L. acidophilus* during the passage through the gastrointestinal tract was determined by exposing the yogurts at 37°C for 420 min to a simulated gastric juice (pH 3.0) containing pepsin and, subsequently, to a simulated intestinal juice (pH 7.0) containing pancreatin, and by monitoring changes in total viable counts. Bile tolerance (1%) at pH 7.0 was evaluated for 300 min. The experiment was repeated 3 times and the results were evaluated by ANOVA and Tukey′s mean comparison tests ($P \leq 0.05$). During the simulation of the passage through gastrointestinal conditions, the counts of viable cells in probiotic yogurts with free *L. acidophilus* reduced 0.38 log cycles while this reduction was 0.17 and 0.18 log cycles when the probiotic was added to the yogurt in moist and rehydrated freeze-dried microcapsules, respectively. After 5 h exposed to a bile solution, there was a reduction of 3.5 log cycles in the viability of *L. acidophilus* when incorporated in yogurt in free form, 1.37 and 0.98 log cycles in yogurt with moist and rehydrated freeze-dried microencapsulated *L. acidophilus*, respectively. Therefore, microencapsulation of *L. acidophilus* La5 can be considered a potentially useful technique for delivering probiotics to the gastrointestinal tract of humans.

Key words: probiotic yoghurt, microencapsulation, gastrointestinal resistance

T91 Viability of free and microencapsulated *Lactobacillus acidophilus* La5 in stirred yoghurt during refrigerated storage. M. C. E. Ribeiro, C. G. M. S.C. Tenório, K. S. Chaves, F. N. Souza, C. R. F. Grosso, and M. L. Gigante*, *State University of Campinas, Campinas, SP/Brazil.*

The aim of this study was to evaluate the characteristics of probiotic stirred yogurt added of *Lactobacillus acidophilus* La5 in both free and microencapsulated forms during refrigerated storage. The probiotic microorganism was microencapsulated by association of ionotropic gelation with Ca^{2+} and complex coacervation using low methoxyl amidated pectin and whey protein as wall materials. The yogurts were manufactured with sterilized, homogenized and standardized milk added of 2.5% (v/v) yogurt starter culture and submitted to the following treatments: 1) addition of 1% (v/v) of free *L. acidophilus*; 2) addition of 10% (w/v) of moist microcapsules containing *L. acidophilus* and 3) addition of 13% (w/v) of rehydrated freeze-dried microcapsules containing *L. acidophilus*. Incubation was carried out at 42°C until fermentation reached pH 4.8 ± 0.05. Yogurts were evaluated once a week over the 5-week storage period for pH and viability of starter culture and probiotic microorganism. A Split-Plot design in a 3 × 6 factorial, in completely randomized blocks, was used with 3 replications. Results were evaluated by ANOVA and Tukey′s mean comparison tests ($P \leq 0.05$). The samples with free cells and with moist microcapsules showed fermentation time of 180 min, while this period was 200 min for microencapsulated rehydrated freeze-dried cells. The yogurts added by microencapsulated *L. acidophilus* showed less post-acidification and higher survival of probiotic microorganism after 35 d storage than yogurt added by free probiotic. The number of free cells was reduced by 0.98 log cycles, while in microencapsulated form for both moist and rehydrated freeze-dried forms the reduction was 0.2 log cycles. The microencapsulation of *Lactobacillus acidophilus* La5 by association of ionotropic gelation with Ca^{2+} and complex coacervation using pectin and whey protein as wall materials provides protection to the microorganism during refrigerated storage of probiotic yogurt.

Key words: probiotic, yoghurt, microencapsulation

T92 In vitro property evaluation of *Propionibacterium* cultures for probiotic applications. W. Y. Yang*, A. Hostetler, C. Nolan, and H. S. Kim, *Culture Systems Inc., Mishawaka, IN.*

The potential use of propionibacteria as a probiotic is getting attention for both humans and animals. Some of the benefits include aiding in the prevention of colon cancer for humans and weight gain for animals. Certain properties are needed for maximizing probiotic effectiveness, such as tolerance in acidic and bile salt conditions and maintenance of viability. The objective of this study was to isolate and screen *Propionibacterium* strains from healthy cow rumen fluid and cheese, and to evaluate their effectiveness as probiotic cultures. The tolerance of propionibacteria strains to acidic environments and bile salt conditions was tested. In addition, the antibiotic susceptibility and growth in a limited fermentation medium were assessed. Finally, the stability of the freeze-dried cultures during storage at 25°C was analyzed. These tests resulted in the selection of 2 *Propionibacterium freudenreichii* strains: *P. freudenreichii* ssp. freudenreichii CS36 and *P. freudenreichii* ssp. shermanii CS39. No loss of viable cell count was observed in any of the *P. freudenreichii* strains during a 3-h treatment at pH 4 or 3. Bile salt at 0.5% concentration had no apparent effect on the cell count of the strains. There was no atypical antibiotic resistance by the 2 strains. The highest concentration of propionic acid was obtained with the limited growth medium. Cell count was stable at 25°C for a one year storage period when strains were properly packaged as a freeze-dried powder. In conclusion, 2 strains of propionibacteria, CS36 and CS39, meet some of the basic property requirements for the use as probiotics or direct fed microbial products.

Key words: propionibacteria, probiotics, colon cancer

T93 Can high quality raw milk have enough microbial load to show a reduction of organisms in a pasteurization adjunct? J. A. Zonneveld*, A. M. Lammert, and R. Jimenez-Flores, *California Polytechnic University, San Luis Obispo.*

UV light treatment may be used in adjunct to pasteurization to further decrease microbial load in milk. The purpose of this study was to determine if fresh raw milk could be used to evaluate the reduction in microbial load of milk treated by pasteurization and UV light and to determine if the microbial reduction was significant. Sixteen hundred gallons of raw Holstein and Jersey milk from the Cal Poly Dairy were standardized to 3.5% fat and stored in a 4.4°C raw milk bulk tank. Control samples were obtained from the raw milk tank and stored without air or agitation. Every 24 h for 4 d milk control samples and raw milk bulk tank samples were analyzed for coliform and aerobic plate counts. The raw bulk tank milk was processed using UV light treatment in addition to pasteurization using traditional pasteurization controls to determine boundaries for future testing. Samples were taken after each variable to determine microbial kill efficiency. After 4 d in the raw milk bulk tank, the aerobic plate counts and coliform counts were >500,000 cfu/mL and 2175 coliforms/mL, respectively, and much higher than the 39,000 cfu/mL and 195 coliforms/mL, respectively, from the initial batch tank samples. Microbial disinfection worked well with all processing methods evaluated. Every variable of treated milk had 0 coliforms/mL and aerobic plate counts below 50 cfu/mL, when combined with pasteurization. Even the minimal UV treatment lowered both aerobic plate counts and coliforms greatly prior to pasteurization; however, the maximum level UV treatment came much closer to traditional pasteurization in effectiveness. When milk was processed with UV light first followed by traditional pasteurization, aerobic plate counts decreased greater than 2 log and coliforms were essentially eliminated from milk leaving only a few cfu/mL if any. This work indicates that 4 d old day raw milk treated with UV light and pasteurization may be used to successfully lower the microbial counts of aerobic bacteria and coliforms.

Key words: fluid milk, ultraviolet light