### M133  **Comparison of the adhesion characteristics of common dairy spore formers and their spores.** S. Jindal and S. Anand*, South Dakota State University, Brookings, SD.

The initial attachment of aerobic spore forming bacteria to the surfaces of dairy processing equipment leads to biofilm formation and fouling. Although spore formers may vary in attachment, various surface modifications are being studied to develop a surface that is less vulnerable to attachment. The aim of this study was to compare the extent of adhesion of spores and vegetative cells of high-heat-resistant spore formers (HHRS) such as *B. sporothermodurans*, and *G. stearothermophilus*, and thermo-tolerant species *B. licheniformis*, and, on both native and modified stainless steel surfaces. Influence of various contact surface and cell surface properties including surface energy, surface hydrophobicity, cell surface hydrophobicity, and zeta-potential on the adhesion tendency of bacteria were compared. The ability of the vegetative cells and spores of different aerobic spore former to attach to native and modified (Ni-P-PTFE) stainless steel surfaces was determined by allowing the interaction between the contact surface, and spores or vegetative cells for an hour at ambient temperature. Hexadecane assay was employed to determine the hydrophobicity of vegetative cells and spores of aerobic spore-forming bacteria, while the surface charge (expressed as zeta potential) was determined using Zeta sizer Nano series instrument. The results indicated higher adhesion tendency of spores over vegetative cells of aerobic spore forming bacteria. On comparing the sporeformers, *B. sporothermodurans* demonstrated greatest adhesion tendency followed by *G. stearothermophilus* and *B. licheniformis*, respectively. As the vegetative cells and spores of *B. sporothermodurans* and *G. stearothermophilus* demonstrated significantly greater attachment as compared with *B. licheniformis* thus it can be interpreted that HHRS show great attachment tendency as compared with thermo-tolerant spore formers. The tendency to adhere varied with the variations in cell surface properties as it decreased with lower cell surface hydrophobicity and higher cell surface charge. On the other hand, modifying the contact surface properties caused the attachment tendency to decrease with the lowering surface energy and increasing surface hydrophobicity.

**Key Words:** aerobic sporeformer, hydrophobicity, zeta-potential

### M134  **Evaluating enzyme formulations for biofilm removal from dairy separation membranes.** N. Garcia-Fernandez1,2 and S. Anand*, 1Midwest Dairy Foods Research Center, Brookings, SD, 2Department of Dairy and Food Science, South Dakota State University, Brookings, SD.

Enzymatic cleaners are generally used during cleaning in place (CIP) processes to improve the cleanability of dairy separation membranes. Many of the commercial enzymatic cleaners, however, contain general action enzymes, not specifically designed to degrade recalcitrant biofilm matrices. In our previous screening, some enzymes showed a greater biofilm removal on reverse osmosis (RO) membranes, as compared with commercial enzyme-based cleaners. This project aims to evaluate the efficacy of a protease (EC 3.4.24.31, named S1), an alkaline phosphatase (EC 3.1.3.1, S2), and a lactase (EC 3.2.1.23, S3) in removing biofilms on diverse dairy separation membranes, for RO (KMS HRX: TFC polyamide), and Ultrafiltration (UF) processes (HFK-131; polyethersulfone, and HFM-180; polyvinylidene difluoride) (Koch membrane systems, Wilmington, MA). Forty-eight-hour-old mixed species biofilms, constituting common dairy sporeformers *Bacillus licheniformis, B. coagulans, B. sporothermodurans, and Geobacillus stearothermophilus*, were developed on the respective membranes (4cm²) under lab conditions. Tryptic soy broth at 37°C served as the immersion medium. All enzymes and buffer solutions used were prepared following manufacturer recommendations (Sigma-Aldrich, Saint Louis, MO). Membranes in triplicates were rinsed with sterile distilled water, followed by separately cleaning for 45 min at 55°C with individual enzyme solutions at 0.2 U/mL (S1), 0.1 DEA/mL (S2), and 0.01 U/mL (S3). All assays were repeated 3 times, and data were statistically analyzed. The residual viable cell numbers were estimated by swabbing, and plating on plate count agar. Percentage reductions in viable counts for S1, S2 and S3, respectively, were 99.93, 99.69 and 90.74% cfu/cm² for biofilms formed on RO, 99.99, 99.91 and 99.40% for UF HFK-131, and 98.23, 0.0 and 48.0% for UF FM-180. In conclusion, S1 was the most effective enzyme for reducing multispecies biofilms on all membrane types. Additionally, the most resistant biofilms were observed on HFM-180. These findings suggest that for better cleaning of any membrane material, it will be critical to design a specific enzyme-based formulation, depending on a particular biofilm matrix.

**Key Words:** membrane cleaning, biofilm, enzyme


In dairy manufacturing plants, biofouling of separation membrane represents a serious quality issue. Besides hydrodynamic conditions, the adhesion of pioneer bacteria and the formation of biofilms during filtration of dairy fluids could be influenced by membrane material properties. Consequently, the objective of this study was to characterize the impact of 3 different membrane materials (polyethersulfone [PES], polyvinylidene fluoride [PVDF], polyacrylonitrile [PAN]) on the diversity of early bacterial communities formed on membranes after ultrafiltration (UF) of dairy fluids. A laboratory-scale crossflow filtration system equipped with parallel modules, each containing with a different membrane of 42 cm², was used for UF of pasteurized skim milk and cheese whey. The UF system was operated at 50°C during 5h- and 20h-periods in the concentration mode with the feed maintained at 10°C between passages in the UF system. Membranes were cleaned with an alkaline solution prior and following each UF experiments. The bacterial diversity was assessed on cleaned membrane coupons and in filtered fluids after UF of 5 h and 20 h through a metabarcoding approach targeting the 16S rRNA gene. The bacteria numeration in samples was also estimated using qPCR targeting the same gene target. Bacterial genus ratios within the biofilms were found dependent of the composition of the membrane material used during UF of milk and whey. Interestingly, the qPCR quantification revealed a similar number of bacteria for each condition ($P > 0.05$). According to a PERMANOVA analysis, the diversity observed on membranes was dependent of the nature of the filtered fluids and the filtration duration, explaining respectively 53.24% and 21.75% ($P = 0.002$) of the variances among bacterial communities. Consequently, this study suggests that the membrane material may affect the biofilm formation on UF membranes, but other operational parameters such as...
while no report has been available for the correlation between a w, pH counts of dehydrated bovine milk may have been studied extensively, the objectives of this study were to determine aw, pH and storage stability, where aw is directly related to bacterial growth, especially concerns over food safety. The objective of this study was to investigate the survivability of Escherichia coli in powdered goat milk (PGM) at 4°C and 22°C during 0, 2 and 4 mo storage. Three different lots of commercial whole goat milk powder products were purchased from a local retail outlet, and the total amount of each lot was divided into 2 equal quantities to assign them to 2 treatment groups as control and E. coli inoculated groups. Ten grams of the experimental PGM samples were inoculated with 50 μL of E. coli K12. Both of the treated and control samples without inoculation of the pathogens were subjected to the 2 temperature and 3 storage treatments. All experimental PGM samples were microbiologically analyzed according to the manufacturer’s procedure (3M Center, St. Paul, MN). The PGM samples in duplicates were serially diluted, plated on the 3M Petrifilm EC plates, and colonies were counted after 48 h incubation at 37°C. The initial inoculation rate was at least 8 log cfu/g for each sample. Results showed that the inoculated experimental PGM contained average 5.01 log cfu/g E. coli in the initial samples. Mean E. coli counts of 4 and 22°C at 0, 2 and 4 mo storages were: 5.01, 4.16, 3.43, 1.85; and 3.77, 1.48 cfu/g, respectively, indicating that E. coli counts significantly (P < 0.01) decreased during 4 mo storage period. There were significant (P < 0.01) differences in E. coli counts between temperatures and between storage periods for both of main factors. E. coli counts of the powder milk samples were not affected by batch effect up to 2 mo, but did affect at 4 mo storage. It was concluded that the survivability of E. coli in the powdered whole goat milk significantly decreased as the storage time advanced.

Key Words: Escherichia coli, powdered goat milk, storage


The objective of this research was to determine the concentration of lactose oxidase (LO) needed to activate the lactoperoxidase system (LPS) in skim milk and assess its ability to inhibit the growth of Pseudomonas fragi, a milk spoilage strain. LO oxidizes the lactose in milk and produces hydrogen peroxide needed for the activation of the antimicrobial system. Seven treatments were evaluated at 4 and 21°C: the control, and three levels of LO or LPS+LO (0.012, 0.12, and 1.2 g/L). The base LPS was obtained adding 30 mg/L of bovine lactoperoxidase and 14 mg/L of NaSCN to ultra-pasteurized skim milk. Three independent trials of the experiment were performed and the microbial reduction was calculated for 1, 4, and 7 d. The effect of treatment, temperature, time, and their interactions was determined through a multi-factorial analysis. Also, for both temperatures, a one-way ANOVA was conducted separately for each day to determine the significance of the treatments followed by a Tukey’s test. The results showed that treatments were more effective at refrigeration temperature (P < 0.001). At 4°C, LO at 0.12 and 1.2 g/L showed a significantly higher reduction than the control (P < 0.001) when added alone and combined with the system for every time point. An increase in the concentration of LO caused higher reductions of P. fragi at d 7, achieving a >2.93 log cfu/mL reduction for the 1.2 g/L treatments. At 21 °C, treatments with a concentration of 1.2 g/L of LO achieved a reduction of >2.93 log cfu/mL, while under the other conditions reductions were not significantly different from the reduction observed for the control (P < 0.05). Results confirm that lactose oxidase can be used to inhibit the growth of P. fragi and represents a new way to extend the shelf-life of dairy products. The application of LO serves as an opportunity to reduce food waste and for the dairy industry to benefit from a longer shelf-life while meeting the consumers’ demand for clean label products. Further research will assess the inhibition of other spoilage microorganisms in different dairy products, as well as the effect of the inoculation level and thiocyanate concentrations.*

Key Words: lactoperoxidase, lactose oxidase, spoilage

*Corrected abstract

M139 Selective primer development for rapid detection of the gas-producing non-starter bacterium Lactobacillus wasatchensis. M. Culumber1, T. Oberg2, T. Allen3, F. Ortakci2, C. Oberg*1, and D.
Lactobacillus wasatchensis is a slow-growing non-starter lactic acid bacterium (NSLAB) recently implicated in gassy defects in aged Cheddar cheese. This organism has been detected in cheeses from 7 cheese processing facilities in different regions of the United States and is of significant concern to cheese producers. Rapid detection of *Lb. wasatchensis* would allow for better control of the organism, and help determine where it is entering the manufacturing process. A set of 16S rRNA primers were developed using NCBI Primer-Blast against the *Lb. wasatchensis* genome and selected based on product length, melting temperature, and primer self-complementarity. In silico analysis against the NCBI database indicated the primers should have high specificity for *Lb. wasatchensis*. PCR optimum conditions were determined experimentally with *Lactobacillus casei* and *Lactobacillus curvatus* DNA as non-target template. To determine specificity, the primers were tested against DNA extracted from 22 different common NSLAB, including strains of *Lb. wasatchensis* isolated from cheese and the original *Lb. wasatchensis* WDC04. Only strains identified previously as *Lb. wasatchensis* amplified with the primers. Even the mostly closely related NSLAB species (such as *Lb. curvatus*) to *Lb. wasatchensis* could be differentiated with these primers. DNA from all isolates amplified using standard bacterial 16S rRNA primers. The new primers, LW86Fa and LW258Ra, will be used in traditional and real-time PCR for rapid detection of *Lb. wasatchensis* in gassy cheeses and the cheese processing environment. Rapid molecular detection will help diagnose and track *Lb. wasatchensis* contamination, and help control the occurrence of gassy-cheese defects.

**Key Words:** *Lactobacillus*, gassy defect, cheese

**M140** Effect of bio-protective lactic acid bacteria cultures on *Lactobacillus wasatchensis*. A. Lavigne, 1 S. Smith, 1 C. Oberg*, 1 I. Bowen, 2 and D. McMahon, 1 Weber State University, Ogden, UT; 2 Utah State University, Logan, UT.

The nonstarter lactic acid bacterium (NSLAB) *Lactobacillus wasatchensis* can cause late gassy defect when it grows to high numbers during Cheddar cheese storage. A potential strategy for preventing such growth is incorporation of specific lactic acid bacteria strains (termed bio-protective LAB) into the cheese during manufacture, which may specifically inhibit growth of *Lb. wasatchensis*. Determination of inhibition by common NSLAB lactobacilli and potential bio-protective LAB (BPLAB) strains against *Lb. wasatchensis* was done using the spot test along with the agar flip method. MRS agar supplemented with 1.5% ribose (MRS-R) was inoculated with each NSLAB or bio-protective LAB using the spread plate method and incubated anaerobically at 25°C for 48 or 72 h. Inoculated agar was then flipped over and either *Lb. wasatchensis* WDC04 or CGL04 swabbed on the newly exposed surface with anaerobic incubation at 25°C for up to 72 h. None of the BPLAB strains produced any more inhibition after 48 h than the general competitive inhibition caused by the NSLAB cultures *Lactobacillus brevis* or *Lactobacillus fermentum* LF7469. When incubation time was extended to 72 h before challenge, BPLAB P200 showed the largest inhibition zones for both *Lb. wasatchensis* WDC04 and CGL04. The next inhibitory BPLAB was LB-3 with the NSLAB, *Lb. fermentum* LF7469, also producing a large inhibition zone. To test for bacteriocin production by the BPLAB, a paper disc assay test was performed using cell free extracts. Results confirmed several BPLAB strains produced a bacteriocin, showing a very small zone of inhibition for *Lb. wasatchensis* around the paper disc. Examining the antagonism between bio-protective cultures and NSLABs for *Lb. wasatchensis* strains allows for selection of lactic acid bacteria strains that could inhibit this problematic bacterium during cheese ripening.

**Key Words:** lactic acid bacteria, *Lactobacillus*, gassy defect

**M141** The antibacterial effect of addition of citrulline in fermented milk against foodborne pathogens. S. W. Ho* and Nengdra P. Shah, The University of Hong Kong, Hong Kong, China.

LAB contribute to antibacterial effect against pathogens by generating antimicrobial agents, however, a sufficient cell concentration is required. Citrulline, a non-protein amino acid, provides extra energy to LAB by arginine deiminase pathway to improve cell growth. Citrulline is also a precursor of nitric oxide (NO), which plays an important role in protecting from enteric pathogens. The aims of this study were (1) to investigate the effect of adding citrulline on NO production by LAB and its antibacterial activity, (2) to investigate the antibacterial mechanisms of LAB against foodborne pathogens, and (3) to examine the stimulating effect of NO production in the intestinal epithelial cells and its anti-adhesive effect. The selected LAB were incubated with 0, 0.1, or 0.2% of citrulline in de Man, Rogosa, Sharpe (MRS) broth and in milk at 37°C for 18–20 h. The antimicrobial activities against the pathogens were determined by measuring the diameter of the zones of inhibition. The NO production ability of LAB was determined by using metmyoglobin supplemented MRS plates and the bacteriocin-like inhibitory substances (BLIS) production ability of LAB was determined by eliminating the effect of acids and hydrogen peroxide. The stimulating effect of NO production by citrulline and LAB and the anti-adhesive effect were evaluated using IPEC-J2 cell line. The selected LAB when fermented with citrulline addition in MRS and fermented milk with *Lactobacillus helveticus* ATCC 511 significantly inhibited the tested pathogens (all *P* < 0.001). Milk with added citrulline when fermented with *L. helveticus* ATCC 511 and *L. bulgaricus* ATCC 756 showed the dose-dependent effect on the inhibitory activity of *Shigella sonnei* ATCC 25931. None of the selected LAB were capable of producing NO for converting metmyoglobin to nitrosomyoglobin in MRS-Mb agar. Addition of citrulline to milk fermented with LAB might enhance the antibacterial effect of LAB against selected pathogens in vitro. The cell culture work has shown some interesting data with regard to stimulation effect of NO production in the intestinal epithelial cells and its anti-adhesive effect.

**Key Words:** lactic acid bacteria, citrulline, antibacterial activity

**M142** Influence of the antimicrobial myrrh on yogurt culture bacteria over yogurt shelf life. M. Alhejaili*, D. Olson, M. Janes, C. Boeneke, and K. Aryana, Louisiana State University Agricultural Center, Baton Rouge, LA.

Myrrh is a natural flavoring substance approved by FDA as a food flavor and essential oil. Also, myrrh has antibacterial and antifungal activity against pathogens. The objective was to determine the effect of myrrh on *Streptococcus thermophilus* and *Lactobacillus bulgaricus* counts, pH and titratable acidity of yogurt during 5 wk of storage. Myrrh dispersion was prepared and incorporated at 1% vol/vol yogurt mix. A control with no myrrh was also prepared. Three replications were conducted. *Streptococcus thermophilus* was enumerated using *Streptococcus thermophilus* agar with aerobic incubation at 37°C for 24 h, and *Lactobacillus bulgaricus* was enumerated using MRS agar adjusted to pH 5.2 with addition of myrrh.
The purpose of our work was to study the technological properties and sensitivity to antibiotics of the 4 strains of Enterococcus faecium isolated from traditional Carpathian cheese. These strains are classified as Enterococcus faecium based on the microbial and genotypic properties (RAPD-PCR, RFLP-PCR, sequence 16S RNA), but are not registered in Gene Bank as a nucleotide sequence. The strains were labeled as SB20, SB18, SB6, SB12. The studies included morphological characteristics, optimal growth temperature, the ability to produce CO₂ from glucose, hydrolysis of arginine, catalase activity and fermentation of spectrum carbohydrates. Technological properties were evaluated as the ability to form lactic acid and the ability to grow in the presence of 2, 4, and 6.5% NaCl. The sensitivity to antibiotics (11 group) was determined by disc diffusion method. It was established that the strains SB20, SB18, SB6, SB12 were gram-positive cocci, grew well on MRS at temperatures of 15–45°C, did not ferment fructose, raffinose, xylose and sorbitol, were catalase negative, did not form CO₂ from glucose, hydrolyzed arginine, grew in the environment of 6.5% NaCl. The acidity of skim milk increased to 80–82 °T and the pH decreased to 5.1 upon 24 h fermentation. It was established that all strains of Enterococcus faecium were sensitive to a wide range of antibiotic (penicillin, makrolides, tetracyclines, fluoroquino-lones, cephalosporins, nitrofurans, chloramphenicol, glycopeptides, polimikany, rifampicin) except for aminoglycosides (gentamicin, streptomycin, kanamycin). The natural resistance to aminoglycosides is explained by the absence of a system transfer of antibiotics through the cell membrane by anaerobic Enterococcus faecium. It was concluded that enterococci strains SB20, SB18, SB6, SB12 are showing good technological properties and high sensitivity to antibiotics of all groups and may be considered as potentially promising for the industry, but further research on their virulence and pathogenicity is required.

Key Words: Carpathian cheese, Enterococcus faecium, antibiotic resistance


During milk fermentation with lactic acid bacteria (LAB) can be produced biologically active peptide sequences known as bioactive peptides. One of the most important biological activities is angiotensin-converting enzyme (ACE) inhibitory activity. The ACE-inhibitory activity depends on the fermentations conditions (temperature, pH, and inoculum). To evaluate the effect of some fermentation conditions on ACE-inhibitory activity a central composite design was used. Previously, 3 probiotic strains Lactobacillus plantarum, L. pentosus and L. acidipiscis were isolated from double cream cheese produced in the state of Chiapas (Mexico). These probiotic bacteria were shown to generate an ACE-inhibitory activity (more than 50%) in vitro tests. Lactobacillus plantarum was chosen for evaluating the effect of temperature (X₁), initial pH (X₂) and inoculum concentration (X₃) on the generation of ACE-inhibitory activity (Y). This study aims to optimize the ACE-inhibitory activity during milk fermentation by Lactobacillus plantarum using response surface methodology (RSM). For the optimization of fermentation process, a central composite design was used. ACE-inhibitory activity (response variable) was measured by the Cushman and Cheung method at initial and final fermentation time (16 h). The equation for the proposed model and model parameters where calculated with NCSS 11 Data Analysis Software. The mathematical model for the generation of

Key Word: probiotic viability, acidifying kinetic parameters, functional food

M144 Properties of Enterococcus faecium strains isolated from traditional Carpathian ewe’s cheese. O. Tsisaryk*, I. Slyvka¹, L. Musiy¹, I. Kushnir1, and T. Bocer², ¹Liviv National University of Veterinary Medicine and Biotechnologies, Liviv, Ukraine, ²Rzeszow University, Rzeszow, Poland.

The consumption of probiotic products, which is proven to provide health benefits, has increased significantly in the last few years. A proper selection of strains and food matrix should be conducted for the processing of probiotic food products, because certain components in the food matrix may interact with these probiotics, altering their functional performance. Therefore, the application of probiotic strains in different food matrices could represent a great challenge to maximize their effectiveness. This research aimed to evaluate the viability of potentially probiotic strains in fermented milk prepared using different matrices. L. casei SJRP38 and L. fermentum SJRP43, previously selected by their good technological features, safety and high probiotic potential, were evaluated and used in coculture with the commercial strain of S. thermophilus TA040 for fermentation. The influence of matrices: M1 - reconstituted skim milk powder (RSMP) + 7% sucrose and M2 - RSMP + 7% sucrose + 5% flaxseed (Linum usitatissimum L.) were evaluated on the acidifying kinetic parameters and viability of the strains under simulated gastrointestinal (GI) conditions during refrigerated storage. Times to reach the maximum acidification rate, pH 5.0, and pH 4.6 (end of fermentation) were influenced by the food matrix. M2 had a negative effect on the fermentation time, causing an increase of up to 3 h for finishing the process. All strains in the M1 matrix survived well (>7 log cfu/mL) during the simulated GI, which is equivalent to the passage of bacteria through the human GI tract. M2 affected the counts of L. casei SJRP38, L. fermentum SJRP43, and S. thermophilus TA040, compared with the M1 matrix, and they had a reduction of up to 3 log cfu/mL after intestinal passage. Additionally, the population reduction after the assay was influenced by the storage period for both matrices. Considering the overall results, Lactobacillus casei SJRP38 in coculture with S. thermophilus TA040 in the M1 matrix presented a high probiotic potential for further application in functional fermented products.

Key Words: fermented, antimicrobial, yogurt

M143 Influence of the food matrix on the viability of Lactobacillus casei and Lactobacillus fermentum strains. B. M. Salotti-Souza, T. F. Borgonovi, and A. L. B. Penna*, São Paulo State University, São José do Rio Preto, SP, Brazil.

The purpose of our work was to study the technological properties and sensitivity to antibiotics of the 4 strains of Enterococcus faecium isolated from traditional Carpathian cheese. These strains are classified as Enterococcus faecium based on the microbial and genotypic properties (RAPD-PCR, RFLP-PCR, sequence 16S RNA), but are not registered in Gene Bank as a nucleotide sequence. The strains were labeled as SB20, SB18, SB6, SB12. The studies included morphological characteristics, optimal growth temperature, the ability to produce CO₂ from glucose, hydrolysis of arginine, catalase activity and fermentation of spectrum carbohydrates. Technological properties were evaluated as the ability to form lactic acid and the ability to grow in the presence of 2, 4, and 6.5% NaCl. The sensitivity to antibiotics (11 group) was determined by disc diffusion method. It was established that the strains SB20, SB18, SB6, SB12 were gram-positive cocci, grew well on MRS at temperatures of 15–45°C, did not ferment fructose, raffinose, xylose and sorbitol, were catalase negative, did not form CO₂ from glucose, hydrolyzed arginine, grew in the environment of 6.5% NaCl. The acidity of skim milk increased to 80–82 °T and the pH decreased to 5.1 upon 24 h fermentation. It was established that all strains of Enterococcus faecium were sensitive to a wide range of antibiotic (penicillin, makrolides, tetracyclines, fluoroquinolones, cephalosporins, nitrofurans, chloramphenicol, glycopeptides, polimikany, rifampicin) except for aminoglycosides (gentamicin, streptomycin, kanamycin). The natural resistance to aminoglycosides is explained by the absence of a system transfer of antibiotics through the cell membrane by anaerobic Enterococcus faecium. It was concluded that enterococci strains SB20, SB18, SB6, SB12 are showing good technological properties and high sensitivity to antibiotics of all groups and may be considered as potentially promising for the industry, but further research on their virulence and pathogenicity is required.

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During milk fermentation with lactic acid bacteria (LAB) can be produced biologically active peptide sequences known as bioactive peptides. One of the most important biological activities is angiotensin-converting enzyme (ACE) inhibitory activity. The ACE-inhibitory activity depends on the fermentations conditions (temperature, pH, and inoculum). To evaluate the effect of some fermentation conditions on ACE-inhibitory activity a central composite design was used. Previously, 3 probiotic strains Lactobacillus plantarum, L. pentosus and L. acidipiscis were isolated from double cream cheese produced in the state of Chiapas (Mexico). These probiotic bacteria were shown to generate an ACE-inhibitory activity (more than 50%) in vitro tests. Lactobacillus plantarum was chosen for evaluating the effect of temperature (X₁), initial pH (X₂) and inoculum concentration (X₃) on the generation of ACE-inhibitory activity (Y). This study aims to optimize the ACE-inhibitory activity during milk fermentation by Lactobacillus plantarum using response surface methodology (RSM). For the optimization of fermentation process, a central composite design was used. ACE-inhibitory activity (response variable) was measured by the Cushman and Cheung method at initial and final fermentation time (16 h). The equation for the proposed model and model parameters where calculated with NCSS 11 Data Analysis Software. The mathematical model for the generation of...
ACE-inhibitory activity of fermented milk with *Lactobacillus plantarum* was the following: $Y = 524.99 - 122.70X_1 + 344.85X_2 + 186.79X_3 + 1.66X_1^2 - 32.33X_2^2 - 0.86X_3^2 + 7.47X_1X_2 + 7.81X_1X_3 - 89.00X_2X_3 - 0.100X_1^2X_2 - 0.10X_1X_2X_3 + 6.03X_2^2X_3$. The results of regression analysis showed that initial pH was the most important factor positively affecting the ACE-inhibitory activity. Other factors significantly affecting the activity were inoculum and temperature (negative correlation). This mathematical model predicted the ACE-inhibitory activity in 86.99% of the cases.

**Key Words:** *Lactobacillus plantarum*, ACE-inhibitory activity, optimization