495 Spore incidence in individual cows and correlation with weather in California. V. Arechiga* and R. Jimenez-Flores, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo.*

Thermophilic endospore-formers have created a growing concern in the maintenance of high quality milk products. The spores of these species can survive many processing environments and for many years. With the ability to germinate when a product is most vulnerable, they represent a variable source of contamination with difficult means of prediction measures or prevention. Therefore, it is ideal to learn the source of these spores to design preventative measures. Standards for spore counts have been established in other countries already, and with its long processing run times the United States stands to benefit from finding means to limit spore presence in milk products. Past studies have examined the various environmental factors in the pasture and the dairy and the effect on bulk tank spore counts. This study aims to look at the environmental factors that affect the milk of individual cows, eliminating equipment contact involved in bulk tank results. Thermophilic spore quantities, which are the most problematic in milk powder, were enumerated in fluid milk samples from individual cows in one herd over a 9-mo period (September through May) during the course of changing weather patterns in California. The milk samples were split and put through a mesophilic (80°C for 12 min) or thermophilic (100°C for 30 min) heat shock treatment, and plated on Tryptic Soy Agar with 1% starch. After incubating for 48 h at 55°C, the colony forming units were counted and recorded along with the weather conditions for the days of milk collection. Statistical analysis was done using Minitab at a significance level of 0.05. Mesophilic spores existed in nearly every milk sample collected ($n = 474$, $\hat{y} = 6.3$ cfu/mL), while the thermophilic spores existed in much smaller quantities and fewer cows ($\hat{y} = 3.0$ cfu/mL). Spore counts were significantly higher in both spore types in wet weather ($\hat{y} = 7.4$ cfu/mL) as compared with dry weather ($\hat{y} = 4.5$cfu/mL). Last, thermophilic spores were significantly decreased in colder months ($\hat{y} = 2.0$ cfu/mL).

Key Words: spore, milk, thermophile

496 Aroma development in relation to microbial growth in milk under Ragusano cheese-making conditions using different wooden vats (tina). S. Carpino*[1], T. Rapisarda[1], I. Schadt[1], G. Belvedere[1], and G. Licitra[1,2], *[1]CoRFiLaC, Regione Siciliana, Ragusa, Italy, [2]DISPA, Catania University, Catania, Italy.*

Ragusano cheese is a brine-salted pasta filata raw-milk cheese. Starters are not added, but the biofilm of the wooden vat (tina) and the natural milk flora are responsible for the milk acidification. The aim of this study was to investigate the development of volatiles' and odor active compounds in relation to microbial growth in milk which has been inoculated with biofilm from different tinas and which has undergone the usual cheese-making procedure except for the rennet addition step. Biofilm samples were obtained from the inner surface of 11 wooden tinas (A-K) with sterile swabs suspended in peptone water. Aliquots of a UHT milk sample were inoculated with the biofilm samples. tina surface area per volume milk was 1.5 times the usual exposure conditions. Incubated samples were first analyzed with Smart Nose®. Four (B, E, F, J) were selected as they were representing the greatest volatiles' variation (PC1 45%;PC2 25%). Samples E and F were similar in profile, but different from B and J. Profiles of B and J differed also. Samples B, E, F, and J were further analyzed by GC/O and GC/MS and for the total bacterial count (TBC), for counts of mesophilic lactobacilli (ME_LB), mesophilic lactococci (ME_LC), thermophilic lactobacilli (TH_LB), thermophilic lactococci (TH_LC) and enterococci (EC). Data were analyzed with principal component analysis. Odor active compounds, which distinguished samples, belonged to 3 groups of compounds that within group had the same presence/absence pattern. Group 1 was composed by pentanol,(Z)-2-nonenal, 2-nonanone and methyl thiophene, group 2 by 2-hexenol, ethyl hexanoate, dimethyl disulfide and (E)-limonene oxide, group 3 by (E)-2-nonenal, octane and methyl geranate. Samples B and E had odors of group 1 and 2, sample F had odors 1 and 3, whereas sample J had no odors of groups 1, 2 or 3. Odors of groups 1 and 2 were negatively associated with TBC and LC_TH. Odors of group 3 were positively associated with LC_ME and with lower effect on LC_ME and negatively with LB_TH. Levels of EC had low influence on odor or volatile differentiations (PC1 28%; PC2 20%).

Key Words: Ragusano, cheese, biofilm

497 Development of enzyme substrate assay for monitoring *E. coli*/*E. coli* O157:H7 in milk and milk products. R. Lawaniya*, N. Kumar, B. Arora, A. Khan, and M. Blahara, *National Dairy Research Institute, Department of Dairy Microbiology, Karnal, India.*

Monitoring the microbiological quality of milk relies largely on examination for indicator bacteria such as coliforms, *E. coli* and enterococci. *E. coli* is used as indicators of fecal contamination of water and food and is of most interest to clinical, food and water microbiologists. Detection of *E. coli*/*E. coli* O157:H7 in dairy products still relies on conventional method requiring at least 3 to 4 d for its identification. Alternative methods based on nucleic acid, fluorescent antibody techniques need expensive devices as well as long enrichment steps. Using chromogenic substrate against specific enzymes produced by *E. coli*/*E. coli* O157:H7 can be a rapid detection strategy and an alternative for conventional methods. Keeping this in mind, a selective enrichment medium with potential inhibition up to 5.6 log cfu/mL of contaminants such as *Salmonella*, *Shigella*, *Yersinia*, *Proteus*, *Citrobacter*, Staph. aureus, and *L. acidophilus* has been developed and MIC of selective agents used in the development of medium are determined at specific cell level; i.e., 3 to 6 log cell/mL based on chromogenic assay. The developed assay can detect the target bacteria i.e., *E. coli* / *E. coli* O157:H7 within 12 ± 1 h compared with a 3- to 4-d protocol of conventional method in ISO-5887 part-1 detection method.

Key Words: *E. coli*, enzyme substrate assay, rapid method

498 Effect of drying methods on microencapsulated bacteria on secondary protein structure and glass transition temperature as studied by FTIR and DSC. D. Dianawati[1] and N. P. Shah*[2], *[1]Victoria University, Melbourne, Australia, [2]The University of Hong Kong, Pokfulam Road, Hong Kong.*

The objective of this study was to examine the effect of drying methods on microencapsulated *L. acidophilus* and *L. cremoris* on secondary protein structure and glass transition temperature. Protective mechanism of casein-based microcapsule containing mannitol on *L. acidophilus* and *L. lactis* ssp. *cremoris* and glass transition of the microcapsules were studied after spray- or freeze-drying and after 10 week of storage in aluminum foil pouch containing different desiccants (NaOH, LiCl or silica gel) at 25°C. An in situ FTIR analysis was carried out to recognize any changes in fatty acids of bacterial cell envelopes; interaction between polar site of cell envelopes and microcapsules, as well as alteration of their secondary protein structures, whereas DSC was used to determine glass transition

(T_g) of microcapsules. Hierarchical cluster analysis based on functional groups of phospholipid bilayers of cell envelopes and secondary protein structures was also carried out to classify the microencapsulated bacteria. The results showed that drying process did not affect fatty acids and secondary protein structures of bacteria, however, those structures were affected during storage depending upon the type of desiccant. Interaction between bacterial cell envelopes and microencapsulant occurred after drying as shown by alteration of wavelength of $P = O$ symmetric of cell envelopes from 1075 cm^{-1} to 1047 and 1048 cm^{-1} (fresh L. acidophilus, freeze-dried and spray dried cells, respectively). Similar phenomenon was demonstrated by *L. cremoris*. However, these structures were maintained after storage in foil pouch containing NaOH. Method of drying and type of desiccants influenced the level of similarities of microencapsulated bacteria. Desiccants and method of drying affected glass transition; yet no $T_g \leq 25°C$ was detected. This study demonstrated that the changes in fatty acids and secondary structures of the microencapsulated bacteria still occurred during storage at T_g above the room temperature indicating that glass state did not completely prevent chemical activities.

Key Words: microencapsulation, FTIR, DSC

499 The relationship between *Streptococcus thermophilus* exopolysaccharide diversity and fermented milk viscosity. H. Yi*, L. Zhang, and L. Zhang, *College of Food Science and Engineering, Harbin Institute of Technology, Harbin, China.*

Exopolysaccharides produced by *Streptococcus thermophilus* fermented milk plays a crucial role in the viscosity of the fermented milk. In this work, the content of exopolysaccharides of 19 different *Streptococcus thermophilus* fermented milk and the corresponding viscosity were studied. Then the exopolysaccharides produced by *Streptococcus thermophilus* were purified by DEAE-52 cellulose chromatography and Superdex 200 chromatography purification. The purified exopolysaccharide were added to fermented milk to detect the effect of purified exopolysaccharide on the yogurt viscosity. And the compositions of exopolysaccharide were determined by Fourier transform infrared spectroscopy. The results showed that strains which produced large amount of exopolysaccharides could improve the viscosity of fermented milk. The exopolysaccharides of 3 *Streptococcus thermophilus*, zlwTM11-EPS, zlwB9–3-EPS and zlwSP1.1-EPS, were selected and detect the effect of exopolysaccharides on fermented milk viscosity. It showed that the contribution of the zlwTM11-EPS on the viscosity of the fermented milk is the largest. Four major purified components were obtained from zlwTM11-EPS: zlwTM11–1, zlwTM11–2, zlwTM11–3 and zlwTM11–4. The purity were 89.35%, 92.49%, 81.03% and 93.37% respectively. These 4 purified components were added to the *S. thermophilus* zlwSP1.1 fermented milk. The 4 purified components could all increase the viscosity of the fermented milk, but the effect of the zlwTM11–2 was significantly higher than the other components. The major structure of zlwTM11–2 produced exopolysaccharide was β-D-pyranose. And the exopolysaccharide of zlwTM11–2 were constituted by rhamnose, mannose, glucose and galactose with the ratio of 9.7: 4.87: 39.14: 46.29. The optimum exopolysaccharides production conditions of zlwTM11–2 in fermented milk were also determined that was 4.49% of the inoculum size, 6.74h of fermentation time, 43°C of fermentation temperature. At last, the actual production of the zlwTM11–2 was 42.947mg/L which was 11.87% higher than before.

Key Words: fermented milk, exopolysaccharide, *Streptococcus thermophilus*

500 The growth and interaction of yeasts and lactic acid bacteria in milk fermentation. X. Han*, L. Zhang, H. Yi, and Q. Yi, *College of Food Science and Engineering, Harbin Institute of Technology, Harbin, China.*

Yeasts affect the quality of fermented milk through the production of flavor compounds and other metabolites. As a specialty product, yeast and lactic acid bacteria combined fermented milk has its own niche in the special places in China. In present study, 5 yeasts were screened and isolated from traditional dairy products throughout the northwestern China according to their fermented milk characteristics. The yeasts which have higher production of CO_2 and acid rate were selected to combine with the yogurt starter culture to fermented milk. The fermented temperature, inoculate and the ratio of yeast and LAB were optimized. Then the changes of viable count of yeast and LAB, the content of CO_2, ethanol and pH were also detected during 10°C storage for 4 weeks. The results showed the total inoculate rate of starter was 4%, the ratio of yeast and LAB were 1:4, fermented temperature was 37°C. The storage test manifest the pH of fermented milk were decreased from 4.21 to 3.83. The counts of LAB were increased from 5.5×10^8 to 8.3×10^8 cfu/mL during the first week storage at 10°C. Then the counts were decreased to the 7.3×10^7 cfu/mL. The viable counts of yeast were increased from 6.8×10^5 to 9.6×10^6 cfu/mL. The content of CO_2 and ethanol produced by yeast was 22.9 mmol/l and 14.57 mg/ml respectively. For the texture of product were better than LAB fermented milk (yogurt). However the apparent viscosity of product was lower than yogurt. For the sensory evaluation, the product has the little milk taste and with the little fresh flavor. These results manifested that the yeast possesses stability enhancing effects on LAB and yeast combined yogurt could provide the new products to the Chinese dairy marketplaces.

Key Words: yeasts, lactic acid bacteria, viable count

813 Cytokine and regulatory T cell responses of lactic acid bacteria and probiotic organisms in human peripheral blood mononuclear cells R. Ashraf*[1], O. N. Donkor[1], S. C. Smith[2], T. Vasiljevic[1], *[1]Victoria University, School of Biomedical and Health Sciences, Werribee Campus, Melbourne, VIC, Australia, [2]Deakin University, School of Exercise and Nutrition Sciences, Faculty of Health, Gut Health SRC Molecular and Medical Research, Burwood, VIC, Australia*

Many strains of lactic acid bacteria (LAB) are believed to have probiotic properties and offer various health benefits. In the current study, the immuno-modulatory effects of probiotic organisms and LAB were assessed following stimulation of peripheral blood mononuclear cells (PBMCs) with seventeen strains of probiotic and lactic acid bacteria. The production of pro- and anti-inflammatory cytokines including interleukin (IL)-2, IL-4, IL-10, IL-12 p70, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β, the expressions of CD25 marker and forkhead box P3 (FoxP3)–regulatory T cell (Treg) marker were examined after stimulation of PBMCs with seventeen LAB strains. The results show that (i) live strains stimulated significant production of all cytokines but in varying concentrations, (ii) the pattern of cytokine production was found strain specific, (iii) TNF-α, IFN-γ, IL-10 and TGF-β were stimulated at high concentrations (iv) the strains of Bifidobacterium stimulated highest levels of TGF-β, IL-10 and IL-4 whereas L. casei 290 gave the highest response for TNF-α and S. thermophilus M5 for IFN-γ, (v) CD3+CD4+CD25+ cell population was significantly increased for all tested strains except Lc. lactis R704 (vi) the number of Treg population was significantly increased for tested strains where S. thermophilus M5, B. animalis subsp. lactis BB12, L. casei 290 and S. thermophilus 1342 showed seven-fold more expression of FoxP3 than unstimulated PBMCs. The study suggests that probiotic bacterial strains differ in their immunomodulatory effects.

Key Words: probiotic bacteria, immunomodulation, cytokine