and 6 h post-LPS injection, whereas there was no change in IL-1 activity at any time postinjection in the BSA-injected birds. TNF- α activity significantly increased at 6 and 24 h post-LPS injection, whereas there was no difference in the TNF- α activity at any time postinjection in the BSA-injected birds. Interleukin-2 activity was decreased significantly at 3 h post-LPS injection compared to base line levels at 0 h. However, IL-2 activity increased at 3 h post-BSA injection compared to 3 h post saline injection. Corticosterone levels significantly increased 1, 3, and 6 h post-LPS injection, whereas there was no change in corticosterone levels at any time postinjection in the BSA-injected birds compared to saline injected birds. Tri-iodothyronine levels significantly decreased 3, 6, and 24 h post-LPS injection, whereas there was no change in T_3 levels at any time postinjection in the BSA-injected birds compared to saline injected birds. The results indicate that although LPS and BSA injection can induce a humoral antibody response in chickens, the mechanism of the initiation of this antibody response involving the activation of the cytokine network and the neuroendocrine system are different for each antigen.

Key Words: Chickens, Anitgens, Cytokines and Hormones

1251 Control of coccoidiosis in chiken by trickle immunisation. Srinivasan K¹, Dilipkumar Garikipati^{*2}, and Venkaram A², ¹Madras Vety College, ²College of Vety Sci, Tirupati.

Immunisation control of coccidiosis caused by Eimeria tenella was carried out by trickle immunisation (TI) method. Immunizing chickens daily with 100 number of oocysts at 1-7 days of age (experiment I), 1-14 days (experiment II), and 8-14 days (experiment III) of age. The corresponding doses were also administered at 1 and 8 days as double dose and at 8 days as single dose in experiment II and III, respectively. Chickens were challenged with 50,000 oocysts at 28 days of age and were sacrificed 7 days after injection. Highly significant increase in ceacal length was observed in experiment I and II, whereas no significant difference was noted in experiment II when compared to control. Trickleimmunized chickens showed lesser lesion score, decreased feacal oocyst output, and ceacal oocyst contents when compared to control. Further trickle immunized groups showed higher immunizing response than their corresponding single or double dosed groups against challenge infection. The results suggested that trickle immunization of chickens continously for 1-14 days of age conferred better protection than trickle immunization for 1-7 days or 8-14 days of age.

Key Words: coccidia, chicken, trickle immunisation

1252 gamma-Interferon and IL-2 activities in supernatant of lymphocytes on chicken splenocytes stimulated with concanavalina A. G. Gomez^{*1}, G. Tellez¹, A. Isibasi³, and V. Ortiz², ¹Departamento de Produccin Animal Aves, FMVZ, UNAM , ²Departamento de Biomedicina Molecular del CINVESTAV del IPN, ³Unidad de Investigacin Medica en Inmunoquimica del hosp. de especialidades del centro medico nacion.

Previous studies have shown a protection for the experimental challenge against infections resulting from Salmonella gallinarumand Salmonella enteriditisin chickens, through the manipulation of birds' immunocompetent system by the use of lymphokines (ILK) raw extract obtained from T lymphocytes of hyper-immunized birds with Salmonella gallinarum (ILK-Sg) or Salmonella enteriditis (ILK-Se), and re-stimulated with concavaline A. In order to identify interleucines present in the ILK, the presence of interleucine-2 (IL-2) and Interferon was assessed. The results obtained show the presence of both interleucines in ILKSe. The chicken IL-2, as in other mammals, participates in the proliferation of T lymphocytes; however, a significant difference compared to other species is that there was a species barrier in its biological activity. The INF induced an increase in the expression of class I and class II molecules of the Major Histocompatibility Complex (MHC). The presence of INF in the supernatant, suggests that this interleucine could be participating in the protection effect which is conferred by the administration of ILK, encouraging macrophages and heterophils activation, which could be responsible for the bacteria elimination. Besides, these studies offer more evidences indicating that the observed immunity in the in vivo studies against Salmonella gallinarum and Salmonella enteriditis is probably induced by these important lymphokines. Studies are in progress to purify and identify the effector lymphocines genes in chickens, as well as a systemic research regarding the mechanism by means of which these lymphokines potentialized their protective effects.

Key Words: interleukines, interferon-gamma, interleucine-2

ADSA Dairy Foods: Microbiology and Cheese Technology

1253 Flavor development of cholesterol-reduced Cheddar cheese slurries. H. S. Kwak*¹, C. S. Chung¹, S. J. Lee¹, and J. Ahn¹, ¹Sejong University.

This study was carried out to find the development of flavor in cholesterol-reduced Cheddar cheese slurries by β -cyclodextrin (β -CD). The cheeses were made by 3 different treatments as followings: 1) control (no homogenization, no β -CD treatment), 2) Treatment A (1000psi homogenization, 10% -CD), and 3) Treatment B (cream separation, 10% β -CD). The cheese slurry was aged for 3 wk. The cholesterol removal was 79.30% (Treatment A) and 91.22% (Treatment B). Among 8 volatile flavor compounds, dimethylsulfide, 2-pentanone and 2-heptanone appeared to be insignificantly increased during 3 wk storage, and no difference was found among treatments as expected. The amounts of acetone and ethylacetate were slightly increased in control at 3 wk, however, no difference was found in others. Ethanol production was dramatically increase at 1 wk and decreased thereafter in all treatments. Most volatile flavor compounds were not significantly different among control and treatment A and B as expected. Based on our results, cheese slurry made by cheese milk after cream separation (36% milk fat) and 10% β -CD treatment showed a highest cholesterol removal. Therefore, this study provided a possibility of cholesterol-reduced Cheddar cheese manufacture without any flavor change.

Key Words: Low cholesterol Cheddar cheese, Flavor, Beta cyclodextrin

1254 Dynamic headspace analysis and sensory characteristics of ewes milk La Serena cheese. Mara Carbonell, Estrella Fernndez-Garca*, and Manuel Nunez, *Instituto Nacional de Investigacin y Tecnologa Agraria y Alimentaria (INIA).*

La Serena cheese is a semi-soft variety, made in Extremadura (Spain) from raw milk of Merino ewes, coagulated with vegetable rennet. The objective of our study was to investigate the composition of the volatile fraction of La Serena cheese, and its correlation with sensory characteristics. Duplicate batches of La Serena cheese were made at 4 dairies during the 4 seasonal periods. Cheese (60-day-old) samples were homogenized with sodium sulfate and an internal standard. An aliquot was extracted in a purge and trap apparatus. Volatiles were concentrated in a Tenax trap at 30C, and desorbed with He during 1 min at 230C directly into the injection port. Gas chromatography was carried out in a HP-6890 apparatus equipped with a HP Innowax capillary column and a HP 5973 mass spectrometer. Peaks were identified by comparison of retention times and ion spectra with those of real standards and of Wiley 275 library spectra. Quantitation was carried out by sum of characteristic ions abundance. Cheeses were scored by 14 trained panelists for quality and intensity of odor and aroma, on a 7point scale. Volatile compounds found in the headspace of 60-day-old La Serena cheeses were 13 aldehydes, 8 ketones, 24 alcohols, 24 esters, 10 hydrocarbons, 14 terpens, 3 sulfur compounds, 3 nitrogen compounds, 7 aromatic compounds and 5 free fatty acids. Most compounds were detected in cheeses made in all seasonal periods, and higher concentrations of most volatiles found in spring cheeses. Some terpens were only detected in winter and spring cheeses. Analysis of variance showed significant seasonal differences of quality and intensity of cheese odor and aroma. Spring cheeses obtained the best quality scores, whereas winter cheeses had a more intense aroma. Odor intensity correlated positively with 2-butanone and 3-methyl-1-butanal, and negatively with terpens, ethyl esters and quality of odor and aroma. A high concentration of 2-butanone was responsible for low quality scores, but high levels of esters resulted in improved quality scores even in the presence of high 2-butanone concentrations, as shown by Principal Component Analysis. Stepwise discriminant analysis of selected volatile compounds achieved a correct classification of 100% cheeses in their respective seasonal periods.

 ${\sf Key}$ Words: Volatile compounds, Sensory characteristics, La Serena cheese

1255 The volatile compounds of raw milk Manchego cheese and their relationship to some sensory attributes. Estrella Fernndez-Garca* and Manuel Nunez, *Instituto Nacional de Investigacin y Tecnologa Agraria y Alimentaria (INIA)*.

Manchego cheese is the best known of Spanish ewes milk cheeses. The objective of this study was to isolate, identify and compare the relative amounts of volatile compounds in 6-month-old raw milk Manchego cheese made throughout the year. The correlation of certain volatile compounds with sensory characteristics is discussed. Cheese (15 g) was homogenized in an analytical blender with sodium sulfate (20 g) and an internal standard. The mixture (2 g) was subjected to helium purge at 50C, 15 min, 10 min previous equilibration. Compounds were concentrated in a Tenax trap at 25C. Desorption was at 230C/0.5 min directly into the injection port at 220C. Chromatography was carried out in a GC-MS system equipped with an HP Innowax capillary column. Main chemical families found in Manchego cheese were aldehydes, methylketones, alcohols and esters. Terpenes, sulfur and aromatic compounds, and hydrocarbons were also detected. Most aldehydes were at higher concentrations in spring cheeses. Major ketones were 2-propanone and 2-butanone. 2,3-Butanedione was more abundant in summer cheeses, possibly due to the higher level of starter inoculation in summer. Alcohols were quantitatively the main chemical family. Large quantities of ethanol, 2-propanol, 2-butanol and 3-methylbutanol were observed, but also of 2-pentanol and 2-heptanol. Branched chain alcohols were at higher concentrations in fall and winter, and at lower concentrations in summer cheeses. Twenty three species of esters were identified, with ethyl, propyl, branched chain alkyl and butyl esters as the most quantitatively important. Branched chain alkyl esters were less abundant in summer cheeses. Abundance of volatile compounds and sensory data were subjected to principal components analysis. Primary and secondary alcohols, 2-methyl-ketones, and branched chain aldehydes and esters showed a high positive correlation with function 1, together with the descriptors rancid and piquant and aroma intensity. Aroma quality together with dimethyl disulfide showed a negative correlation coefficient with function 1. Branched chain alcohols, 2-butoxy-ethanol, butyl glycol acetate, dimethyl sulfide and isoamyl butyrate showed a high positive correlation with function 2, together with the aroma descriptor fruits-flowers.

Key Words: Volatiles, Sensory attributes, Manchego cheese

1256 Effect of long term frozen storage on Manchego-type cheese proteolysis. Esther Sendra^{*1}, Jose Pons¹, Jordi Saldo², Reyes Pla², Montserrat Mor-Mur², and Ventura Guamis², ¹Universidad Miguel Hernndez, ²Universitat Autonoma de Barcelona.

Two batches of Manchego-type cheese were manufactured. After press, curds were distributed in 2 groups of 8 units each; control and treated (which were packaged in polyethylene bags, frozen at an ice front velocity of 1.5 cm/h). Control analysis were run in 1993: before salting (C_0) and after salting and 60 days of ripening (C_{60}) . Treated groups were kept at -20° C during 7 years, defrosted at 0° C and sampled: just defrosted (day 0), after salting (days 1, 30 and 60 of ripening). Overall composition, nitrogen (nt) and nitrogen soluble fractions (ncn: at pH 4.6. nTCA: in 12% trichloroacetic acid) were determined (in duplicate) in all curds and cheese units and their relative indexes were calculated (nnc/nt, nTCA/nt). Moisture significantly decreased during frozen storage. Salt content dramatically increased. An increase in ncn/nt and nTCA/nt was observed in defrosted curds (P<.05). Therefore, rennet and bacterial induced proteolysis still go on at freezing conditions. Proteolysis was more intense in cheese obtained from frozen curds than in control.

	dry matter ¹		salt^1		$nitrogen^1$		ncn/nt		npn/nt	
	X	std	Ā	std	Ā	std	Ā	std	Ā	std
\mathbf{C}_{0}	47.87^{a}	.39	$.29^{a}$.06	2.81^{a}	.03	6.35^{a}	.32	2.42^{b}	.23
0	52.29^{b}	1.13	$.48^{b}$.07	3.37^{b}	.32	9.04^c	.14	5.18^{a}	.25
1	54.18^{c}	.96	2.62^{c}	.19	3.26^{b}	.10	7.35^{b}	.39	5.84^{a}	.26
30	61.36^{d}	.82	2.57^{c}	.19	3.67^{c}	.05	16.59^{d}	.68	17.82^{d}	.77
60	68.54^{e}	.63	3.22^{d}	.04	3.95^{d}	.05	20.99^{e}	.95	16.68^{d}	.68
C_{60}	61.17^{d}	.31	2.10^c	.26	3.32^{b}	.10	21.40^e	.71	15.03^c	.50

 1 (% in wet basis); a,b,c,d,e values with the same letter within columns do not differ significantly (p<.05) by oneway ANOVA analysis.

Key Words: cheese, proteolysis, freezing

1257 Texture of artisan Spanish fresh goat's milk cheese. Esther Sendra^{*1}, Laura Alenda¹, Casilda Navarro¹, Estrella Sayas¹, Juana Fernndez-Lpez¹, and Jos Angel Prez-Alvarez¹, ¹Universidad Miguel Hernndez.

Servilleta is an artisan fresh goat's milk cheese that has not been typified or characterized. Distinctive characteristics are pasteurization conditions (82°C 10s), wrapping and pressing in cotton gausses with no use of molds and mild salting. Cheeses were sampled from 3 manufacturers, 3 times at monthly intervals (total: 9 samples per manufacturer). Overall composition (triplicate), stress relaxation test (4 replicates) and 80% uniaxial compression test (6 replicates) were run. Oneway ANOVA analysis was run (P<.05). There is no uniformity in the textural and compositional characteristics of commercial artisan Servilleta cheese. Its moisture content is higher than that of other fresh goat's milk cheeses. It is soft, and its viscoelastic parameters showed high elasticity and a great fluid behavior. The high pasteurization temperature may affect moisture retention and textural properties.

	$dry matter^1$		salt^1		fracture $force^2$		$fracture point^3$		r	
	Х	std	Х	std	Х	std	Х	std	Х	std
1	37.91^{a}	.77	$.27^{a}$.02	16.18^{a}	1.79	44.65^{a}	1.34	$.07^{a}$.0
					15.92^{a}				$.07^{a}$.0
3	43.69^{b}	.58	$.72^{c}$.02	32.43^{b}	1.79	54.91^{c}	1.33	$.08^{b}$.0

¹ (% in wet basis), ² (Newtons), ³ (distance in % of deformation); r reflects the #rate# at which the stress relaxes (r=0 in an ideal elastic body where stress does not relax); ^{a,b,c} values with the same letter within columns do not differ significantly (p<.05) by oneway ANOVA analysis.

Key Words: texture, cheese, goat

1258 Survivability of Probiotic Cultures in Symbiotic Goat's Milk Yogurt. Patricia Buldo*, Velitchka Gotcheva, and Mingruo Guo, University of Vermont, Burlington VT.

The ingestion of prebiotics and probiotics is proved to have a positive effect on maintaining healthy balance of the microflora in human digestive system. In this study, symbiotic goat's milk vogurt prototypes were prepared by combining probiotic cultures (Lb. paracasei ssp. paracasei, Lb. acidophilus, and Bifidobacteria) with prebiotic substances (fructooligosaccarose, FOS, and inulin). Changes in pH, titratable acidity (TA), and survivability of the probiotic cultures in six prototype yogurt products, i.e., control, fortified with goat's milk powder (4%), FOS (1 and 3%), and inulin (1 and 3%) were evaluated during nine-week refrigerated storage (4°C). Titratable acidity and pH were within the ranges of 1.11 to 0.89% and 4.26 to 4.06, respectively, throughout the period. Selective media (LP-MRS and B-MRS) were used to enumerate the viable cells of the probiotic strains during the storage. The effect of the prebiotics on the cultures was also studied. In all of the yogurt combinations, the three probiotic strains showed good survivability, starting at levels of 10^9 CFU/g and decreasing to about 10^7 CFU/g after 9 weeks of storage. The addition of milk powder seems to improve the viability of the problem to the other combinations. The survivability of the three strains varied, but, in general Lb. acidophilus and Bifidobacteria maintained higher concentrations than Lb. paracasei ssp., except for the sample fortified with milk powder, in which Lb. acidophilus and Lb. paracasei ssp. showed better viability than Bifidobacteria. Chemical composition and the mineral profiles (Ca, K, Mg, Na, and Zn) of the six yogurt prototypes were also analyzed. There are no considerable differences in concentrations of all the minerals analyzed between the control and the samples fortified with FOS, and inulin but the sample fortified with milk powder. In conclusion, the probiotic cultures in all prototypes remained at acceptable level $(>10^7)$ for more than two months under refrigerated conditions. The addition of the prebiotic substances in the goat's milk yogurt seemed to have no positive impact on the survivability of the probiotic strains. The addition of milk powder contributed a better texture of the yogurt, as well as higher values of CFU/g.

Key Words: Pre-, probiotics, yogurt, goat's milk

1259 Protein profiles and rheological properties of fresh goat milk cheese. D. L. Van Hekken^{*1}, M. H. Tunick¹, and Y. W. Park², ¹USDA-ARS-ERRC, ²Fort Valley State University.

Soft fresh goat milk cheese is a highly valued dairy food, yet its production is limited by seasonal milk supply and its availability by short shelf-life. Protein profiles and rheological properties of the fresh cheese were determined to examine their variation during refrigerated storage. The cheeses were purchased from a commercial manufacturer within 24 hr of packaging and stored at 4°C for 7, 14, and 21 d. Protein extracts were prepared using either SDS or urea buffers, and protein profiles were examined using SDS-PAGE. Rheological properties were measured using a universal testing machine and a small strain oscillatory analyzer. Over the three week storage time, β -case decreased approximately 10% with a concomitant increase in the $\beta\text{-case$ in fragments. During thethree weeks of storage, rheological properties averaged 2.9 \pm 0.5 N for hardness, 9.9 ± 0.4 mm for springiness, 21 ± 3 kPa for elastic modulus, $6.5\,\pm\,0.9$ kPa for viscous modulus, and $0.22\,\pm\,0.03$ kPa-s for complex viscosity. Only cohesiveness significantly changed over that time with a decrease from 0.25 to 0.12. Storage of fresh soft goat cheese at $4^{\circ}C$ for up to 21d showed minimal changes. Protein profiles and cohesiveness were the best indicators of change in fresh soft goat milk cheese as it aged and may be useful in monitoring shelf-life.

Key Words: Goat Cheese, Rheology, SDS-PAGE

1260 Characterization of potential probiotic and milk fermenting properties of lactic acid bacteria strains. Velitchka Gotcheva*², Ely Hristozova³, Tsonka Hristozova⁴, Angel Angelov², Zlatka Roshkova², and Mingruo Guo¹, ¹University of Vermont, Burlington 05405, ² Higher Institute of Food and Flavor Industries, Plovdiv, Bulgaria, ³Medical Academy, Plovdiv, ⁴Institute of Microbiology, Plovdiv.

Several species lactic acid bacteria were isolated from a traditional Bulgarian cereal-based fermented beverage. These strains have been consumed for ages and proved to be safe. Two strains Lb. plantarum (B25 and B28), and one Lb. casei ssp. pseudoplantarum(B29) were studied in the present work for selected probiotic properties. Acid and bile resistance of the strains were evaluated. Results showed that the LAB tested are tolerant to low pH values (2.0-3.0) and bile concentration within 0.2-0.4%. Further, tests for antagonistic activity against some of the most common pathogens (Salmonella, Pseudomonas, E. coli, and Enterococcus) were carried out. Lb. plantarum B28 has a good antagonistic activity towards almost all the test-pathogens. Resistance of the LAB strains to the most commonly used antibiotics was also evaluated. Thirty-nine antibiotic compounds were used for the experiment. Strain Lb. plantarum B25 was resistant to 13 of them, and Lb. plantarum B28 was unaffected by 18 of the antibiotics. The third strain, Lb. casei ssp. was resistant to 16 of the substances tested. The potential use of the three LAB strains in milk fermentation was studied as well. They were tested for their ability to coagulate milk singly or in combinations. Fermentation trials showed that LAB at concentration 10 5 CFU/ml coagulated milk in about 10 h at 43° C. Incubation time varied for the three single strains and the combinations to clot the milk samples. Lb. plantarum strains had a faster rate of acid production than Lb. casei spp.. The combination of the three strains resulted in fermented milk with good texture and flavor. In conclusion, the LAB strains isolated from the cereal-based product have the potential to be probiotics. Further investigations are needed to evaluate other probiotic properties of the LAB strains and their milk coagulation characteristics.

1261 Melt and proteolysis of Mozzarella cheese as affected by starter culture and coagulating enzymes. P. Sharma*¹, R. I. Dave¹, K. Muthukumarappan¹, D. J. McMahon², and J. R. Broadbent², ¹MN-SD Dairy Center, South Dakota State University, Brookings, SD 57007, ²Western Dairy Center, Utah State University, Logan, UT 84322.

Low moisture part-skim Mozzarella cheeses were made to elucidate if the melt characteristics of mozzarella cheese were affected by starter cultures, or coagulating enzymes and how they were correlated with the proteolysis. Cheeses were prepared using single culture (SC) of Streptococcus thermophilus, or mixed culture (MC) of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. helveticus using $1\times$ or $6\times$ of cheese coagulants, Rennet or Sure curd. Cheeses were analyzed for total solids, fat, protein, ash, salt, and calcium on day 1. Changes in melt characteristics and proteolysis during storage (4°C) were monitored on 1,7,15, and 30 days (d). The melt characteristics were measured using modified Schreiber test and SDSU-meltmeter (Creep test). Proteolysis was measured using capillary electrophoresis and soluble nitrogen. After 30 d storage, melt area as measured by modified Schreiber test increased only by ${\sim}2$ times in cheeses made with ST as against ${\sim}3\text{-}4$ times in the cheeses made with MC. On d 30, creep test showed that the fall in height increased by \sim 4-5 times in the SC cheeses but \sim 7-9 times in the cheeses made with MC, when compared with the values obtained on d1 for SC cheeses. Melt characteristics on d 7 for cheeses prepared with MC almost corresponded to that of d 30 cheeses made with SC; suggesting faster ripening and better improvement in the melt of cheeses made with MC. Soluble nitrogen was also higher in MC cheeses as compared to those made with SC only. The highest % breakdown of α_s case in was ${\sim}75\%$ on d 30 in cheese samples prepared with MC using $6 \times$ rennet enzyme and that of β case in was ~50% in cheese samples prepared with MC using $6 \times$ sure curd. Results clearly indicated that selection of starter culture followed by type and concentration of coagulants were crucial in deciding the melt and rheology of part-skim Mozzarella cheese.

Key Words: Mozzarella cheese, Melt characteristics, Proteolysis

1262 Effects of Refrigerated Storage on Proteolytic and Lipolytic Properties of Soft Goat Milk Cheeses Manufactured in a Southern U.S. State. Aref Kalantari^{*1}, Young W. Park¹, and Diane Van Hekken², ¹Agricultural Research Station, Fort Valley State University, Fort Valley, GA, ²Eastern Regional Research Center, USDA/ARS, Wyndmoor, PA.

Information on shelf-life of commercial goat milk cheeses is extremely limited. Fresh soft goat cheeses were purchased from a commercial goat dairy manufacturer in a southeastern state, and stored at $4^{\circ}C$ for 0, 7, 14, and 21 days for evaluation of shelf-life of the cheeses in relation to proteolytic and lipolytic parameters. One batch of the soft cheeses was frozen, stored for 3 months, thawed, and compared for the differences in shelf-life and storage quality between the fresh and frozen-stored products. Total N, percent water soluble N (WSN), pH and acid degree values (ADV) were determined, and protein profiles of fresh cheeses were examined using SDS-PAGE and densitometric analysis. The percent WSN ranged from 4.4 to 11.1 in the fresh cheeses for 3 weeks storage, and WSN increased with storage period, which occurred with the concomitant decrease in beta-casein as revealed in the SDS-PAGE and densitometric results. There was a greater increase in WSN in frozen-stored cheeses than that in the fresh counterparts, suggesting that frozen-storage and thawing accelerated protein degradation in the frozen thawed cheeses, presumably due to the denaturation of cheese proteins as well as activation of some proteinase enzymes. The mean ranges of pH for the testing period in both fresh and frozenstored cheeses were 4.14 to 4.81, and tended to decrease with storage times. ADVs were significantly increased with time, especially at 21 days. ADVs were higher in the frozen-stored cheeses than fresh ones. It was concluded that the food quality of the soft goat cheeses had begun to deteriorate significantly at 21 days refrigerated storage especially when the sealed packages were open and stored, as evidenced by the elevated proteolytic and lipolytic indices.

Key Words: Soft goat cheese, storage, shelf-life, proteolysis, lipolysis

 $\ensuremath{\mathsf{Key}}$ Words: Lactic acid bacteria, probiotic properties, milk fermentation

1263 Fluid milk quality: Microbiological analysis of fluid milk at the carton encoded sell by date. T.J. Pritchard^{*1} and P.S. Kindstedt¹, ¹Northeast Dairy Foods Research Center, University of Vermont.

The objectives of our study were to evaluate the temperature of dairy display cases and to determine the microbiological load of fluid milk at the carton-encoded sell by date. A total of 211 reduced fat fluid milk samples were obtained from 174 dairy display cases throughout Northern New England. Stores evaluated ranged from small mom and pop stores to large multi-state chain stores. Dairy display case temperatures were obtained by recording the temperature of product present in the case. Samples were packaged in ice during transport back to the lab. Milk samples were maintained at 38F or below until the sell by date. Milk samples were diluted in a peptone-NaCl solution and evaluated utilizing both 3M APC Petrifilms and 3M coliform Petrifilms. The temperature of the display cases ranged from 33F to 53F. One hundred forty-two of the coolers registered temperatures at or below the US Food Code designated upper limit of 45F while 32 of the coolers registered temperatures above 45F. The aerobic plate count of the samples ranged from a low of <1 to a high of $>10^8$ CFU/ml. Sixty-eight of the samples were still within Pasteurized Milk Ordinance standards at their sell by date. Two-thirds of the samples, 143 of 211, failed to meet the requirements for fluid milk outlined in the PMO. The majority of the sample which failed PMO standards, 111 of 136, contained 10^6 CFU/ml or more. Eight of these samples contained greater than 10^8 CFU/ml. Analysis of 210 of the milk samples for the presence of coliform bacteria indicated that 90% of the samples, 189 of 210, were at or below the PMO limit for pasteurized fluid milk. Twenty-one samples contained >10CFU/ml of coliform bacteria. Within the samples that failed, counts ranged from 11CFU/ml to $>10^3$ CFU/ml. The results of our survey indicate that dairy display cases are a potential site for temperature abuse of fluid milk which require further scrutiny. Furthermore, the sell by date encoded on packages of fluid milk does not necessarily indicate the microbiological quality of the product contained within.

Key Words: Milk Quality, Microbiology, Shelf-life

1264 Linoleic acid isomerase activity in Lactobacillus acidophilus and Propionibacterium freudenreichii subsp. shermanii. T Lin^{*1}, C Lin², and Y Wang², ¹Chinese Culture University, ²National Taiwan University.

Crude protein extracts of Lactobacillus acidophilus (CCRC14079) and Propionibacterium freudenreichii subsp. shermanii (CCRC11076) were reacted with linoleic acid at 50°C for 10min at pH 5, 6, 7, and 8, and the levels of conjugated linoleic acid (CLA) isomers formation were determined by high performance liquid chromatography. The activity of linoleic acid isomerase was observed in >100 Kd crude protein filtrates of both culture extracts, as evidenced by the formation of conjugated linoleic acid isomers after the reaction. The filtrate of L. acidophilus produced more CLA (1125.63 μ g /mg protein) than P. freudenreichii subsp. Shermanii (620.89 μ g /mg protein). Higher levels of CLA isomers were formed at pH 5 of L. acidophilus treatment. However, no significant difference in CLA formation was observed among four pH treatments of P. freudenreichii subsp. Shermanii. While the amounts of eight CLA isomers produced varied with culture and pH, more c10,t12-CLA was formed, followed by c9,t11-CLA, in most of the treatments.

Key Words: Conjugated linoleic acid, Isomerase, Lactobacillus acidophilus

1265 Comparison of effect of vacuum condensed and ultrafiltered milk on Cheddar cheese quality. M. R. Acharya* and V. V. Mistry, *MN-SD Dairy Foods Research Center, South Dakota State University, Brookings.*

Raw whole milk was separated to approximately 0.4% fat, pasteurized and split in 3 parts. One part (175 kg) was vacuum condensed to 11-12% protein (CM) and another (175 kg) was ultrafiltered to 15-16% protein (UF). The third part, pasteurized cream and CM or UF were blended to case to fat ratio of 0.7 and protein of 4.5% (UF1 and CM1) and 6.0% (UF2 and CM2). No concentrate was added to the control (C), 3.2% protein. Total quantity of blended milk was 100 to 105 kg for C, 72.5 kg for UF1 and CM1 and 54.5 kg for UF2 and CM2. Cheddar cheeses (5 replicates) were made using frozen concentrated starter (7g/kg protein in cheese milk) and rennet (9ml/45.4 kg milk for C and 6ml/45.4 kg milk for the concentrates). At 1 wk, the moisture content of Cheddar cheeses was 39.2 (C), 37.8 (UF1), 36.5 (UF2), 38.5 (CM1) and 36.7% (CM2). At 12 wk it had dropped to 38.8 (C), 37.3 (UF1), 35.6 (UF2), 37.7 (CM1) and 35.5% (CM2). The fat content ranged from 31.5 to 32.4% and salt from 2.05 to 2.14% with no significant differences between treatments. Calcium content was higher in UF cheeses (0.75%-UF1 and 0.79%-UF2) than CM cheeses (0.71%-CM1 and 0.76%-CM2) followed by C (0.68%) and it increased with protein content in cheese milk. UF milk produced cheese with higher protein content (25.6%-UF1 and 26.4%-UF2) than CM milk (24.7%-CM1 and 25.4%-CM2). The soluble protein content of all cheeses increased during 12 wk of ripening. Control cheeses exhibited the highest extent of proteolysis followed by treatments with 4.5 and 6.0% protein in cheese milk, respectively. CM cheeses exhibited higher level of proteolysis than UF cheeses. Counts of lactic starters were in the range of 9-10 logs at 1 wk, which reduced to approximately 6-7 logs after 12 wk, and the counts of non-starter lactic acid bacteria were about 2-3 logs at 1 wk and increased to 4-5 logs after 12 wk, but there were no differences in the counts among treatments. These cheeses will be used for process cheese manufacture.

 $\textbf{Key Words: } Vacuum \ condensing, \ Ultrafiltration, \ Cheddar \ cheese$

1266 Comparison of rennet curd formation characteristics of milk concentrated by vacuum condensing and ultrafiltration. V. V. Mistry*, P. Upreti, and M. R. Acharya, *MN-SD Dairy Foods Research Center, South Dakota State University, Brookings.*

Raw whole milk was separated to 0.4% fat, pasteurized and split into three parts. One part was vacuum condensed to 11-12% protein (CM), and another ultrafiltered to 15-16% protein (UF). The third part, pasteurized cream, and CM or UF were blended to case in to fat ratio (CF) of 0.7 and protein of 4.5% (CM1 and UF1) and 6% (CM2 and UF2). The control (C) was a blend of milk and cream with no concentrate and CF of 0.7 and 3.2% protein. A Formagraph was used to determine rennet clotting time (RCT), amplitude (or curd firmness) at 40 min, (A40), and time to achieve amplitude of 20 mm (k20). Rennet was added at 9-mL/45.4 kg milk and with no starter added. The RCT for C was 33.3 min. It was lower for CM than for UF and for 6% protein than for 4.5%. The k20 for 3.2, 4.5 and 6% protein was 43.5, 8.4 and 4.3 min, respectively, and was not affected by method of concentration. The A40 was dependent on protein (3, 35 and 54 mm for 3.2, 4.5 and 6% protein, respectively) and milk (46 mm, CM and 44 mm, UF). A Brookfield viscometer equipped with a UL adapter was used under a constant rate of strain of 1/306 s to determine viscosity every minute for 40 min at 32° C after addition of starter (same rate as in cheese making) and rennet at 6 and 9-mL/45.4 kg milk. There were three distinct phases. In the first phase there was no change in viscosity. This initial viscosity increased with protein and was higher for the CM than for the UF milk for the same protein content. In the second phase there was a sharp increase until a maximum was reached. In the third phase there was a decline in viscosity. Viscosity began to increase approximately 7 min earlier for samples with 9 mL rennet than with 6 mL. The time at which the maximum viscosity occurred was affected by amount of rennet and occurred earlier in UF than in CM. The maximum viscosity depended on protein content and method of concentration (23.2-CM2, 16.5-UF2, 10-CM1, 8.5-UF1, 5.1 mPa.s- C). For the same protein content maximum viscosity was lower for UF milks than CM.

Key Words: vacuum condensing, ultrafiltration, rennet coagulation

1267 Standardization of cheesemilks using cold ultrafiltration retentates for the manufacture of Parmesan cheese. J.J. Jaeggi^{*1}, S. Govindasamy-Lucey¹, M.E. Johnson¹, and J.A. Lucey², ¹Wisconsin Center for Dairy Research, University of Wisconsin, Madison, Wisconsin/USA, ²Department of Food Science, University of Wisconsin, Madison, Wisconsin/USA.

Milk for Parmesan cheese is generally standardized by cream removal. This method of standardization and the low fat and moisture contents of Parmesan cheese results in low yield efficiency. The addition of NFDM is not usually practiced because of increased concentration of residual sugar in the cheese. One solution to the problem of lower cheese yield efficiency, and one that would overcome the problem of excessive residual sugar, is the use of UF milk. Milks concentrated by cold UF (<7C) were used to manufacture Parmesan cheese. Milks for cheesemaking were made by blending cold (whole milk) UF retentates (26% solids)

with partially skimmed milk (1.0% fat) to obtain blends with 14.5%solids and a casein:fat ratio of 1.1. A second approach was used where skim milk was concentrated by cold UF to 26% solids and blended with partially skimmed milk (3.4% fat) to obtain blends with 14.5% solids at a casein:fat ratio of 1.1. Control milks were partially skimmed to a casein:fat ratio of 1.1 and total solids of 11.3%. Traditional Parmesan manufacturing protocol was followed. Setting times were $16~{\rm and}~22$ min for experimental and control milks, respectively. Following brining for 70 h, the cheeses were dried at 60% relative humidity and 12C in a commercial Parmesan drying facility until a target moisture of around 32% was reached. As expected, cheese yields were higher in the higher solids milks (12-13%) compared to control cheese (7.5%) due to higher protein and fat levels in the cheesemilk. However, there were higher fat losses in the whey, derived from milk blended with whole milk UF retentates, possibly due to fat damage during the UF process. Higher protein recoveries were obtained in cheeses manufactured using cold UF retentates. There was no difference in the pH(5.14-5.15) and moisture contents (40.1-40.6%) of the cheeses prior to brining. No residual lactose or galactose was left in the cheeses. Experimental cheeses had higher moisture contents (33.0-35.2%) than control cheeses (32%) after drying for 9 weeks. It appeared to be more difficult to dry cheeses made from the higher solids cheesemilks, probably due to an altered structure in these cheeses. The use of milk concentrated by cold UF appears to be a promising way of improving the yields of Parmesan cheeses.

$\textbf{Key Words: } Ultrafiltration, Parmesan \ cheese, \ Standardization$

1268 Modulation of colonic microbiota with sweet bifidus milk. Elisa Teshima¹, Celia L. L F. Ferreira^{*1}, Neuza M. B. Costa¹, Ferlando L. Santos¹, and Izabele D. P. Marliere¹, ¹Federal University of Viosa.

The human gastrointestinal tract constitutes a complex microbial ecosystem and colon is the most heavily colonized region. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have both positive and negative effects on the gut physiology. Therefore, there is some interest in manipulation of the composition of the gut microbiota towards a more salutary regimen, increasing the numbers and activities of bacterial groups such as bifidobacteria and lactobacilli. One of approaches to increase the number of these organisms in the gut is through the oral administration of live, beneficial microbes, termed probiotics. Dairy products are the most common carriers of probiotic organisms, mainly in fluid milk, yogurt or fermented milk. Although many products have been commercialized, few probiotics strains were analyzed for its ability of modification on the microbiota composition. New isolates of Bifidobacterium breve from neonates were selected in our laboratory, with high resistance to gastric juice and bile salts, therefore suitable candidates for use as probiotics. The purpose of this study was to elucidate effects of sweet bifidus milk containing a pool of these bacteria in cecal and colonic microbiota levels in rats. Twenty female adults Wistar rats were randomly assigned to one of two treatments: 1) control diet (AIN-93 diet for rodents) or 2) control diet + sweet bifidus milk (Log 8 cfu/ml). The duration of the study was 28 days. The cecal and colonic contend was analyzed for total anaerobes, bifidobacteria, total aerobes, lactobacilli and E. coli using selective media. The consumption of sweet bifidus milk resulted in higher bifidobacteria levels in cecum (Log 8.28 cfu/ml) and colon (Log 8.49 cfu/ml) when compared to levels in cecum (Log 6.55 cfu/ml) and colon (Log 7.06 cfu/ml) of control group (P<0.01). The total anaerobes and aerobes levels were not affected (P>0.05) by the treatment in both segments of the gut. The numbers of E. coli were lower (P < 0.05) in cecum, but not in the colon of probiotic feed animals. Inversely, the numbers of lactobacilli were lower (P < 0.05) in the colon but not in the cecum. Therefore the consumption of sweet bifidus milk may be beneficial in improving gastrointestinal health.

Key Words: Bifidobacteria, Microbiota, Rats

1269 Structural and functional properties of a small cryptic plasmid of *Streptococus thermophilus*. G.A. Somkuti and D.H. Steinberg, *Eastern Regional Research Center, ARS-USDA*.

A small cryptic plasmid designated as pER13 was isolated from the lactic starter bacterium *Streptococcus thermophilus* strain ST113. The complete sequence of pER13 was 4137 bp with five distinct open reading frames (ORFs), some sharing a high level of homology with ORFs of the pMV158 family plasmids found in *S. suis* (SS) and *S. agalactiae* (SA), clinical streptococci which cause diseases in domestic animals and humans. In *S. thermophilus*, ORF1 encodes a 45-residue protein that is identical to the CopG protein in plasmids of SS (pSSU-1, 4.9 kb) and SA (pMV158, 5.5 kb). The ORF2 (rep) encodes a 179-residue protein, shorter than but with high level of homology to the RepB proteins found in the other two species. In addition, the 500-amino acid protein encoded by ORF3 is highly homologous to the mobilization proteins (Mob) encoded on the SS and SA plasmids. The presence of a mob gene in pER13 allows its mobilization and transfer into new hosts by functions of conjugative plasmids in the pIP501-pAM β 1family. When one of these plasmids is electrotransformed into ST113, the subsequent co-transfer of pER13 and pIP501 into lactococci occurs by a conjugative process, possibly involving the formation of a cointegrate as shown by agarose gel electrophoresis. This permits the development of pER13 as a cloning vector and offers another approach to introducing new genetic functions into lactic fermentation bacteria. The presence of several highly homologous ORFs in S. thermophilus, SS and SA also implies the possibility of horizontal gene transfer between clinical and nonclinical species of streptococci.

Key Words: Plasmid, Streptococcus thermophilus

1270 Production and functional properties of dairy products fermented with probiotic bacteria. Sharareh Hekmat*, *Brescia College at University of Western Ontario*.

The concept of probiotics which is the use of bacteria to enhance health has gained increased interest in the dairy food industry. *Lactobacillus acidophilus* and *Bifidobacterium bifidum* are two examples of probiotic bacteria that could be delivered to consumers through fermented dairy products. Some of the potential benefits of these bacteria are maintaining optimal balance of microbial organisms, and producing antibiotics, organic acids and *B*-galactosidase in the intestine. Many dairy products such as milk, yogurt, frozen yogurt, carbonated liquid yogurt, and ice cream could be fermented with these bacteria. This paper reviews the production of different probiotic dairy foods, the survival rate of these bacteria during manufacture and storage, the enumeration techniques, and the optimum growth condition in terms of pH, nutrients, and temperature. It will also examine *B*-galactosidase activity during storage and sensory properties of these fermented dairy products.

Key Words: Probiotic bacteria, Fermented dairy products

1271 Changes in functionality of Mozzarella cheese produced from bovine and caprine milk during refrigerated storage. E. J. Oh¹, J. Y. Imm^{*2}, K. S. Han¹, J. S. Kim², S. Oh³, and S. H. Kim¹, ¹Korea University, ²Korea Food Research Institute, ³Korea Yakult Co. Ltd.

Up to present, only limited information is available for functionality of caprine Mozzarella cheese (MC). The objective of this study was to compare functionality of caprine MC with bovine MC during refrigerated storage. Low moisture part skim MC was prepared from fresh bovine and caprine milk using commercial starter and rennet. Changes in meltability, free oil, browning, texture characteristics and microstructure was monitored for 8 weeks. Proteolysis was determined by measuring 12% TCA and pH 4.6 soluble nitrogen and relative degradation of major caseins was quantified by SDS-PAGE and densitometric scanning. The data were analyzed by ANOVA using PROC GLM of SAS and Duncan's multiple comparison test. Largest increase in meltability was found at early refrigerated storage from 1 to 7 days both for bovine and caprine MC. Except the first day, meltability did not show significant difference between bovine and caprine MC during storage. Bovine MC formed significantly higher amount of free oil than that of caprine MC throughout storage. The major decrease of TPA hardness in bovine MC occurred between 2 and 4 weeks storage, but hardness of caprine MC gradually decreased throughout the storage. Although bovine MC appeared to have denser protein matrix at early storage (2 weeks), degradation of protein matrix in bovine MC was much greater than caprine MC for more than 4 weeks storage. Significantly higher soluble nitrogen content of bovine MC in 12% TCA or pH 4.6 acetate buffer indicated that greater extent of proteolysis was occurred in bovine MC during the storage. Breakdown of α_{s1} -case in was dominant in the storage of bovine MC and only 65% of α_{s1} -case in was remained after 8 weeks storage. For caprine MC, β O-casein showed major change and 77% of initial $\beta \text{O}\text{-case$ in was remained intact. When MC was bakedbovine MC showed significantly higher brown color intensity than that of caprine MC throughout the storage. The intensity of brown color was not significantly affected by storage period for caprine MC.

Key Words: Mozzarella cheese, caprine milk, functionality