820 Addition of probiotic microorganisms to improve proteolysis, sensory evaluation and the release of antihypertensive peptides in Cheddar cheeses ripened at 4 and 8ºC. L. Ong[1], N. P. Shah*[1], and A. Henriksson[2], [1]*Victoria University*, *Werribee, Victoria, Australia*, [2]*DSM Food Specialties*, *Moorebank, NSW, Australia*.

Our objectives were to assess the influence of probiotic microorganisms and ripening conditions on proteolysis, organic acid production and sensory evaluation of Cheddar cheeses, and to examine the release of angiotensin-converting enzyme (ACE)-inhibitory peptides during ripening. Cheddar cheeses were made using starter culture and probiotic microorganisms and ripened at 4 and 8°C for 24 wk. Proteolytic patterns were examined using SDS-PAGE and by monitoring water soluble nitrogen, PTA-soluble nitrogen and TCA soluble nitrogen. Organic acids were analysed using HPLC. Semi-trained panellists (n=15) were used in sensory evaluation. ACE-inhibitory activity was measured using spectrophotometric method. Peptides were purified using RP-HPLC and identified using MALDI-TOF. Addition of probiotic organisms and higher ripening temperature increased proteolysis and the concentration of lactic, acetic and propionic acids in Cheddar cheeses ($P< 0.05$). Acceptability of probiotic cheeses during sensory evaluation was not affected by the increased proteolysis and organic acids concentration. The ACE-inhibition of the cheeses stored at 8°C was not significantly different to those stored at 4°C. The ACE-inhibitory activity of the probiotic cheeses (IC_{50}, 0.15-0.20 mg/mL) was higher compared to the cheeses without probiotic (IC_{50}, 0.22 mg/mL) after 24 wk of ripening. The lowest value of the IC_{50} (0.15 mg/mL), and therefore the highest ACE-inhibitory activity, corresponded to cheeses made with the addition of *Lb. acidophilus* LAFTI® L10 ripened at 8°C. Various ACE-inhibitory peptides from the water-soluble extract of the cheeses corresponding to α_{s1} -casein [(f1-6), (f1-7), (f1-9), (f24-32) and (f102-110)], and β-casein [(f 47-52) and (f193-209)] were identified. Probiotic organisms used in this study can be added successfully in Cheddar cheeses for their health benefits while simultaneously producing bioactive peptides for further health attributes.

Key Words: Probiotic, Cheese, Angiotensin Converting Enzyme

821 Ras cheesemaking using starter cultures and nonstarter lactic acid bacteria isolated from the Pharos land. M. El Soda*, S. Awad, and N. Ahmed, *Faculty of Agriculture, University of Alexandria*, *Alexandria, Egypt*.

Consumers make their choice of cheeses primarily based on flavor characteristics. Cheese flavor results mainly from the biochemical activities of the starter and nonstarter lactic acid bacteria. Ras cheese is the main hard cheese made in Egypt; it is also appreciate in many neighbor countries. The cheese has been manufactured for a long time from raw milk. Nowadays Egyptian regulations require the cheese to be made from pasteurized milk for hygienic reasons and for consistency purposes. The aim of the present communication was to develop cheese culture mixtures for Ras cheesemaking. Cultures isolated, identified and selected in the Laboratory of Biochemistry of Dairy Microorganisms were evaluated during Ras cheesemaking. The cheese was made according to the conventional method and analyzed for chemical composition. Free fatty acids (FFA) and free amino acids (FAA) were determined to follow the ripening process. The resultant cheese was also evaluated for organoleptic properties. The variations were statistically analyzed during cheesemaking and ripening. Starter or non-lactic acid bacteria did not influence cheese composition during manufacture or ripening. On the other hand, levels of FFA, FAA correlated well with the rate of ripening and flavor intensity of Ras cheese. Several of the tested mixtures produced the typical flavor and texture of Ras cheese. The highest overall score of flavor intensity, flavor and texture acceptability was detected in the cheese made using a commercial starter in addition to an adjunct mixture isolated from the Egyptian dairy environment composed of several lactobacilli in addition to *Enterococcus faecium*.

Key Words: Ras Cheese, Starter Culture, Lactobacilli

822 Microbiological evaluation of commercial cream cheese. A. Losambe* and P. S. Kindstedt, *University of Vermont*, *Burlington*.

Currently, the literature contains very little information relating to the microbiological characteristics of commercial Cream cheese. Furthermore, little is known about the potential for microbial-induced syneresis in commercial Cream cheese. The objective of this research was to evaluate microbiological characteristics of commercial Cream cheese samples during refrigerated storage and under temperature abuse conditions. Retail samples of Cream cheese representing three different brands were obtained from local sources. Three retail samples of each brand, each with a different expiration date, were obtained and stored at 4°C. Split samples were evaluated for aerobic plate count (APC) and aerobic spore count (ASC) immediately upon receipt (≥60 d before expiration) and again on the expiration date printed on the package. Also, split samples were subjected to temperature abuse at 20°C for 14 d and then enumerated for APC and ASC. Effects of storage time (at receipt v. at expiration) and temperature abuse on microbial counts were evaluated ANOVA using a repeated measures design. Upon receipt, all 3 brands of Cream cheese had very low APC values (1.70±1.56 log CFU/g) which did not differ significantly among the 3 brands. Similarly, initial ASC values were also very low (1.50±1.47 CFU/g) and did not differ among brands. APC and ASC values at the expiration date did not differ significantly from their initial values, indicating little or no microbial growth during storage at 4°C for ≥60 d. Similarly, APC and ASC values after temperature abuse at 20°C for 14 d did not differ significantly from the initial values. None of the samples displayed any visible syneresis at the expiration date or after temperature abuse. In summary, all of the samples resisted syneresis and had very low microbial counts, comprised mostly of aerobic spore formers, which remained low throughout product shelf life and during temperature abuse.

Key Words: Cream Cheese, Microbiology, Syneresis

823 Microbiological and sensory characteristics of Prato cheese obtained from milk with different levels of somatic cells. P. C. B. Vianna[1], G. Mazal[1], M. V. Santos*[2], H. M. A. Bolini[1], and M. L. Gigante[1], [1]*State University of Campinas*, *Campinas, São Paulo, Brazil*, [2]*University of São Paulo*, *Pirassununga, São Paulo, Brazil*.

Mastitis, an inflammatory reaction of the mammary gland, is characterized by increased somatic cell count (SCC) and by the higher concentrations of antimicrobial substances in milk. Antimicrobial

substances, originating from blood or secreted by somatic cells, may influence the growth and metabolism of starter bacteria used in cheese production. The objective of this research was to evaluate the effect of two levels of raw milk SCC on microbiological and sensory characteristics of Prato cheese, during ripening. Two groups of animals were selected to obtain milk with low (≤200,000 cells/ml) and high (≥700,000 cells/ml) SCC. Prato cheese was manufactured by traditional method and evaluated after 3, 9, 16, 32 and 51 days of storage for lactic bacteria, psychrotrophs, and yeasts and moulds counts. A 2x5 factorial design with four replications was performed. The sensory evaluation of cheeses with low and high SCC was carried out for firmness and taste attributes using nine points just right scale and for general acceptance using hedonic scale of nine points after 8, 22, 35, 50 and 63 days of storage. The SCC level only affected the lactic bacteria count of the cheeses. Cheeses produced with the low-SCC milk presented, in average, higher lactic bacteria count than cheeses produced with the high-SCC milk. A negative effect of time of storage was observed in lactic bacteria and psychrotrophic counts, while yeast and moulds counts were increased during storage time. Interaction of SCC x storage time significantly affected the lactic bacteria count. The presence of antimicrobial factors may be responsible for lower counts and the behavior of the lactic bacteria in cheeses produced with high SCC milk. Cheeses produced with the low-SCC milk had better overall acceptance in sensory evaluation by panelists, which may be explained by differences in firmness and flavor of the cheeses during storage time.

Key Words: Somatic Cell, Prato Cheese, Microbiological Characteristics

824 Effect of temperature abuse on water-holding capacity and microbiological characteristics of commercial cream cheese and cream cheese spread. A. Losambe* and P. S. Kindstedt, *University of Vermont*, *Burlington*.

Previous laboratory-scale studies have suggested that microbial growth in Cream cheese during refrigerated storage or as a result of temperature abuse may contribute to the development of syneresis defect, a costly problem for the industry. Cream cheese spread is higher in moisture than Cream cheese and typically has sorbic acid added as a preservative. Little is known about the potential for microbial-induced syneresis in commercial Cream cheese and Cream cheese spread. The objective of this research was to evaluate the water-holding capacity and microbiological characteristics of commercial Cream cheese and Cream cheese spread samples before and after temperature abuse. Three retail samples of Cream cheese and Cream cheese spread representing 3 different brands were obtained from local supermarkets. All samples had ≥60 d remaining before expiration. Split samples were stored at 20°C for 14 d and evaluated before and after storage for aerobic plate count (APC), aerobic spore count (ASC), and expressible serum (ES), obtained by centrifugation at 12,500 x g for 75 min at 25°C. Typical colonies were picked from APC and ASC and identified using the RiboPrinter® system. Effects of cheese type (Cream cheese v. Cream cheese spread) and temperature abuse on ES and microbial counts were evaluated by ANOVA using a repeated measures design. Upon receipt, Cream cheese and Cream cheese spread samples had very low APC (1.80±1.49, 1.66±.32 log CFU/g) and ASC (1.53±1.0, 1.43±0.9 log CFU/g) values, which did not differ significantly. APC, ASC and ES values before and after temperature abuse did not differ significantly. Colonies picked from APC and ASC were identified as Bacillus pumilus, Bacillus cereus, Bacillus subtilis and Bacillus lichenformans. In summary, all of the samples had very low microbial counts, comprised mostly of aerobic spore formers, which remained low despite temperature abuse. Temperature abuse did not cause a loss of water-holding capacity in these microbiologically stable samples.

Key Words: Cream Cheese, Microbiology, Syneresis

825 New alternative approaches to study cheese microstructure. M. Caccamo*[1], G. Impoco[2], F. Zanini[3], G. Tromba[3], P. Campo[1], S. Carpino[1], and G. Licitra[1,4], [1]*CoRFiLaC, Regione Siciliana*, *Ragusa, Italy*, [2]*IPLAB, University of Catania*, *Italy*, [3]*Sincrotrone Trieste S.C.p.A.*, *Trieste, Italy*, [4]*D.A.C.P.A. University of Catania*, *Italy*.

Scanning Electron Microscopy (SEM) is nowadays widely used to study and characterize cheese microstructure. SEM imagery offers the advantage of high resolution digital scanning. Several recent studies have applied computerized systems of image analysis to SEM images in order to obtain quantitatively valuable features of cheese microstructure. Nevertheless SEM maps a 3D structure of the scanned surface to a 2D. It does not allow therefore direct in-depth measurements. Two alternative approaches are proposed to overcome the problem of lack of information and creation of artifacts due to sample preparing techniques for SEM analysis. The microstructures of 9 Sicilian cheeses (5 pressed and 4 pasta filata) were analyzed using stereoscopy applied to SEM images and X-ray microtomography techniques. Stereoscopy mimics the characteristics of the human visual system by reconstructing 3D information from 2D images that are required to partially overlap. Two images of the same sample were taken with a small displacement (3mm vertically, 4mm horizontally). These scans are accurately aligned such that the structures in small overlapping areas match between different images. 3D models of the scanned cheese surface were generated using a software system that mimics human binocular vision. X-ray microtomography does not require a special sample preparation. This technique allows to obtain a 3D representation of the inner structure of a sample from a set of projection measurements recorded from a certain number of points of view. In the common case of X-ray absorption tomography, the structure of the object under study is represented by the linear attenuation coefficient, which is proportional to the mass density. The microtomographic investigations were carried out using the SYRMEP beamline of the Elettra Laboratory in Trieste, Italy. The samples, mounted on a rotation stage, were illuminated by monochromatic radiation (E = 16 KeV). For each tomographic set 1440 projections of the sample were acquired for equally spaced rotation angles and measurement times of 1 s over a total rotation of 180 degrees. The final result was visualized by 3D rendering by 2D slices using the ImageJ software.

Key Words: Microstructure, Stereoscopy, X-ray

826 Enhancement of flavour profile of cheddar cheese using microencapsulated enzymes. K. Kailasapathy* and S. Seneweera, *University of Western Sydney*, *NSW, AUSTRALIA*.

To accelerate cheese ripening and to improve cheese flavour, exogenous enzymes are used in the cheese industry. Direct addition of free enzymes however, leads to premature proteolysis, glycolysis, and

lipolysis. The aim of our study was to develop an efficient hydro-gel enzyme encapsulation process for the controlled release of flavour-enhancing enzymes into the cheese matrix, and to study proteolysis during cheese ripening using a reverse-phase HPLC. Flavourzyme (commercial protease-peptidase enzyme mixture from Novozymes Pty. Ltd) when encapsulated using 1.8% (w/v) alginate and polymerised in 0.1M calcium chloride solution and 0.1% (w/v) chitosan solution, produced 72% encapsulation efficiency. When the hydro-gel capsules containing enzymes were incorporated during cheese manufacture, the enzymes were released and accelerated cheese ripening. Four batches of cheddar cheese were prepared without enzyme (control) and with encapsulated enzyme. Sampling was carried out during various stages of cheese ripening (0, 1, 7 and 10 weeks) to assess ripening. Free amino acid, water soluble and insoluble fractions of nitrogenous compounds in the ripening cheese were separately analysed by RP-HPLC and SDS-PAGE. Addition of encapsulated enzyme accelerated the cheese proteolysis by producing a large number of low to medium size molecular mass peptides in the water soluble nitrogenous fraction, some of which were unique for the enzyme treated cheese. In contrast, analysis of water insoluble nitrogenous fraction showed that hydrolysis of casein after 70 days of maturity was greater in enzyme treated cheese. Particularly, beta-casein degradation was rapid compared to alpha-casein and kappa-casein in the enzyme treated cheese. This study showed that alginate microcapsules can be effectively used to release flavour enhancing enzymes into cheese matrix to accelerate ripening and improve the flavour profile of cheddar cheese

Key Words: Cheddar Cheese, Microencapsulation, Accelerated Cheese Ripening

Dairy Foods: Milk Proteins and Enzymes: Proteomics and Milk

827 Recent developments in proteomics: Implications for dairy protein research. P. Qi*, *USDA-ARS-ERRC*, *Wyndmoor, PA*.

Proteomics, the systematic study of the identities, quantities, structures, and biochemical and cellular functions of all proteins in a cell, tissue or organism, promised a rapid transformation for biological and medical research in the post-genomic era. Tremendous progress has been made over the past decade in this highly mass spectrometry dependent discipline of systems biology. Proteomics is regarded as a comprehensive research tool not only capable of identifying and quantifying large sets of proteins but also can be used to determine their localization, interactions, modifications, activities, and functions. Recent developments in proteomics research that include protein separation methods, mass spectrometric instrumentation, computational analysis, and integrative databases will be reviewed with the focus on post-translational modifications. In light of the current status and the perspectives of proteomics applications, we will discuss the implications for future dairy protein research suc as structural and functional studies of milk fat globule membrane proteins and other low abundance yet biologically important proteins in milk and dairy products.

Key Words: Proteomics, Post-translational Modification, Milk Proteins

828 Quantitative proteomic analysis of bacterial enzymes released in cheese during ripening. V. Gagnaire, D. Molle, J. Jardin, and S. Lortal*, *INRA*, *Rennes, France*.

Bacterial ecosystems contribute to the cheese ripening not only during their growth but also after their lysis, through the release of numerous proteins in the cheese curd, including enzymes. A prefractionation method allowing the isolation of bacterial proteins from cheese extracts was previously developed as well as a proteomic qualitative survey during Swiss cheese ripening (Gagnaire et al., 2004. Int. J. Food Microbiol.,94,185). Numerous peptidases coming from the lactic starters were identified on 2D-gel electrophoresis as well as many glycolytic enzymes and stress proteins. This new insight even if very informative provided only qualitative data. The aim of this work, using new iTRAQ technique (isobaric tagging reagent for quantitative proteomic analysis), was to provide quantitative proteomic data about the bacterial proteins released at different stages of ripening. This technique is based upon chemically tagging the N-terminus of peptides generated from protein digests, which are then fractionated by nanoLC and directly analysed by tandem mass spectrometry.

Experimental Swiss cheeses were performed in our laboratory using microfiltered milk in order to remove the initial bacterial raw milk contamination. Thermophilic lactic acid bacteria (L. helveticus LH1 and S. thermophilus ST20) and propionibacteria (P. freudenreichii P23) were used as starters. At four times of the ripening (day one, entrance, middle and end of the warm room ripening), cheese aqueous extracts were prepared and fractionated to separate bacterial proteins. To standardize the protein content of each sample before proteomic analysis, a total amino acid analysis was performed. The standardized samples were i) analysed by 2D-electrophoresis for qualitative analysis and ii) submitted to trypsinolysis. Each tryptic hydrolysate was labelled with a specific iTRAQ tag (one tag per ripening time) and submitted to a LC-ESI-MS/MS analysis. This technique provided the identification of the bacterial proteins released and their respective abundance at different times of the ripening.

Key Words: Proteomic, Cheese, Enzyme