192 Proteolysis and texture development in Prato cheese made with different coagulants. C. Merheb-Dini*[1], L. S. Alves[1], E. Gomes[2], R. da Silva[2], and M. L. Gigante[1], *[1]Faculty of Food Engineering, University of Campinas - UNICAMP, Campinas, SP, Brazil, [2]Instituto de Biociências, Letras e Ciências Exatas, UNESP - Univ Estadual Paulista, São José do Rio Preto, SP, Brazil.*

This work had the aim of comparing the effect of different coagulants on the ripening of a typical Brazilian cheese. Prato cheese was made using the following coagulants: laboratory obtained protease, from the fungus Thermomucor indicae-seudaticae N31, recently isolated in Brazil (Thermomucor cheese) and commercial coagulant from *Rhizomucor* sp. (Alternative, Bela Vista) (Control cheese). Fifty liters of milk were enzymatic coagulated in vats with heating-cooling jacket, stirrers and speed control. After cutting, the mass was cooked (42°C) and washed and, after whey drainage, chesses were pressed. For both chesses the amount of enzyme added was calculated to achieve milk clotting in ≈35 min. Cheese ripening was periodically monitored for 50 d. A 2 × 6 factorial design with 3 replications was performed and the results were evaluated by ANOVA and mean values were compared by Tukey's test ($P < 0.05$). Proteolysis (NS pH 4.6 (%NT)) and firmness were affected by coagulant type and by ripening time. Thermomucor cheese exhibited lower proteolysis and higher firmness and Control cheese exhibited higher proteolysis and lower firmness. For both cheeses, proteolysis increased and firmness decreased throughout ripening. However, the interaction between coagulant type × ripening time was not significant for the evaluated parameters. The capillary electropherograms showed that the protein hydrolysis profile of both cheeses was very similar exhibiting degradation of casein fractions α_{S1}-CN 8P, α_{S1}-CN 9P, β-CN A^1, β-CN A^2 and formation of hydrolysis products α_{S1}-I-CN 8P, α_{S1}-I-CN 9P and γ-CNs. This behavior was equivalent to the classic and expected profile of chymosin made cheeses: hydrolysis of α_{S1}-CN, by the residual coagulant during initial stages of ripening on the bond Phe23-Phe24, resulting in the formation of α_{S1}-I-CN and hydrolysis of β-CN by plasmin, resulting in the formation of γ-CNs. The data showed that ripening developed in the same way for both cheeses suggesting the potential of protease from Thermomucor indicae-seudaticae N31 as milk clotting agent for industrial scale cheese production. Acknowledgments: FAPESP, CNPq.

Key Words: ripening, capillary electrophoresis

193 Application of an improved powder X-ray diffraction method to evaluate cheese crystals. G. Tansman*[1], P. S. Kindstedt[1], and J. M. Hughes[2], *[1]Department of Nutrition and Food Sciences, University of Vermont, Burlington, [2]Department of Geology, University of Vermont, Burlington.*

Cheese crystals have been studied for over a century, often with the goal of eliminating visible crystals. With the resurgence of artisan cheese making in America and globally, the incidence and morphological diversity of visible crystals appear to be on the rise. During the 1930s through 1970s several investigators used powder x-ray diffraction (PXRD) to identify cheese crystals. However, attempts to optimize analyses using newer advanced PXRD instrumentation are limited. The objectives of this research were to develop an improved PXRD method for cheese crystal analysis and to demonstrate test capabilities by identifying major and minor components of crystal complexes in Cheddar, Gouda and Asiago cheeses. Cheese samples were obtained from commercial sources. Crystals were physically removed from the cheese surface or cheese interior using a dissecting needle, razor blade, and tweezers. Crystalline species were fractionated and purified through differential solubility in water and acetone, which enabled the identification of minor crystal components that might otherwise go undetected due to a lack of instrumental resolution and baseline noise. Purified crystals were dried in a desiccator for 12 h and ground into power using a mortar and pestle for analysis using a Rigaku MiniFlex II x-ray diffractometer. Surface crystals from aged Cheddar cheese contained crystalline calcium lactate pentahydrate, tyrosine, calcium phosphate and an unidentified crystalline component (UCC). Crystals from the interior of Cheddar contained only calcium lactate pentahydrate. In aged Gouda, interior crystals consisted mostly of tyrosine with lesser amounts of the same UCC found on Cheddar; crystals from the surface of interior eyes contained both tyrosine and the UCC. Interior crystals from Asiago were exclusively tyrosine. Powder x-ray diffractometry, combined with crystal separation though differential solubility, provides a rapid and sensitive means to identify crystalline species that comprise complex visible deposits in cheese. Such information may help to inform future studies aimed at elucidating mechanisms of co-crystallization in cheese.

Key Words: crystal, cheese, X-ray diffraction

194 The effect of the exopolysaccharide producing cultures and adjunct cultures isolated from the Egyptian dairy environment on the texture and sensory characteristics of fat-free Cheddar cheese. M. El Soda* and N. Ahmed, *Faculty of Agriculture, Alexandria University, Alexandria, Egypt.*

Low fat cheeses often suffer from undesirable texture and flavor properties, the exopolysaccharide producing cultures were used in the manufacture of low fat cheeses to increase yield and improve texture. The objective of this communication is to assess the effect of EPS producing cultures and adjunct cultures isolated from the Egyptian dairy environment on the texture and flavor characteristics of fat-free Cheddar cheese. Pilot-scale fat-free Cheddar cheeses were manufactured using commercial Cheddar starter in addition to selected EPS producing cultures and adjunct cultures obtained from our culture collection. Modifications to the cooking temperature, cheddaring conditions and pressing were also performed. The obtained results revealed that the best cheese was obtained using 2 mesophilic lactobacilli producing exopolysaccharides in addition to a 1:1 ratio of 2 strains of *Lactobacillus paracasei* exhibiting debittering activity. The obtained yield for the experimental fat free Cheddar was 8.3%, the values for moisture; fat and protein were 52% ± 0.5, 0.7% and 38% ± 0.8 respectively after 6 mo of ripening. The rheological parameters of full-fat Cheddar were compared with those of the experimental fat-free Cheddar. The obtained data reveal 80% similarity between the 2 cheeses for hardness, chewiness and gumminess, whereas fat-free cheeses showed more cohesiveness and springiness when compared with the full-fat cheese. The use of *Lactobacillus paracasei* strains led to a considerable reduction of bitterness and to the development of the characteristic Cheddar notes in the fat-free cheese. In conclusion, the use of EPS producing lactobacilli, *Lactobacillus paracasei* adjunct and the different modifications to the make procedure led to the production of a fat free Cheddar cheese exhibiting flavor and texture characteristics comparable to the full fat product.

Key Words: fat-free Cheddar, adjunct, exopolysaccharide

195 Effect of milk protein concentration on the microstructure and properties of full-fat Cheddar cheese during ripening. K. Soodam*[1,2], L. Ong[1,2], S. E. Kentish[1], and S. L. Gras[1,2], *[1]Department of Chemical and Biomolecular Engineering, The University of Melbourne, Melbourne, Victoria, Australia, [2]Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria, Australia.*

Ultrafiltered retentate (UF) can be used to standardize cheese milk and can potentially affect cheese yield but few studies have examined the effect of added UF on the microstructure of the cheese and linked these to the properties observed during ripening. This study investigated the effect of increasing the milk protein concentration on the microstructure, as well as the biochemical changes that occur during the ripening of Cheddar cheese. Advanced microscopy techniques, including confocal scanning laser microscopy and cryo-scanning electron microscopy (cryo-SEM) were coupled with textural, sensory and chemical analyses and applied during ripening. The milk protein level was found to significantly affect the hardness of the cheese. A possible link was also established between the protein level, stretchability and the microstructure of the cheese, as observed with cryo-SEM. Several changes were also observed during ripening for all protein treatments. The protein network within cheese contained fewer branches, as observed by the number of intersections (vertices), as a result of ripening. A significant correlation was also found between the microstructure of the cheese and level of proteolysis as well as the microstructure of the cheese and the cheese texture. Our findings provide new insights into the effect of processing conditions and the effect of the maturation process on the development of cheese microstructure and functional properties.

Key Words: cheese ripening, microstructure

196 Evaluation of an alternative method for the rapid and direct determination of sodium in cheese. J. A. Stankey*, C. Akbulut, J. Romero, and S. Govindasamy-Lucey, *Wisconsin Center for Dairy Research, Madison.*

Currently, Na in cheese is routinely determined via an indirect method using the Cl Analyzer. A direct and rapid method of Na detection is needed in the industry due to the increasing use of Na replacers. An x-ray fluorescence spectroscopy (XRF) method for the determination of Na in cheese was developed and compared with inductively coupled plasma-optical emission spectroscopy (ICP) and Cl Analyzer. Sodium quantification was performed by multi-point calibration with standard cheeses (n = 7) (Cheddar, Gouda, Mozzarella, pizza, nonfat and processed) over the range of 0–5% Na (wt/wt). Amount of Na in the each of cheese standard (cheese discs: 7 × 30 mm) was quantified with the XRF equipment. Method validation was performed and the results for linearity, precision, limit of detection and limit of quantification were determined. Linearity ($R^2 \geq 0.99$) was observed in calibration curves obtained for different cheese standards. Procedure was tested by quantifying the amount of Na in a wide range of commercial cheese samples. Na data obtained by XRF were in good agreement with those from ICP and Cl Analyzer for most commercial cheeses. Lowest concentration of Na (LOQ) that can be determined with an acceptable level of repeatability, precision, and trueness was about 245 mg/100g cheese. Calibration graph for Na quantification in the presence of increasing K levels was created using natural cheeses made with different ratios of Na:K and it was able to predict Na content in cheeses manufactured with K-based salt replacers. The Cl analyzer was less accurate than the XRF when Na-replacers may be present, due to the indirect quantification of Na (by the Cl Analyzer). If quantifying Na in the presence of K using XRF method, it was critical that the calibration plot should be created with standards in the presence of K. The XRF method enables the rapid and direct measurement of Na content in a variety of cheeses.

Key Words: Na, X-ray fluorescence spectroscopy, cheese

197 Proteolysis and microstructure of salt-reduced Cheddar cheese. A. Sheibani*, M. M. Ayyash, T. Vasiljevic, and V. Mishra, *Victoria University, Melbourne, Victoria, Australia,*

The effects of salt reduction on proteolysis and microstructure of Cheddar cheese was investigated. Four levels of dry salting including control (2.5%) and 3 treatments (1, 1.5 or 2% salt) were applied followed by storage for 8 weeks at 4°C. Samples were taken at fortnightly intervals and subjected to chemical composition, proteolysis rate and microstructure analysis. Except 12% trichloroacetic acid-soluble nitrogen (TCA-SN), water-soluble nitrogen (WSN) and 5% phosphotungstic-soluble nitrogen (PTA-SN) significantly ($P < 0.05$) differed among cheeses samples. There was no significant ($P > 0.05$) difference in the release of major peptides among samples during storage, whereas hydrophilic peptides differed substantially. Total free amino acids (TFAA) showed significant difference between experimental cheeses at the same storage time ($P < 0.05$). Significant differences in chemical composition except fat content were observed between cheese samples at every sampling point. There was a significant difference between pH of control cheese comparing with other experimental cheeses. pH values of all treatments increased significantly from about 4.8 to 5.1 after first 2 weeks of storage. In compare with control, microstructures of treatments 1, 2, and 3 were smooth, small voids and close structure. During storage, the structure of Cheddar cheeses became more close and homogenous. Salt reduction treatment significantly influenced cheddar cheese characteristics during storage period.

Key Words: salt reduction, proteolysis, microstructure