556 Release of bioactive peptides and essential amino acids as affected by sodium chloride reduction and substitution in Akawi cheese. Akanksha Gandhi* and Nagendra P. Shah, *The University of Hong Kong, Hong Kong.*

The aim of this study was to evaluate the effects of sodium chloride reduction and its substitution with potassium chloride on selected probiotic bacteria and their functionality in Akawi cheese during storage for 30 d at 4°C. The survival of selected probiotic bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium longum*) and starter bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*), the angiotensin-converting enzyme-inhibitory and antioxidant activities, and the concentrations of essential amino acids as affected by storage in different brine solutions [10% NaCl, 7.5% NaCl, 7.5% NaCl+KCl (1:1), 5% NaCl and 5% NaCl+KCl (1:1)] were investigated. Peptide profiles of cheese extracts, from different salt concentrations, were observed by RP-HPLC and in vitro bioactivities of the peptide fractions were evaluated using Caco-2 cell line. Survival of probiotic bacteria increased significantly in cheeses with low salt after 30 d, and after 20 d for starter bacteria. No significant difference in texture profile of cheeses during 30-d storage was observed except for fracturability and gumminess, which was found to be lesser in cheeses brined with only NaCl solutions. No significant changes were observed in ACE-inhibitory activity and antioxidant activity of the cheeses during storage. Interestingly, concentrations of 4 essential amino acids (phenylalanine, tryptophan, valine and leucine) increased significantly during storage in brine solutions containing 7.5% (w/v) total salt. The study revealed that at total salt concentration of 7.5% (w/v), 50% substitution of NaCl with KCl in Akawi cheese would not affect the activities of potential bioactive peptides, and would lead to increased release of essential amino acids.

Key Words: salt reduction, cheese, essential amino acids

557 Generation of highly antioxidative peptides from purified bovine α_{S2}-casein. Zahur Z. Haque* and Xue Zhang, *Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS.*

Alpha$_{s2}$-casein is particularly rich in π electron rich aromatic amino acids that, in the absence of structural constraints, partake in π-π stacking and interact with positively charged residues (cation-π) resulting in electronic continuity required for rapid quenching of lone-pairs of oxidative radicals. This study investigated isolation of α_{s2}-casein from bovine milk, standardization of its chymotryptic hydrolysis, visualization of resulting peptide profiles by tricine-SDS-polyacrylamide gel electrophoresis (TSDS-PAGE) and size-exclusion high performance liquid chromatography (SE-HPLC), and determination of the antioxidative efficacy of the fractions. Alpha$_{s2}$-casein was isolated from whole casein by a 2-step 1-proponal-precipitation method and hydrolyzed using chymotrypsin (EC 3.4.21.1) because it selectively cleaves peptide bonds adjacent to aromatic residues, increasing the chances for inter-peptide aromatic-stacking. Five mg/mL of α_{s2}-casein was hydrolyzed with chymotrypsin (50 μg/mL) for 5–60 min at 37°C. A novel fixing method with sodium hypochlorite was used to fix the highly amphipathic peptides after TSDS-PAGE and visualization was by silver staining. The original protein was complete hydrolyzed to smaller peptides within 5 min. Peptide profiles determined by SE-HPLC, using a Superdex Peptide 10/300 GL column and 30% acetonitrile (v/v) as the eluent, substantiated the effectiveness of the peptide fixation following TSDS-PAGE peptide profiling. Average molecular weight of each fraction was determined by comparing its retention time with those of molecular weight standards. Data depicted an inverse relationship between molecular weight and the duration of enzymatic hydrolysis. Average molecular weight of the smallest fraction at 5 min of hydrolysis was 981 Da, and it was gradually reduced to 130 Da after 60 min of hydrolysis. As expected all hydrolyzates had oxygen radical absorbance capacity values (2000–3000 μ*M* TE) that were significantly ($P < 0.05$) higher than that of the original protein (580 μ*M* TE). Correlation of the antioxidative efficacy with residual secondary structural constraints and intensity of association tendency of the peptide fractions is presently being investigated.

Key Words: milk protein, reactive oxygen species, oxygen radical absorbance capacity

558 Efficacy of Cheddar whey combined with chitosan in edible coatings to reduce protein-fat oxidation and accumulation of reactive oxygen species in retail-cut catfish fillet. Zahur Z. Haque* and Dipaloke Mukherjee, *Department of Food Science, Nutrition and Health Promotion, Mississippi State University, Mississippi State, MS.*

Retail-cut muscle foods are especially susceptible to oxidative degradation and formation of harmful reactive oxygen species (ROS) owing to exposure to oxygen, bright lights and contact with metal counters. Due to extended cooking at temperatures near the pH optima of most of starter culture proteases during its Cheddar cheese manufacture, the resulting whey (CW) is naturally rich in antioxidative peptides and Maillard reaction products. In the present study, we report the antioxidative efficacy of CW when used in an edible coating, with and without chitosan (Ch), to reduce protein and fat degradation in stored farm-raised retail-cut catfish and the accumulation of ROS in the muscle. Fresh catfish fillet of uniform thickness were cut into cubes of unvarying weight (5g) and geometry, and immersed in dispersions of CW (2% w/v), Ch (1%, w/v) or mixture of both (CW+Ch) in 0.2 *M* McIlvaine's iso-ionic buffer (pH 7) at 22°C for 2 min. Protein and lipid oxidation [evident from carbonyl contents (CC) and peroxide values (PV)] of the samples were determined after storage at 4°C for nil, 1, 3, 5 and 7 d . Accumulation of ROS at cellular level was studied using a fluorogenic molecular probe (CellROX Deep Red Reagent, Life Technologies). Results showed significantly ($P < 0.05$) superior efficacy of the coatings in reducing overall oxidative degradation of samples for most of the storage periods compared with control (immersed only in the buffer). The CW+Ch mixture showed dramatic effects in alleviating lipid oxidation and ROS accumulation. It also reduced CC by ~60% compared with control after 3 d of storage, thus indicating a considerable extension in shelf life. The PV was reduced to ~22 and 16% in the samples immersed in CW+Ch and CW, respectively, after 5 d of storage compared with control. The same samples also exhibited 28 and 21% reduction in ROS accumulation relative to control after the same number of days of storage. The results indicated a vivid potential of this combined approach for combating oxidative degradation of foods and prevention of the formation/accumulation of harmful ROS in foods.

Key Words: reactive oxygen species, muscle food, sweet whey.

559 Influence of fish oil alone or in combination with hydrogenated palm oil on sensory characteristics and fatty acid composition of bovine cheese. Einar Vargas-Bello-Pérez*[1], Gonzalo Íñiguez-González[1], Karen Fehrmann-Cartes[1], Paula Toro-Mujica[1], and Philip C. Garnsworthy[2], *[1]Pontificia Universidad Católica de Chile, Santiago, Chile, [2]The University of Nottingham, Loughborough, UK.*

The objective of the present study was to evaluate the effect of dietary supplementation of fish oil (FO) alone or in combination with hydrogenated palm oil (FOPO) on the fatty acid (FA) profile of milk and cheese from dairy cows and the sensory characteristics of cheese. Nine Holstein cows (173 ± 21 DIM) were used in a replicated 3 × 3 Latin square design with 21-d periods. Dietary treatments consisted of a basal diet (Control; no fat supplement), and fat-supplemented diets containing fish oil (FO; salmon oil; 500 g/d/cow) and FO (250 g/d/cow) + hydrogenated palm oil (PO; palm oil; 250 g/d/cow). Milk collected on d 21 was pooled from 3 cows within the same treatment and period and made into cheese. Three cheeses per treatment per period at 14 d of aging were used for sensory evaluation. Cheese color measurements were determined according to the CIE L*, a*, and b* LAB color system. Sensory evaluation considered the following attributes: color homogeneity, holes, overall odor, ripe cheese odor, cow milk odor, salty, acid, bitter, overall flavor, ripe cheese flavor, sharpness, toughness, graininess, screeching, moisture and greasiness. Except for milk lactose yield, milk and cheese components were not affected by dietary treatments. Milk and cheese contents of C6:0, C8:0, C10:0 and C14:0 and atherogenicity index were lower ($P < 0.05$) with FO and FOPO diets than control diet. Compared with control and FOPO, FO increased ($P < 0.05$) C18:1 trans 11 and C22:6n3 content in milk and cheese. The color obtained from cheeses elaborated from cows given FO resulted in a more ($P < 0.05$) intense yellowness than control and FOPO. Compared with control and FOPO, FO resulted in higher ($P < 0.05$) notes for color homogeneity, whereas in comparison with control and FO, FOPO resulted in higher ($P < 0.05$) tough texture. In conclusion, supplementation of dairy cow diets with FO alone or in combination with hydrogenated palm oil can enhance the FA profile of milk and cheese without deleterious effects on sensory characteristics of cheese. This study was sponsored by a research grant from FONDECYT 11121142 (Fondo Nacional de Desarrollo Científico y Tecnológico, Chile).

Key Words: cheese, fatty acids, fish oil

560 Comparison between whey dilution during cheese-making and standardization of milk lactose by ultrafiltration on the properties of low and reduced fat Gouda cheese. Rodrigo A. Ibáñez*[1,2], Selvarani Govindasamy-Lucey[3], John J. Jaeggi[3], Mark E. Johnson[3], Paul L. H. McSweeney[1], and John A. Lucey[2,3], *[1]University College Cork, Cork, Ireland, [2]University of Wisconsin-Madison, Madison, WI, [3]Wisconsin Center for Dairy Research, Madison, WI.*

In recent years, the consumption of cheeses with reduced fat content has experienced growth due to health concerns. However, these products are associated with a hard and rubbery texture, poor melting properties and excessive development of acidity. In the manufacture of Gouda cheese, some whey is removed and replaced with water to decrease the residual lactose content and hence to control pH. This step is also known as whey dilution (WD) and may be a cause of high variability depending on the amount of water added, temperature, holding times and stirring rates. As an alternative, the standardization of lactose content in cheesemilk by ultrafiltration (UF) prior cheese manufacture could potentially reduce the residual lactose content in cheese and thus control the final pH. This study aimed to compare the effect of WD during cheese manufacture, with the alternative approach of adjustment of the lactose content of cheesemilk using UF, on the composition, texture, functionality and sensory properties of reduced-fat (RF) and low-fat (LF) Gouda-type cheeses during 6 mo of ripening. A stirred curd direct-salted cheese manufacture was used, differing in the levels of WD at 30, 15 and 0% (WD_{30}, WD_{15} and WD_0, respectively). The RF and LF milks used in WD_{30} and WD_{15} had a lactose-to-casein (L:C) ratio of ~1.8, which is the typical ratio found in milk. The WD_0 treatments were made with UF standardized milks to L:C ratio of ~1.1. Similar trends between treatments were observed in both RF and LF treatments. WD_0 exhibited lower residual lactose and lactic acid contents than WD_{30} and WD_{15}, leading to higher pH values ($P < 0.05$). WD_0 had softer texture and were more meltable ($P < 0.05$), probably due to a lower proportion of insoluble Ca caused by the addition of water required to achieve the lower L:C ratio in UF milks. Sensory analysis also indicated that WD_0 cheese had lower acidity and softer texture. These results suggest that UF standardization of the L:C ratio of cheesemilk could be a useful alternative to WD to reduce the acidity, improve texture and functionality of reduced- and low-fat Gouda cheese.

Key Words: Gouda cheese, lactose standardization, whey dilution

561 Growth and gas formation by a novel obligatory heterofermentative nonstarter lactic acid bacterium in cheese made using a *Streptococcus thermophilus* starter. Fatih Ortakci*[1], Jeffery Broadbent[1], Craig Oberg[2,1], and Donald McMahon[1], *[1]Utah State University, Logan, UT, [2]Weber State University, Ogden, UT.*

A novel slow-growing obligatory heterofermentative nonstarter lactic acid bacterium, *Lactobacillus wasatchii* sp. nov, was studied for growth and gas production in Cheddar cheese made using a *Streptococcus thermophilus* starter. Cheesemaking trials were conducted using starter *St. thermophilus* alone or in combination with *Lb. wasatchii* deliberately added to cheese milk at a level of ~10^4 cfu/ml. Then cheeses were ripened at 6 or 12°C. At d 1, starter streptococcal numbers were similar in both cheeses (~10^9 cfu/g) and nonstarter lactic acid bacteria (NSLAB) counts were below detectable levels (<10^2 cfu/g). As expected, *Lactobacillus wasatchii* counts were 3×10^5 cfu/g in cheeses inoculated with this bacterium. Starter streptococci decreased over time at both ripening temperatures but fell more rapidly at 12°C, especially in cheese with *Lb. wasatchii* ($P < 0.05$). Populations of NSLAB and *Lb. wasatchii* reached 5×10^7 and 2×10^8 cfu/g, respectively after 16 wk of ripening at 12°C, and their emergence was correlated with ~0.5% reductions in galactose concentrations. Levels of galactose at 6°C had also similar decrease after 16 wk storage. Gas formation and textural defects were only observed in cheese with added *Lb. wasatchii* ripened at 12°C. Results demonstrate that, *Lb. wasatchii* can contribute to late gas blowing in Cheddar cheese made with *St. thermophilus*, especially when the cheese is ripened at elevated temperature.

Key Words: cheese late blowing, nonstarter lactic acid bacteria, *Streptococcus thermophilus*

562 Late blowing of Cheddar cheese induced by accelerated ripening and ribose and galactose supplementation in presence of a novel obligatory heterofermentative nonstarter lactobacilli species. Fatih Ortakci*[1], Jeffery Broadbent[1], Craig Oberg[2,1], and Donald McMahon[1], *[1]Utah State University, Logan, UT, [2]Weber State University, Ogden, UT.*

A novel nonstarter lactic acid bacterium, *Lactobacillus wasatchii* sp. nov. has been studied for growth and gas formation in a control Cheddar

cheese and in cheese supplemented with 0.5% ribose, 0.5% galactose, or 0.25% ribose plus 0.25% galactose using regular and accelerated cheese ripening temperatures of 6 and 12°C. Cheese milk along with starter lactococci was inoculated with *Lb. wasatchii* at a level of 10^4 cfu/mL, whereas a control vat was inoculated with starter lactococci only. Starter numbers in both cheeses decreased from 10^7 to ~10^3 cfu/g at 23 wk of ripening at both temperatures, except the control cheese at 6°C which had one log higher final cell counts. Unlike starter bacteria, nonstarter lactic acid bacteria started at <10^2 cfu/g in the cheese and reached 10^6 to 10^7 cfu/g with higher numbers observed at 12°C. *Lactobacillus wasatchii* grew to ≥10^8 cfu/g in cheese supplemented with ribose (alone or with galactose) at elevated temperature which was ~1-log higher compared with the control and galactose-supplemented cheeses. In all cheeses with adjunct *Lb. wasatchii*, highest growth and gas formation was observed at 12°C although most gas production occurred at ≥16 wk. Adding both ribose and galactose provided substantially higher growth and gas formation because of the ability of *Lb. wasatchii* to co-utilize both sugars; producing gas from galactose as a result of the obligatory heterofermentative nature of the bacterium. Even without sugar supplementation, gas was observed in the presence of adjunct *Lb. wasatchii* after 16 wk. We have observed that *Lb. wasatchii* can grow to high cell densities when grown in carbohydrate-restricted broth containing lactococcal cell lysate. During cheese ripening, lysis of starter bacteria would provide sufficient substrate (such as ribose) to allow growth of *Lb. wasatchii* during cheese ripening and the presence of any hexoses in cheese would allow *Lb. wasatchii* to produce gas. We conclude that *Lb. wasatchii* is a previously undetected contributor to late gas formation in Cheddar cheese and the defect is more pronounced when elevated ripening temperatures are used.

Key Words: nonstarter lactic acid bacteria, gas, cheese

563 Demonstration of pH micro-heterogeneity in cheese matrices by fluorescence microscopy. Zuzana Burdikova[1], Zdenek Svindrych[2], Jan Pala[3], Cian D. Hickey[1,4], Martin G. Wilkinson[4], Jiri Panek[5], Mark A. E. Auty[1], Ammasi Periasamy[2], and Jeremiah J. Sheehan*[1], *[1]Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland, [2]Department of Biology, University of Virginia, Charlottesville, VA, [3]Third faculty of Medicine, Charles University, Prague, Czech Republic, [4]Dept of Life Sciences, University of Limerick, Ireland, [5]Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.*

Cheese, a product of microbial fermentation, may be defined as a protein matrix entrapping fat, moisture, minerals and solutes as well as dispersed bacterial colonies. The growth and physiology of bacterial cells in these colonies may be influenced by the microenvironment around the colony, or alternatively the cells within the colony may modify the microenvironment (e.g., pH, redox potential) due to their metabolic activity. To date there remains a significant knowledge gap relating to the degree of micro-heterogeneity in compositional and biochemical parameters such as pH within the cheese matrix and its relationship with microbial, enzymatic and physiochemical parameters and ultimately with cheese quality, consistency and ripening patterns. The objective of this study was to investigate whether pH micro-heterogeneity exists in cheese matrices. For the first time and arising from the development of a method using fluorescent lifetime imaging (FLIM) microscopy with Oregon green 488 dye, it is now possible to examine cheese for localized differences in pH. A ripened, semi-hard, dry salted cheese manufactured with thermophillic cultures was analyzed. Our study showed micro-heterogeneity in pH within the matrix of that cheese with pH ranging between 4.0 and 5.5. This is particularly interesting as it shows, contrary to previous assumptions, that the pH of a cheese matrix is not homogenous at micro-scale but contains localized variation. This may be due to localized differences in the aqueous phase or concentrations of constituents of the aqueous phase including lactose, lactate, minerals or salt. It may also be influenced by variations in buffering capacity of the surrounding cheese matrix. It is envisaged that future work using this method will focus on determining whether manufacture processes influence pH at local level within different cheese matrices and whether different cheese types may have different patterns of micro heterogeneity. It is also envisaged that this methodology will be employed to examine a broad range of food matrices in differing food products.

Key Words: pH, micro-heterogeneity, cheese

564 Evaluation of X-ray fluorescence spectroscopy for determination of minerals in process cheese. Catherine Shawl*[1], Jordan S. Rose[2], and David R. McCoy[3], *[1]Kraft Foods Group, Glenview, IL, [2]Oxford Instruments, Concord, MA, [3]Dairy Management Inc., Rosemont, IL.*

X-ray fluorescence spectroscopy (XRF) has been demonstrated to have the ability to rapidly and directly measure sodium in a variety of cheeses by Stankey et al. Their rapid and simple method used a disk of cheese as the sample. However, inaccuracies may result from the formation of a thin layer of butter oil forming between the cheese and the surface of the holder and from using cheese samples that do not have a uniform surface such as shredded or ground cheeses. It is also important in process cheese manufacture to control the concentration of chloride, phosphorus and potassium as well as sodium. This study investigated various modifications to the sample preparation procedures for XRF technology that would remove the mentioned limitations, expand the minerals assayed, and evaluate the feasibility of using XRF in a manufacturing situation. The optimum sample preparation was created by homogenizing process cheese into 0.1 *N* nitric acid at 50o C. The homogenate was then centrifuged for 2 min in a microcentrifuge to separate the sample into a solids, an aqueous, and an oil layer. Two to 3 mL of the aqueous layer was removed from the centrifuge tube and assayed in an Oxford Instruments X-Supreme8000 Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer. Instrument assay time was 3 min, which allowed simultaneous measurement of the minerals of interest. Total time from sample submission to result was 15 min, making it rapid enough for in-plant quality control use. Relative standard deviation for multiple trials with the same block of process cheese product was 3.0% for sodium, 6.5% for phosphorus, 0.9% for chlorine, and 1.4% for potassium in preliminary studies. The instrument calibration to a reference ICP method has an R2 of 0.915 for sodium using a straight line fit. The modified XRF method extends the range of minerals assayed and addresses the causes of the inaccuracies that had limited the desirability for industrial use.

Key Words: sodium, cheese, assay

565 Novel sample preparation for smear ripened cheese rinds evaluated by powder X-ray diffractometry. Gil F. Tansman*[1], Paul S. Kindstedt[1], and John M. Hughes[2], *[1]Department of Nutrition and Food Sciences, University of Vermont, Burlington, VT, [2]Department of Geology, University of Vermont, Burlington, VT.*

Recently we developed methods to evaluate surface crystals in Cheddar and hard Italian-style cheeses, and internal crystals in Cheddar, Gouda, and Parmigiano Reggiano cheeses by powder X-ray diffraction (PXRD). However, new sample preparation techniques are needed to analyze

surface crystals of washed rind cheeses because of the complex nature of the surface smear. Our objectives were to develop repeatable sample preparation protocols for smear evaluation by PXRD and to validate the protocols by single crystal X-ray diffraction (SCXRD). Initial efforts to evaluate crystalline inclusions in smears involved scraping the surfaces of 3 washed rind cheeses with a spatula and loading them onto PXRD slides. Fresh samples were used deliberately because ikaite ($CaCO_3 \cdot 6H_2O$), which in cheese has thus far only been observed in surface smears, decomposes in the presence of acetone. Diffraction patterns showed limited repeatability between repeated measures when data collection was performed in succession at roughly 25 min intervals. The lack of repeatability was found to be an artifact of sample desiccation during data collection. To prevent drying during data collection, 2 modifications were investigated; sample slides were coated in a thin layer of immersion oil, and alternatively allowed to air-dry before pulverization in a mortar. PXRD data collected using both PXRD method modifications revealed that all 3 smears contained ikaite and that one of the smears also contained struvite ($NH_4MgPO_4 \cdot 6H_2O$). The presence of ikaite and struvite was confirmed by single crystal x-ray diffraction (SCXRD) in the corresponding cheeses. It appears that smear material used in PXRD requires additional prep due to its extensive loss of volume upon drying, which causes diffraction artifacts. As confirmed by SCXRD, both modified PXRD methods provide valid diffraction data on the identities of crystals present in smear samples. Although SCXRD is a superior method for identifying the single crystals from surface smears, a reliable PXRD method is useful because powder diffractometers are typically less expensive, more widely available, and require less training to operate.

Key Words: cheese, crystals, PXRD