

## Dairy Foods: Cheese

**720 Microbial and sensory evaluation of fresh Mozzarella cheese.** B. Ganesan\*, D. Irish, C. Brotherson, and D. J. McMahon, *Western Dairy Center, Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan.*

Commercial fresh Mozzarella cheese is made by direct acidification and stored dry or in water without salting. The cheese has a shelf life of ~6 wk, but usually develops off flavor and loses textural integrity by 4 wk, potentially due to the lack of salt that allows outgrowth of undesirable bacteria. We hypothesized that low salt promotes unwanted bacterial survival in fresh Mozzarella cheese thereby altering its quality and safety. To study the impact of salt on the microbial and sensory quality and the ability of pathogen-related bacteria to survive during refrigerated storage, we made fresh mozzarella cheese with high (2%) and low (0.5%) salt. Both cheeses were stored dry and the low salt cheese also in 0.5% brine of which one part was used for sensory evaluation and the rest for microbial studies. Consumers preferred the 1% salt dry cheese to the other cheeses ( $P < 0.05$ ), but found no off flavors at 14 d storage in any cheese and also preferred a salad-style serving (cheese served with a cherry tomato marinated in balsamic vinegar) to fresh cheese. Descriptive panelists perceived higher levels ( $P < 0.05$ ) of bitter, umami, brothy, and salty attributes in 1% salt dry cheese. Coliforms and psychrophiles were not detected in cheeses or brine over 9 wks. Standard plate counts remained at 100–300 cfu/gm up to 2 wks, but increased by 1,000–10,000-fold ( $P < 0.05$ ) between 4 and 6 wks in all cheeses independent of salting, and coincides with the observed reduction in cheese quality. This showed that 2% salt was insufficient to control bacterial growth, and that slow-growing, cold- and salt-tolerant bacteria may survive and spoil fresh mozzarella cheese. *E. coli* and *Enterococcus faecalis* added to the 3 cheeses also increased by 100-fold ( $P < 0.05$ ) over 90 d of storage. Interestingly, *E. coli* added to the cheese brine first grew in the brine by 100-fold ( $P < 0.05$ ) before attaching to the cubes, whereas *Ent. faecalis* attached to the curd within 24 h and grew only in the cubes. These observations suggest that incident bacteria may survive and attach to curd by different mechanisms in fresh mozzarella cheese that need to be collectively addressed to reduce pathogen survival and spoilage.

**Key words:** fresh Mozzarella, safety

**721 CheddarCyc: A database of Cheddar cheese flavor reactions and pathways.** B. Ganesan\* and K. Brown, *Western Dairy Center, Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan.*

Cheese flavor is a summation of all microbial enzymatic and chemically spontaneous reactions that sugars, proteins, and lipids and their degradation products undergo during cheese aging. To understand cheese flavor development mechanisms attributable to bacteria we currently host and actively maintain metabolic pathway databases for LAB involved in cheese flavor generation at the ProCyc webserver ([www.usu.edu/westcent/procyc](http://www.usu.edu/westcent/procyc)). However the flavor-related pathways are scattered among the individual bacterial databases, which also contain metabolic routes that do not generate flavor compounds. Alternately, a large number of chemical reactions that are not captured in bacterial metabolic databases, either spontaneous or yet unattributed to enzymes, also participate in flavor compound production. In the current format, metabolic databases do not unify the diverse routes of cheese flavor compound generation. To that end, we created a cheese flavor enzymatic and chemical reaction database that links unchar-

acterized compounds and their reactions to flavor development and microbial metabolism and additionally contains selected flavor compound-generating metabolic pathways of bacteria. The database called “CheddarCyc” contains information on ~100 known Cheddar cheese flavor compounds based on current knowledge from literature. Key mechanisms of flavor generation such as carbohydrate metabolism, amino acid metabolism related to Arg, aromatic, sulfur, and branched chain amino acids, mechanisms of free fatty acid generation, and individual reactions demonstrated in literature are connected together. The database provides a chemical context in our ongoing effort to define flavor profiles of Cheddar cheese. Similar databases that are specific to flavor compounds found in other cheeses can be reconstructed relatively quickly using this mechanism. This provides us a resource to comprehensively tackle flavor generation routes and discover solutions to preexisting issues on Cheddar cheese flavor chemistry and enzymology. CheddarCyc will be publicly available at ProCyc and will eventually house the vast majority of Cheddar cheese flavor compound reactions proposed to date.

**Key words:** Cheddar cheese, flavor, metabolism

**722 New approaches to understand cheese ripening.** S. Lortal<sup>1,2</sup>, V. Gagnaire<sup>1,2</sup>, S. Jeanson<sup>1,2</sup>, J. Floury<sup>1,2</sup>, and M.-N. Madec<sup>1,2</sup>, <sup>1</sup>INRA, UMR1253, STLO, Rennes, France, <sup>2</sup>Agrocampus-Ouest, UMR1253, STLO, Rennes, France.

Cheese ripening is usually described in terms of kinetics of proteolysis, lipolysis and aroma compound production, which provides an averaged view of ripening. However, after milk inoculation and rennet action, bacteria are immobilized as colonies into the curd and grow as such. This colonial distribution is a reality in all kind of cheeses. The efficiency of bacteria within these colonies to act on dairy matrices (gene expression and activity of the enzymes produced) will thus depend on local physico-chemical conditions, matrix structure, diffusion limitations and inter-colony distance. The ambition of our team is to understand the ripening mechanisms in situ at a microscopic scale level, considering the colonial distribution. For that purpose, the following fields were explored: i) to characterize the spatial distribution of bacterial colonies according to the level of inoculation by mathematical modelization and by in situ validation using confocal laser microscopy (Jeanson et al., 2011 Appl. Environ. Microbiol. doi:10.1128/AEM.02233-10); ii) to estimate the diffusion rate of small solutes in dairy matrices differing in composition and microstructure. Indeed diffusion of water and salt were extensively characterized (Floury et al., 2010 Dairy Sci. Technol. 90:477–508) but not the diffusion of small solutes which are crucial for the bacterial metabolic activity and the ripening reactions. Moreover, the impact of cheese composition and microstructure on this diffusion is far from being well understood. The diffusion coefficient of a small solute was estimated for the first time by a noninvasive way within a cheese matrix using new imaging approaches, the Fluorescence Recovery After Photobleaching and using fluorescent 4 and 20 kDa dextran molecules. iii) to reveal in situ bacterial enzymes within and around the colonies immobilised in cheese. Cell wall protease and intracellular peptidases were first detected in situ by immunofluorescent antibodies and confocal microscopy.

**Key words:** cheese ripening, bacterial colony, diffusion of solutes and enzymes

**723 In situ proteolytic activity of *Lactobacillus helveticus* and stretchability of Swiss-type cheese.** L. Sadat-Mekmene<sup>1,2</sup>, R. Richoux<sup>3</sup>, L. Aubert-Frogerais<sup>3</sup>, M.-N. Madec<sup>1,2</sup>, C. Corre<sup>1,2</sup>, M. Piot<sup>1,2</sup>, J. Jardin<sup>1,2</sup>, S. Lortal<sup>1,2</sup>, and V. Gagnaire<sup>1,2</sup>, <sup>1</sup>INRA, UMR1253, STLO, Rennes, France, <sup>2</sup>Agrocampus Ouest, UMR1253, STLO, Rennes, France, <sup>3</sup>Actilait, Rennes, France.

In contrast to other lactic acid bacteria, *Lactobacillus helveticus* can possess 2 cell-envelope proteinases (CEPs) called PrtH2 and PrtH (Genay et al. 2009, Appl. Environ. Microbiol. 75:3238–3249; Sadat et al. 2011a, in press Int. J. Food Microbiol.). These CEPs exhibit different cleavage specificity on pure  $\alpha$ s1-casein (Sadat-Mekmene et al., 2011b, Appl. Environ. Microbiol. 77(1) 179–186). The aim of this work was to investigate the proteolytic activity of *L. helveticus* strains in cheese matrix according to the number of their CEPs and the cheese stretchability. Two strains were selected, ITGLH77 and ITGLH1 which possess one CEP, PrtH2, and 2 CEPs, PrtH and PrtH2, respectively. The proteolysis was monitored during ripening: i) casein hydrolysis by urea-PAGE; ii) peptide pattern of the cheese aqueous extracts first by SDS-PAGE and second by RP-HPLC. Three chromatographic fractions were collected and peptides identified by RP-HPLC coupled on-line with tandem mass spectrometry. In parallel, the dynamic of stretchability of Emmental cheese was measured to determine the contribution of CEPs in its generation, since *L. helveticus* is known to enhance stretching properties in contrast to other lactobacilli, such as *L. delbrueckii* ssp. *lactis*. The microstructure of the strands of Emmental cheeses was observed by confocal laser scanning microscopy. The stretchability of Emmental was significantly higher in cheese manufactured with strain ITGLH77. By using Principal Component Analyses, the stretching properties were found to be correlated with the presence of peculiar peptides derived from primary proteolysis, hydrophobic peptides, containing more than 20 amino acid residues and derived from  $\alpha$ s1-casein, which were accumulated in cheese manufactured using strain ITGLH77. The presence of these peptides suggest their less hydrolysis by this strain, in agreement with results observed in vitro (Sadat-Mekmene et al., 2011b), namely different cleavage specificity of CEPs PrtH et PrtH2 on pure  $\alpha$ s1-casein.

**Key words:** cell envelope proteinase, *Lactobacillus helveticus*, Swiss-type cheese stretchability

**724 Influence of Hofmeister salts on the textural and rheological properties of nonfat process cheese.** J. A. Stankey\* and J. A. Lucey, *University of Wisconsin, Department of Food Science, Madison.*

Hofmeister salts (HS) weaken or strengthen hydrophobic interactions of proteins resulting in salting in or salting out, respectively. Hydrophobic interactions also play a key role in casein particles and gels. In process cheese manufacture emulsifying salts (ES) are used to chelate Ca and disperse the insoluble casein matrix while heating. Since HS modify hydrophobic interactions we investigated the impact of HS on textural and rheological properties of nonfat process cheese (NFPC) made without ES. A directly acidified nonfat cheese base was made with citric acid (pH 5.6) and ripened for 7 d. Cheese was shredded and frozen until use. Two levels (0.1 or 0.3 M) of chaotropic (NaSCN) or kosmotropic (Na<sub>2</sub>SO<sub>4</sub>, NaCl) types of HS were blended with thawed, nonfat cheese. Cheese and HS were heated in a waterbath to 85°C and mixed using an overhead stirrer (6 min). Molten cheese was poured into plastic molds, sealed and stored (4°C) for 7 d before analysis. Small amplitude oscillatory rheological properties were measured while heating from 5 to 85°C. Moisture contents and pH of NFPC were ~55% and ~5.8, respectively. Calcium concentrations (475 mg/100 g)

were similar in all NFPC. Sulfur content increased in NFPC made with increasing concentrations of NaSCN or Na<sub>2</sub>SO<sub>4</sub>. The NFPC made with 0.3 M NaSCN were shiny, stickier, had lower hardness, higher LT values (indicating higher meltability) and melt occurred at lower temperatures than other treatments. The NFPC made with 0.3 M Na<sub>2</sub>SO<sub>4</sub> were tough, very viscous, had increased hardness values and lower LT values (less meltable). At 0.3 M concentrations, hardness increased in cheeses in the following order: NaSCN < NaCl < Na<sub>2</sub>SO<sub>4</sub>. Cheeses made with 0.3 M NaCl or Na<sub>2</sub>SO<sub>4</sub> lost water during processing. Hydrophobic interactions increase with temperature during manufacture; the addition of kosmotropic HS strengthened these interactions resulting in “salting out” (water loss) and a firmer (less meltable) NFPC. Addition of chaotropic HS reduced hydrophobic interactions and resulted in weakened protein-protein bonds and thus created a softer (more meltable) NFPC network.

**Key words:** rheology, Hofmeister series, nonfat process cheese

**725 Impact of reforming on low-fat cheese texture as influenced by pH.** C. Akbulut\* and J. A. Lucey, *Department of Food Science, University of Wisconsin, Madison.*

Grating of cheese and repressing it to “reform” its shape is an approach that could be used to improve the texture of excessively firm low-fat cheese. Our objective was to study the impact of pH on the properties of low-fat cheese that was reformed after grinding. Low-fat cheese bases having 4 different pH values were produced by direct acidification of milk to pH 6.2 with citric acid and altering the final pH by the addition of glucono- $\delta$ -lactone (0, 0.1, 0.3 and 0.4%) to curd, to obtain cheeses with pH values 6.2, 5.8, 5.5 and 5.3, respectively. Cheeses were stored for 2 wk at 4°C, and then ground and reformed by pressing ground cheese into plastic cups using a laboratory hydraulic press for 1 h at 20°C. They were kept at 4°C overnight and then removed from plastic cups, vacuum-sealed and stored for 1 wk before analysis. Hardness was determined at 80% compression. Dynamic moduli and loss tangent was measured during heating from 5 to 85°C. Melt profiles were obtained by the UW-Melt Profiler. Acid-base titrations were used to determine the amount of residual insoluble Ca in cheese. Reforming low-fat cheese reduced its hardness at all pH levels, however the decrease in hardness of high pH cheese was much greater than the decrease obtained when low pH cheese was reformed. As a result, the relatively harder texture of high pH low-fat cheese was softened to a level that could also be accomplished by pH reduction. Meltability increased slightly as a result of reforming. Fluorescence microscopy was used to observe the fusion in the contact region between 2 cheese slices that were held together at 4°C for 1 wk. The pH 6.2 cheese slices remained intact (little fusion). Reducing the cheese pH resulted in a greater fusion between slices. With a decrease in cheese pH, there was a decrease in the insoluble Ca content; cheese became more meltable and softer making it easier to reform. These results showed that reforming cheese with a lower pH didn't alter the texture as much as at high pH since most bonds appeared to reform on storage at low pH. The rubbery and firm texture of the low-fat cheese made at high pH was improved by reforming as the texture became shorter and softer.

**Key words:** low-fat cheese, texture, pH

**726 Recovery of  $\omega$ -3 fatty acids in Cheddar cheese curd and long-term stability of  $\omega$ -3 fatty acids in whey powder.** B. Ganesan\*, C. Brothersen, and D. J. McMahon, *Western Dairy Center, Depart-*

Full-fat Cheddar cheese was made with milk fortified with  $\omega$ -3 fatty acids and the partitioning of  $\omega$ -3 fatty acids into curd and whey was determined, along with flavor attributes of  $\omega$ -3 fatty acid-containing whey powder over storage.  $\omega$ -3 fatty acids from both animal and plant sources were incorporated into the milk. The encapsulated powder was added to cheese milk or the milled curd during dry salting. The oil form of  $\omega$ -3 fatty acids was either mixed or homogenized oil in milk or cream, wherein the homogenized milk and cream constituted 10% of fat in cheese milk. The recovery of  $\omega$ -3 fatty acids from the animal source was 90.5%, significantly higher ( $P < 0.05$ ) than that of the plant source (80.3%). Homogenization provided similar recoveries of  $\omega$ -3 fatty acids (90–93%,  $P > 0.05$ ) for the 2 sources, whereas homogenized treatments were significantly different from non-homogenized treatments (79–83%,  $P < 0.05$ ). Addition of encapsulated  $\omega$ -3 fatty acids to the cheese curd during salting provided higher recovery (91–99%,  $P < 0.05$ ) for both animal and plant sources and was the most efficient method of fortifying Cheddar cheese with  $\omega$ -3 fatty acids. Whey collected during cheese making from the various fortification treatments was further dried into powder using a drum dryer and stored for 9 mo at 4°C or room temperature (18–22°C) to study changes in attributes perceived by a descriptive panel. Sweet taste was perceived higher ( $P < 0.05$ ) at 3, 6, and 9 mo than at 0 mo and perceived at varying levels across the different cheese milk treatments. Bitter, salty, sour, brothy, cooked, and lactone/fatty acid flavor attributes were only perceived higher at 0 and 3 mo; whereas fishy flavor was discerned only at 6 and 9 mo, with all  $\omega$ -3 fatty acid-incorporated whey powders scoring higher than the unincorporated control ( $P < 0.05$ ). Notably, panelists scored the whey powders similarly for other attributes despite the whey powders containing different levels of  $\omega$ -3 fatty acids. Commercial preparations of  $\omega$ -3 fatty acids appear to be prone to develop off flavors in whey powder and lose acceptability during long-term storage.

**Key words:** Cheddar cheese, omega-3 fatty acids, whey powder

**727 Rheology, microstructure and quality of curd made from buffalo milk: A comparison with ultrafiltered cows' milk.** I. Hus-sain\*, A. S. Grandison, and A. E. Bell, *Department of Food and Nutritional Sciences, University of Reading, Reading, Berkshire, UK.*

Rennet induced curds were made from buffalo, cows' and ultrafiltered cows' milk (UF cows' was concentrated such that it has chemical composition approximately equivalent to buffalo milk). These milk samples were compared on the basis of rheology, physicochemical characteristics and curd quality. The ionic and soluble calcium contents were similar in all milk samples. The total and casein bound calcium were higher in UF cows' milk than untreated cows' milk, and found to be lower than in buffalo milk. This is due to concentration of colloidal part of milk (casein) during the ultrafiltration process. The rennet coagulation time was almost the same in UF cows' and buffalo milk while it was higher in cows' milk. The dynamic moduli ( $G'$ ,  $G''$ ) values were higher in buffalo and UF cows' milk than cows' milk 90 min after chymosin addition. The loss tangent was the same in UF cows' and buffalo milks and has a lower value as compared with cows' milk. The frequency profile (0.1–10Hz) was recorded 90 min after the enzyme addition; all of treated samples found to be weak viscoelastic frequency dependent gel. The cows' curd was weaker (dynamic moduli) than buffalo and UF cows' curds. The yield stress was measured 95 min after the enzyme addition, and attained a higher value in buffalo milk as compared with other milk samples. The curd yield, curd moisture and curd fat retention were found to be higher in UF cows' milk than for buffalo and cows' milk. The minimum whey fat losses occurred in UF cows' milk. The total mineral contents were also higher in UF cows' milk than in the buffalo and cows' milk. The electron micrographs showed that sizes of casein micelles and fat globule were different in the 2 types of milk. The curd structure appeared to be more "dense" in buffalo milk than cows' milk.

**Key words:** rheology, curd, ultrafiltration