Cheese has generally been considered ready-to-eat food allowing the growth of *Listeria monocytogenes*, although cheeses unable to support this growth were already observed. Consequently, the absence of the pathogen in 25 g of cheese has to be guaranteed, before placing it on the market, while up to 100 cfu/g are tolerated for food not allowing the growth of *L. monocytogenes*. The presence of *L. monocytogenes* in cheese can lead to harmful economic consequences for producers. Defining more accurately cheeses allowing or not the growth of *L. monocytogenes* is thus a priority. Predictive microbiology is not an optimal solution, since artisanal cheeses present specific characteristics that are not taken into account by current models. Challenge-tests seem more accurate. The goal of this study was to assess the growth potential of *L. monocytogenes* in cheeses from 32 artisanal factories using challenge-tests. Were considered: (a) unripened cheeses (12), (b) mold-ripened soft cheeses (4), (c) smear-ripened soft cheeses (4) and (d) ripened semi-hard cheeses (12). The number of batches to test was determined using SymPrevi-us, an online tool for growth predictions in food microbiology. A cocktail of 3 strains was inoculated in cheeses, targeting a contamination of 100 cfu/g. Cheeses were stored at refrigeration temperature during the whole shelf-life. Growth potentials were calculated as the difference between median contaminations at the use-by date and at the first day of storage, as recommended by the European Union Reference Laboratory for *L. monocytogenes*. Twenty-three cheeses out of 32 did not allow the growth of *L. monocytogenes*, i.e., the growth potential was ≤0.5 log<sub>10</sub> cfu/g. It was the case of all unripened cheeses (~1.0 ± 0.3 log<sub>10</sub> cfu/g on average), meaning that Belgian unripened cheeses should not represent a threat for food safety. On the opposite, soft cheeses allowed growth of *L. monocytogenes* up to 4.5 log<sub>10</sub> cfu/g. Regarding semi-hard cheeses, a huge inter- and intra-batch variability was observed. In the latter case, recommended method for growth potential calculation underestimated the growth and led to inaccurate conclusions concerning product safety.

**Key Words:** *Listeria monocytogenes*, cheese, challenge test

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**Efficacy of bioengineered nisin derivatives H27/31K in combination with phage endolysin PlyP100 to eliminate *Listeria monocytogenes* in queso fresco**, L. A. Ibarra-Sánchez*, W. Kong, T. Lu, and M. J. Miller, University of Illinois at Urbana-Champaign, Urbana, IL. *L. monocytogenes* is a food-borne pathogen of concern in fresh Hispanic-style cheeses, such as queso fresco (QF). Nisin’s ability to inhibit *L. monocytogenes* is well-known, but its activity is limited in QF. The objective of this study was to evaluate the antilisterial efficacy of bioengineered nisin H27/31K, alone and in combination with endolysin PlyP100 in QF. Nisin H27/31K (histidine at position 27 and 31 replaced with lysine) was produced in *Lactococcus lactis* MG1363, an engineered strain having the nisin biosynthetic pathway. Commercial nisin (nisin A) and H27/31K were evaluated to determine their minimum inhibitory concentration (MIC) and residual nisin after 24 h exposure to pH 7 ± 22% milk fat at 37°C. His-tagged PlyP100 was overexpressed in *Escherichia coli* and subsequently purified. Nisin A, H27/31K and PlyP100 were added to QF at the following concentrations: 250 µg/g H27/31K with or without 2.5 U/g PlyP100, and 250 µg/g nisin A. Cheese curds were inoculated with ~3.5 log cfu/g of *L. monocytogenes* cocktail of 5 different foodborne outbreak-associated strains. *L. monocytogenes* cells were enumerated by spread plating on PALCAM (polymyxin-acylative-LiCl-ceftazidime-esculin-mannitol) agar supplemented with cef- tazidime, across 28 d of storage at 4°C. All experiments were repeated 2 times with duplicated samples. H27/31K showed reduced antilisterial activity (MIC range: 12.5 - 50 µg/mL) compared with nisin A (MIC range: 1.56 - 6.25 µg/mL). After 24 h of exposure to pH 7 ± 22% milk fat, >96% residual nisin H27/31K was observed, but residual nisin A was not detected. H27/31K reduced initial viable counts of *L. monocytogenes* in QF by up to ~1.5 log cfu/g. PlyP100 exhibited a strong listeriostatic effect in QF over 28 d of cold storage. The treatment combining H27/31K and PlyP100 in QF achieved *L. monocytogenes* reduction below the detection limit of plating, and, the pathogen was not recovered after enrichment in all QF samples treated with that antimicrobial mixture. In conclusion, our results demonstrate that nisin H27/31K combined with PlyP100 can be used to eradicate *Listeria* in QF.

**Key Words:** queso fresco, *Listeria monocytogenes*, nisin bioengineering

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**Physical and sensorial characteristics of raw milk cheeses and pasteurized milk cheeses from sheep supplemented with sunflower seed silage**, E. Cardoso-Gutiérrez*, A. C. Narvaez-López1, L. E. Robles-Jiménez*, M. d. l. A. Colin-Cruz*, M. González-Ronquillo*, and E. Vargas-Bello-Pérez*, 1Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México, 2Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark.

The objective of this study was to evaluate the physical and sensorial characteristics of raw milk cheeses (RM) and pasteurized milk (PM) cheeses from sheep supplemented with sunflower seed silage (SFS). Six East Friesian ewes were supplemented with SFS (5% of dry matter) for 8 weeks. Three cheeses (1 kg/each) per treatment (RM and PM) were manufactured every 2 weeks (12/treatment), and allowed to mature for 180 d. After maturation times, sensory analyses were performed with a panel composed of 50 untrained judges. Evaluations on odor, color, texture, general taste and overall acceptability used a 5-point hedonic scale (1 = lowest and 5 = highest; intensities). Likewise, pH, colorimetric, shear force and quantification of mesophylls, coliforms, fungi and yeasts were determined. Data were analyzed in a completely randomized design using GLM procedure from SAS. Scores for odor (3.10 ± 0.20), color (3.57 ± 0.38), texture (3.32 ± 0.58), flavor (3.12 ± 0.24), and acceptability (3.12 ± 0.21) were similar. The pH was more acidic (P < 0.01) for RM cheeses (4.78) compared with PM (5.51). Treatments were similar in shear force (2.19 ± 0.16 kg). Lightness (L*: 73.9 ± 2.21), redness (a*: -1.36 ± 0.78), chroma (C*: 14.13 ± 9.13) and hue (H*: 94.26 ± 2.78) were similar between treatments. Compared with PM, the yellowness was higher (P < 0.05) in RM cheeses (b*: 16.82 vs. 24.89). Counts for mesophylls, coliforms, and yeasts were similar between treatments. Overall, RM cheeses from sheep supplemented with SFS have similar sensory characteristics than PM cheeses but lower pH and more intense yellowness.

**Key Words:** raw milk, sheep, sunflower silage

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**Manufacture of culture-based acid curd using micellar casein concentrate**, A. R. A. Hammam* and L. E. Metzger, Dairy and Food Science Department, South Dakota State University, Brookings, SD.

Micellar casein concentrate (MCC) is a high protein ingredient produced by microfiltration of skim milk. It has an elevated level of casein as a percentage of total protein as compared with skim milk. Acid curd is a protein concentrate, which is produced by precipitating the casein at the isoelectric point (pH = 4.6) using starter cultures or acids without rennet. Acid curd is typically manufactured from skim milk in a process similar

Dairy Foods: Cheese
to Cottage cheese manufacture. However, this results in the production of acid whey which is difficult to utilize. We theorize that acid curd could be produced from MCC instead of skim milk which could improve the efficiency of manufacture and allow for removal of the whey protein before acid curd manufacture. The objective of this study was to utilize MCC to manufacture of acid curd using starter cultures. The MCC (pH~6.6) was prepared and standardized by mixing milk permeate, water, and MCC powder to produce a solution with 13.0% solids, 9.0% protein, and 2.0% lactose. Thermophilic cultures were added (0.005%) to the MCC and incubated at 43°C. The fermentation time was approximately 15 h to decrease the pH to 4.6. After reaching pH 4.6, the curd was cut and mixed gently during heating to 50°C in 1 h. The whey was subsequently drained and the curd was washed with water and then pressed. After pressing, the curd was analyzed for solids, protein, lactose, lactic acid, ash, and mineral profile. The moisture adjusted yield efficiency was also calculated. This trial was repeated 4 times. The mean was 24.9%, 23.0%, 0.90%, and 97.4% for solids, protein, ash, and moisture adjusted yield efficiency, respectively. The whey had 0.58% lactose and 1.43% lactic acid. The calcium and phosphate content of the acid curd was 0.19% and 0.12%, respectively. No significant differences (P > 0.05) were detected in the composition of the acid curd made from the 4 replicates of the MCC. We conclude that MCC can be utilized in manufacture of acid curd with starter cultures. The culture-based acid curd will be utilized as an ingredient in imitation Mozzarella type cheese in subsequent studies.

**Key Words:** micellar casein concentrate, acid curd, imitation mozzarella cheese

### 335 Design of manufacturer milk powder for recombined cheeses

S. Sen*, S. Govindasamy-Lucey, J. J. Jaeggi, M. E. Johnson, J. A. Lucey, M. Molitor

Our ultimate goal is to minimize whey drainage in cheesemaking by boosting total protein content in rehydrated milk. Commercial milk protein powders pose 2 major challenges in preparing high protein recombined milk – poor solubility at high protein and slower cheese ripening in semi-hard cheeses due to significant amount of whey proteins. Thus, we made a whey-protein depleted designer powder (WDSCC) with higher lactose and lower calcium (Ca) to aid powder hydration and solubility and compared with 7 commercial powders (MPC 80, MPC 85, MPI 85, MPI 85 Low Lactose, MPI 90 and 2 micellar casein powders) in terms of composition, solubility and rennet coagulation properties. All experiments were replicated (n = 3) and multiple comparison (α = 0.05, Duncan test) were used for statistical analyses. The WDSCC powder was made by microfiltration/diafiltration (MF/DF) of pasteurized skim milk at 24°C. The MF retentate (casein, CN) was acidified and ultrafiltered/diafiltered (UF/DF) at pH 5.5 to get CN with reduced colloidal Ca phosphate. The UF permeate was nanofiltered (NF) to retain whey proteins and permeate lactose and monovalent minerals. Edible-grade lactose, NF permeate and, calcium-depleted CN were blended together, and spray dried to obtain the designer powder - soluble casein concentrate. The WDSCC powder manufacture was replicated twice. WDSCCs have CN: true protein ratio of 0.95 as compared with 0.86–0.92 in commercial powders. WDSCC contained higher lactose (24% versus 2–6%, P < 0.05) and lower total Ca/g protein (15mg versus 21–25mg, P < 0.05) than commercial high-CN powders. Powders were rehydrated to 7.5, 10 and 12.5% total protein using a magnetic stirrer at 800 rpm for 1 h. Solubility and rennet coagulation (using small amplitude oscillatory rheology) were measured at each protein content. Rehydration at 20°C for 10% protein, resulted in commercial powders having lower solubility (average ~60%) than WDSCCs (83–91%) (P < 0.05). As protein concentration was increased, all commercial powders immediately gelled during rehydration but, WDSCCs were easily rehydrated to 12.5% and 15% protein. As WDSCCs were Ca-depleted, no rennet gels formed at ≤ 12.5% protein without adding Ca. 15% WDSCC coagulated regardless of Ca addition. At higher protein, WDSCCs have superior solubility, hydration and rennet coagulation than commercial powders but it has higher lactose content. In future studies, we will evaluate the potential of WDSCC powder in preparing high protein recombined milk for wheyless cheesemaking.

**Key Words:** block Gouda cheese, lactose standardization, curd type

### 336 Impact of lactose standardization and curd types on the properties of direct-salted Gouda cheese

Y. Gong*, S. Govindasamy-Lucey, J. J. Jaeggi, M. E. Johnson, and J. A. Lucey

Direct-salted Gouda cheese allows cheese manufacturers to produce Gouda cheese using existing Cheddar equipment. Our previous survey on commercial US Gouda cheeses found that block Gouda was more acidic and lacked desirable melt attributes compared with the traditional Gouda. To improve the sensory and functional properties of direct-salted Gouda cheese, we studied the impact of lactose standardization (LS) and curd types (stirred curd (SC) or milled curd (MC)) on cheese properties. Milk with 2 different lactose contents (~4.4% (control) and ~2.2% (LS)) were prepared using ultrafiltration and water was added to the LS milk. Four types of cheese (controlSC, controlMC, LSSC and LSMC) were made (n = 5) using milk containing similar casein content (~3.1%) and casein-to-fat ratio (0.7). They were ripened at 10°C for 10 d and then 4°C for 3 mo. Composition, textural and sensory analyses were performed after 1-d, 2-wk, 1-mo and 3-mo of ripening. Cheese functionality was assessed using texture profile analysis (TPA) and dynamic low-amplitude oscillatory rheology. Sensory Spectrum and quantitative descriptive analysis were conducted with 10 trained panelists to evaluate flavor, texture, shred attributes, and pizza performance. Multiple comparison (α = 0.05, Duncan test) and split-plot design were used for statistical analyses. The 4 cheeses had similar composition except that LS cheeses had slightly lower moisture by 1%. LS Gouda had higher pH values, lower lactic acid contents and lower TPA hardness values than control Gouda during ripening. Rheological parameters, maximum loss tangent (MLT) values were impacted by both LS and curd type. Sensory acid scores were lower in unmelted LS cheeses than unmelted control cheeses at 2 wk. When baked on pizza at 1 mo, all 4 cheeses melted completely and had similar blister quantity, strand thickness, and strand length. The use of LS effectively controlled the pH values and reduced the acid flavor in block Gouda cheese. LS had a greater effect on cheese flavor and functionality than curd type.

**Key Words:** block Gouda cheese, lactose standardization, curd type

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J. Dairy Sci. 103 (Suppl. 1)