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**SELECTIVE SEPARATION OF THE MAJOR WHEY PROTEINS USING ION EXCHANGE MEMBRANES**

*S. Goodall, A. S. Grandison, P. J. Jauregi and J. Price*

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Synthetic microporous membranes with functional groups covalently attached were used to selectively separate  $\hat{\alpha}$ -lactoglobulin, BSA, and  $\acute{\alpha}$ -lactalbumin from rennet whey. The selectivity and membrane performance of strong (quaternary ammonium) and weak (diethylamine) ion-exchange membranes were studied using breakthrough curves, measurement of binding capacity, and protein composition of the elution fraction to determine the binding behavior of each membrane. When the weak and strong anion exchange membranes were saturated with whey, they were both selective primarily for  $\hat{\alpha}$ -lactoglobulin with less than 1% of the eluate consisting of  $\acute{\alpha}$ -lactalbumin or BSA. The binding capacity of a pure  $\hat{\alpha}$ -lactoglobulin solution was in excess of 1.5 mg/cm<sup>2</sup> of membrane. This binding capacity was reduced to approximately 1.2 mg/cm<sup>2</sup> when using a rennet whey solution (pH 6.4). This reduction in protein binding capacity can be explained by both the competitive effects of other whey proteins and the effect of ions present in whey. Using binary solution breakthrough curves and rennet whey breakthrough curves, it was shown that  $\acute{\alpha}$ -lactalbumin and BSA were displaced from the strong and weak anion exchange membranes by  $\hat{\alpha}$ -lactoglobulin. Finally, the effect of ionic strength on the binding capacity of individual proteins for each membrane was determined by comparing model protein solutions in milk permeate (pH 6.4) and a 10 mM sodium phosphate buffer (pH 6.4). Binding capacities of  $\hat{\alpha}$ -lactoglobulin,  $\acute{\alpha}$ -lactalbumin, and BSA in milk permeate were reduced by as much as 50%. This reduction in capacity coupled with the low binding capacity of current ion exchange membranes are 2 serious considerations for selectively separating complex and concentrated protein solutions.

**INFLUENCE OF EMULSIFYING SALTS ON THE TEXTURAL PROPERTIES OF NONFAT PROCESS CHEESE MADE FROM DIRECT ACID CHEESE BASES**

*C. A. Brickley, S. Govindasamy-Lucey, J. J. Jaeggi, M. E. Johnson, P. L. H. McSweeney and J. A. Lucey*

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The objective was to investigate the influence of several types of emulsifying salts (ES) on the texture of nonfat process cheese (NFPC). Improperly produced nonfat cheese tends to ex-

hibit several problems upon baking including stickiness, insufficient or excessive melt, pale color upon cooling, formation of a dry skin (skinning) often leading to dark blistering, and chewy texture. These attributes are due to the strength and number of interactions between and among casein molecules. We propose to disrupt these interactions by using suitable emulsifying salts (ES). These ES chelate Ca and disperse caseins. Stirred curd cheese bases were made from skim milk using direct acidification with lactic acid to pH values 5.0, 5.2, and 5.4, and ripened for 1 d. Various levels of trisodium citrate (TSC; 0.5, 1, 1.5, 2, 2.5, 3, and 5%), disodium phosphate (DSP; 1, 2, 3, and 4%), or trisodium phosphate (TSP; 1, 2, 3, and 4%) were blended with the nonfat cheese base. Cheese, ES, and water were weighed into a steel container, which was placed in a waterbath at 98°C and then stirred using an overhead stirrer for 9 min. Molten cheese was poured into plastic containers, sealed, and stored at 4°C for 7 d before analysis. Texture and melting properties were determined using texture profile analysis and the UW-Melt-profiler. The pH 5.2 and 5.4 cheese bases were sticky during manufacture and had a pale straw-like color, whereas the pH 5.0 curd was white. Total calcium contents were approximately 400, 185, and 139 mg/100 g for pH 5.4, 5.2, and 5.0 cheeses, respectively. Addition of DSP resulted in NFPC with the lowest extent of flow, and crystal formation was apparent at DSP levels above 2%. The NFPC manufactured from the pH 5.0 base and using TSP had reduced melt and increased stickiness, whereas melt was significantly increased and stickiness was reduced in NFPC made with pH 5.4 base and TSP. However, for NFPC made from the pH 5.4 cheese and with 1% TSP, the pH value was >6.20 and crystals were observed within a few days. Use of TSC increased extent of flow up to a maximum with the addition of 2% ES for all 3 types of cheese bases. Addition of high levels of TSC to the pH 5.2 and 5.4 cheese bases resulted in increased stickiness. Similar pH trends for attributes such as extent of flow, hardness, and adhesiveness were observed for both phosphate ES but no consistent pH trends were observed for the NFPC made with TSC. These initial trials suggest that the pH 5.0 cheese base was promising for further research and scale-up to pilot-scale process cheese making, because cheeses had a creamy color, reasonable melt, and did not have high adhesiveness when TSC was used as the ES. However, the acid whey produced from the pH 5.0 curd could be a concern.

#### **DIFFERENCES BETWEEN CHEDDAR CHEESE MANUFACTURED BY THE MILLED-CURD AND STIRRED-CURD METHODS USING DIFFERENT COMMERCIAL STARTERS**

*Shakeel-ur-Rehman, M. A. Drake and N. Y. Farkye*  
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Traditionally, Cheddar cheese is made by the milled-curd method. However, because of the mechanization of cheese making and time constraints, the stirred-curd method is more commonly used by many large-scale commercial manufacturers. This study was undertaken to evaluate quality differences during ripening (at 2 and 8°C) of Cheddar cheese made by the milled-curd and stirred-curd methods, using 4 different commercial starters. Twenty-four vats (4 starters x 2 methods x 3 replicates) were made, with ~625 kg of pasteurized (72°C x 16 s) whole milk in each vat. Fat, protein, and salt contents of the cheeses were not affected by the starter. Starter cell densities in cheese were not affected by the method of manufacture. Non-

starter lactic acid bacteria counts at 90, 180, and 270 d were influenced by the manufacturing method, with a higher trend in milled-curd cheeses. Proteolysis in cheese (percentage of water-soluble N) was influenced by the starter and manufacturing method (270 d). Sensory analysis by a trained descriptive panel (n = 8) revealed differences in cooked, whey, sulfur, brothy, milk fat, umami, and bitter attributes caused by the starter, whereas only brothy flavor was influenced by storage temperature. The method of manufacture influenced diacetyl, sour, and salty flavors.

### **THE EFFECT OF REFRIGERATED AND FROZEN STORAGE ON BUTTER FLAVOR AND TEXTURE**

*A. J. Krause, R. E. Miracle, T. H. Sanders, L. L. Dean and M. A. Drake  
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Butter is often stored for extended periods of time; therefore, it is important for manufacturers to know the refrigerated and frozen shelf life. The objectives of this study were to characterize the effect of refrigerated and frozen storage on the sensory and physical characteristics of butter. Fresh butter was obtained on 2 occasions from 2 facilities in 113-g sticks and 4-kg bulk blocks (2 facilities, 2 package forms). Butters were placed into both frozen ( $-20^{\circ}\text{C}$ ) and refrigerated storage ( $5^{\circ}\text{C}$ ). Frozen butters were sampled after 0, 6, 12, 15, and 24 mo; refrigerated butters were sampled after 0, 3, 6, 9, 12, 15, and 18 mo. Every 3 mo, oxidative stability index (OSI) and descriptive sensory analysis (texture, flavor, and color) were conducted. Every 6 mo, peroxide value (PV), free fatty acid value (FFV), fatty acid profiling, vane, instrumental color, and oil turbidity were examined. A mixed-model ANOVA was conducted to characterize the effects of storage time, temperature, and package type. Storage time, temperature, and package type affected butter flavor, OSI, PV, and FFV. Refrigerated butter quarters exhibited refrigerator/stale off-flavors concurrent with increased levels of oxidation (lower oxidative stability and higher PV and FFV) within 6 mo of refrigerated storage, and similar trends were observed for refrigerated bulk butter after 9 mo. Off-flavors were not evident in frozen butters until 12 or 18 mo for quarters and bulk butters, respectively. Off-flavors in frozen butters were not correlated with instrumental oxidation measurements. Because butter is such a desirable fat source in terms of flavor and textural properties, it is important that manufacturers understand how long their product can be stored before negative attributes develop.

### **IMPACT OF FLAVOR ATTRIBUTES ON CONSUMER LIKING OF SWISS CHEESE**

*R. E. Liggett, M. A. Drake and J. F. Delwiche  
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Although Swiss cheese is growing in popularity, no research has examined what flavor characteristics consumers desire in Swiss cheese, which was the main objective of this study. To this end, a large group of commercially available Swiss-type cheeses (10 domestic Swiss cheeses, 4 domestic Baby Swiss cheeses, and one imported Swiss Emmenthal) were assessed both by

12 trained panelists for flavor and feeling factors and by 101 consumers for overall liking. In addition, a separate panel of 24 consumers rated the same cheeses for dissimilarity. On the basis of liking ratings, the 101 consumers were segmented by cluster analysis into 2 groups: nondistinguishers (n = 40) and varying responders (n = 61). Partial least squares regression, a statistical modeling technique that relates 2 data sets (in this case, a set of descriptive analysis data and a set of consumer liking data), was used to determine which flavor attributes assessed by the trained panel were important variables in overall liking of the cheeses for the varying responders. The model explained 93% of the liking variance on 3 normally distributed components and had 49% predictability. Diacetyl, whey, milk fat, and umami were found to be drivers of liking, whereas cabbage, cooked, and vinegar were drivers of disliking. Nutty flavor was not particularly important to liking and it was present in only 2 of the cheeses. The dissimilarity ratings were combined with the liking ratings of both segments and analyzed by probabilistic multidimensional scaling. The ideals of each segment completely overlapped, with the variance of the varying responders being smaller than the variance of the non-distinguishers. This model indicated that the Baby Swiss cheeses were closer to the consumers' ideals than were the other cheeses. Taken together, the 2 models suggest that the partial least squares regression failed to capture one or more attributes that contribute to consumer acceptance, although the descriptive analysis of flavor and feeling factors was able to account for 93% of the variance in the liking ratings. These findings indicate the flavor characteristics Swiss cheese producers should optimize, and minimize, to create cheeses that best match consumer desires.

#### **ENHANCED FUNCTIONALITIES OF WHEY PROTEINS TREATED WITH SUPERCRITICAL CARBON DIOXIDE**

*Q. Zhong and M. Jin*

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The functionality of whey proteins can be modified by many approaches; for example, via complexation with carbohydrates, enzymatic cross-linking, or hydrolysis, and the objective of this work was to research the effects of supercritical carbon dioxide (scCO<sub>2</sub>) treatments on the functionalities of commercial whey protein products including whey protein isolates (WPI) and whey protein concentrates (WPC). The WPI and WPC powders and a 10% (wt/vol) WPI solution were treated with scCO<sub>2</sub>. The WPI solution was treated at 40°C and 10 MPa for 1 h, whereas WPI and WPC powders were treated with scCO<sub>2</sub> at 65°C and 10 or 30 MPa for 1 h. Dynamic rheological tests were used to characterize gelation properties before and after processing. Compared with the unprocessed samples and samples processed with N<sub>2</sub> under similar conditions, scCO<sub>2</sub>-treated WPI, whether dispersed in water or in the powder form during treatments, formed a gel with increased strength. The improvement in gelling properties was more significant for the scCO<sub>2</sub>-treated WPC. In addition, the scCO<sub>2</sub>-processed WPI and WPC powders appeared to be fine and free-flowing, in contrast to the clumps in the unprocessed samples. Proximate compositional and surface hydrophobicity analyses indicated that both compositional and structural changes may have contributed to enhanced whey protein functionalities. The results suggest that functionalities of whey proteins can be improved by scCO<sub>2</sub> treatment to produce novel ingredients.

**EFFECT OF BUTTERMILK MADE FROM CREAMS WITH DIFFERENT HEAT TREATMENT HISTORIES ON PROPERTIES OF RENNET GELS AND MODEL CHEESES**

*P. Morin, Y. Pouliot and M. Britten*

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In this study, buttermilk was manufactured from raw cream and pasteurized cream, as well as from a cream derived from pasteurized whole milk. Skim milks with the same heat treatments were also manufactured to be used as controls. Compositional analysis of the buttermilks revealed a pH 4.6-insoluble protein content approximately 10% lower than that of the skim milk counterparts. Milk fat globule membrane (MFGM) proteins remained soluble at pH 4.6 in raw cream buttermilk; however, when heat was applied to cream or whole milk before butter making, MFGM proteins precipitated with the caseins. Rennet gel characterization showed that MFGM material in the buttermilks decreased the firmness and increased the set-to-cut time of rennet gels, but this effect was amplified when pasteurized cream buttermilk was added to cheese milk. The microstructure of gels was studied, and it was observed that gel appearance was very different when pasteurized cream buttermilk was used, as opposed to raw cream buttermilk. Model cheeses manufactured with buttermilks tended to have a higher moisture content than cheeses made with skim milks, explaining the higher yields obtained with buttermilk. Superior retention of MFGM particles was observed in model cheeses made from pasteurized cream buttermilk compared with raw cream buttermilk. The results from this study show that pasteurization of cream and of whole milk modifies the surface of MFGM particles, and this may explain why buttermilk has poor coagulation properties and therefore yields rennet gels with texture defects.

**WHEY STARTER FOR GRANA PADANO CHEESE: EFFECT OF TECHNOLOGICAL PARAMETERS ON VIABILITY AND COMPOSITION OF THE MICROBIAL COMMUNITY**

*M. Santarelli, M. Gatti, C. Lazzi, V. Bernini, G. A. Zapparoli and E. Neviani*

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This work aimed to investigate the effects of thermal treatments and yeast extract addition on the composition of the microbial community of natural whey starters for Grana Padano cheese. Different natural whey starter samples were held at 4°C for 24 h (cooling treatment), or at -20°C for 24 h (freezing treatment) to evaluate the possibility of conservation, or at 54°C for 1 h (heat treatment) to evaluate the effect of the temperature commonly used during curd cooking. Separately, another set of samples was enriched with 0.3, 0.5, and 1.0% (wt/vol) of yeast extract to study its effect on the growth of lactic acid bacteria (LAB) in the starter. The new approach in this study is the use of 2 culture-independent methods: length heterogeneity (LH)-reverse transcription (RT)-PCR and fluorescence microscopy. These techniques allowed us to easily, quickly, and reproducibly assess metabolically active LAB in the control and treated samples. The LH-RT-PCR technique distinguished microorganisms based on natural variations in the length of 16S rRNA amplified by RT-PCR, as analyzed by using an automatic gene sequencer. Fluorescence microscopy counts were performed by using a Live/Dead BacLight bacterial viability kit. The repeatability of LH-RT-PCR showed that this technique has great

potential to reveal changes in the microbial community of natural whey starters for Grana Padano cheese. All species showed low sensitivity to cold (4°C). However, after the freezing (-20°C) and heating (54°C) treatments, different behaviors of the species were reported, with significant changes in their viability and relative composition. Heating treatment during curd cooking profoundly affected the viability and composition of the community that remained in the cheese and that consequently modified the microbial population. At the same time, this treatment produced the selection of LAB in whey and could be considered as the first step in natural whey starter production. Addition of yeast extract stimulated the growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *lactis* to the detriment of *Lactobacillus helveticus* species. Because the yeast extract altered the microflora balance, whey starter conservation at -20°C and yeast extract addition cannot be suggested as technological innovations.

### **SHORT-WAVE NEAR-INFRARED SPECTROSCOPY OF MILK POWDER FOR BRAND IDENTIFICATION AND COMPONENT ANALYSIS**

*D. Wu, S. Feng and Y. He*

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The aim was to provide new insight into the short-wave near-infrared (NIR) spectroscopic analysis of milk powder. Near-infrared spectra in the 800- to 1,025-nm region of 350 samples were analyzed to determine the brands and quality of milk powders. Brand identification was done by a least squares support vector machine (LS-SVM) model coupled with fast fixed-point independent component analysis (ICA). The correct answer rate of the ICA-LS-SVM model reached as high as 98%, which was better than that of the LS-SVM (95%). Contents of fat, protein, and carbohydrate were determined by the LS-SVM and ICA-LS-SVM models. Both processes offered good determination performance for analyzing the main components in milk powder based on short-wave NIR spectra. The coefficients of determination for prediction and root mean square error of prediction of ICA-LS-SVM were 0.983, 0.231, and 0.982, and 0.161, 0.980, and 0.410, respectively, for the 3 components. However, there were less than 10 input variables in the ICA-LS-SVM model compared with 225 in the LS-SVM model. Thus, the processing time was much shorter and the model was simpler. The results presented in this paper demonstrate that the short-wave NIR region is promising for fast and reliable determination of the brand and main components in milk powder.

### **RHEOLOGICAL PROPERTIES OF RENNET GELS CONTAINING MILK PROTEIN CONCENTRATES**

*M. A. Ferrer, A. R. Hill and M. Corredig*

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Different milk protein concentrates (MPC), with protein concentrations of 56, 70, and 90%, were dispersed in water under different treatments (hydration, shear, heat, and overnight storage at 4°C), as well as in a combination of all the treatments in a factorial design. The particle

size distribution of the dispersions was then measured to determine the optimal conditions for the dispersion. Heating at 60°C for 30 min with 5 min of shear was chosen as the best condition to dissolve MPC powders. The samples were also characterized for composition, presence of protein aggregates, and ratio of calcium to protein. The total calcium present in MPC increased with increasing concentration of protein; however, the total calcium-to-protein ratio was lower in MPC90 than in MPC56 and MPC70. The level of whey protein denaturation, the presence of  $\beta$ -casein-whey protein aggregates in the supernatant after centrifugation, and the amount of caseins dissociated from the micelle increased as the protein concentration in the powder increased. The total amount of casein macropeptide released was lower in samples from powders with a higher protein concentration than for MPC56 or the skim milk control. The gelation behavior of reconstituted MPC was tested in systems dispersed in water (5% protein) as well as in systems dispersed in skim milk (6% protein). The gelation time of MPC dispersions was considerably lower and the gel modulus was higher than those of reconstituted skim milk with the same protein concentration. When MPC dispersions were dialyzed against skim milk, a significant decrease in the gelation time and modulus were shown, with a complete loss of gelling functionality in MPC90 dispersed in water. This demonstrated that the ionic equilibrium was key to the functionality of MPC.