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SONICATION INCREASES THE HEAT STABILITY OF WHEY PROTEINS

M. Ashokkumar, J. Lee, B. Zisu, R. Bhaskarcharya, M. Palmer and S. Kentish
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The thickening or gelling of protein-based dairy streams and ingredients upon exposure to heat has been an ongoing problem in dairy processing for many decades. This phenomenon can restrict the range of dairy product options and reduce manufacturing efficiencies by limiting the type and extent of heat treatment that can be used. In this report, we outline a novel approach to overcoming this problem. The use of preheating treatments to induce whey protein aggregate formation in whey products is well known in the field. However, we show that the application of ultrasound for a very short duration after such a heating step breaks down these aggregates and prevents their reformation on subsequent heating, thereby reducing the viscosity increase that is usually associated with this process. This novel technique has the potential to provide significant economic benefit to the dairy manufacturing industry.

ISOLATION OF CASEINS FROM WHEY PROTEINS BY MICROFILTRATION MODIFYING THE MINERAL BALANCE IN SKIM MILK

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The objective was to study the effect of different salts and salt concentration on the isolation of casein micelles from bovine raw skim milk by tangential flow microfiltration. Tangential flow microfiltration (0.22 μm) was conducted in a continuous process adding a modified buffer to maintain a constant initial sample volume. This buffer contained calcium chloride ($CaCl_2$), sodium phosphate (Na_2HPO_4), or potassium citrate ($K_3C_6H_5O_7$) in concentrations ranging from 0 to 100 m*M*. The concentrations of caseins and whey proteins retained were determined by sodium dodecyl sulfate-PAGE and analyzed using the Scion Image software (Scion Corporation, Frederick, MD). A complete isolation of caseins from whey proteins was achieved using sodium phosphate in the range of 10 to 50 m*M* and 20 times the initial volume of buffer added. No whey proteins were detected at 50 m*M* but this was at the expense of low caseins being retained. When lower sodium phosphate concentrations were used, the amount of caseins retained was higher but a small amount of whey proteins were still detected by sodium dodecyl sulfate-PAGE. Among the salts tested, calcium chloride at 50 m*M* and all volumes of buffer showed the higher retention of casein proteins. The highest casein:whey protein ratio was found at 30 m*M* $CaCl_2$, but no complete casein micelle isolation was achieved. Potassium citrate was the most ineffective salt because a rapid loss of

caseins and whey proteins was observed at all concentrations and with low quantities of buffer added during the filtration process. The results show the potential of altering the mineral balance in milk for isolation of casein micelles from whey proteins in a continuous tangential flow microfiltration system.

PHYSICOCHEMICAL CHARACTERIZATION OF MOZZARELLA CHEESE WHEYS AND STRETCHWATERS IN COMPARISON WITH SEVERAL OTHER SWEET WHEYS

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To better understand the origins of the problems occurring during Mozzarella cheese whey concentration, lactose crystallization, and spray-drying steps, a physicochemical characterization was achieved. For this purpose, Mozzarella cheese wheys were sampled and their content in different compounds such as total nitrogen, noncasein nitrogen, nonprotein nitrogen, lactate, citrate, chloride, sulfate, phosphate anions, calcium, magnesium, potassium, sodium cations, and the sugars glucose and galactose were measured. In a second step, the results were compared with the corresponding content in Cheddar cheese wheys, Raclette cheese wheys, soft cheese wheys, and Swiss-type cheese wheys. At the end of this survey, it was shown that Mozzarella cheese wheys were more concentrated in lactate and in minerals—especially phosphate, calcium, and magnesium—than the other cheese wheys and that they contained galactose. These constituents are known to be hygroscopic. Complementary surveys are now necessary to compare the hygroscopicity of galactose and lactate and discover whether the amounts of these compounds found in Mozzarella cheese wheys are a factor in the problems encountered during the concentration, lactose crystallization, and spray-drying steps.

AN ADJUVANT-FREE MOUSE MODEL TO EVALUATE THE ALLERGENICITY OF MILK WHEY PROTEIN

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Milk allergy is the most common type of food allergy in humans with the potential for fatality. An adjuvant-free mouse model would be highly desirable as a preclinical research tool to develop novel hypoallergenic or nonallergenic milk products. Here we describe an adjuvant-free mouse model of milk allergy that uses transdermal sensitization followed by oral challenge with milk protein. Groups of BALB/c mice were exposed to milk whey protein via a transdermal route, without adjuvant. Systemic IgG1 and IgE antibody responses to transdermal exposure as well as systemic anaphylaxis and hypothermia response to oral protein challenge were studied. Transdermal exposure resulted in a time- and dose-dependent induction of significant IgE and IgG1 antibody responses. Furthermore, oral challenge of sensitized mice resulted in significant clinical symptoms of systemic anaphylaxis within 1 h and significant hypothermia at 30 min postchallenge. To study the underlying mechanism, we examined allergen-driven spleen cell T-helper 2 cytokine (IL-4) responses. There was a robust dose- and time-dependent activation of memory IL-4 responses in allergic mice but not in

healthy control mice. These data demonstrate for the first time a novel transdermal sensitization followed by oral challenge mouse model of milk allergy that does not use adjuvant. It is expected that this model may be used not only to study mechanisms of milk allergy, but also to evaluate novel milk products for allergenic potential and aid in the production of hypo- or nonallergenic milk products.

THE EFFECT OF AGING ON LOW-FAT, REDUCED-FAT, AND FULL-FAT CHEDDAR CHEESE TEXTURE

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This study investigated the effects of aging and fat content on the texture of Cheddar cheese, both mechanical and sensory aspects, over a 9-mo aging period. Cheeses of 6, 16, and 33% fat were tested at 0.5, 3, 6, and 9 mo of aging. Cheeses were evaluated by a trained sensory panel using an established texture lexicon as well as instrumental methods, which were used to probe cheese structure. Sensory analysis showed that low-fat cheeses were differentiated from full-fat cheeses by being more springy and firm and this difference widened as the cheeses aged. In addition, full-fat cheeses broke down more during chewing than the lower fat cheeses and the degree of breakdown increased with aging. Mechanical properties were divided by magnitude of deformation during the test and separated into 3 ranges: the linear viscoelastic region, the nonlinear region, and fracture point. These regions represent a stress/strain response from low to high magnitude, respectively. Strong relationships between sensory terms and rheological properties determined in the linear (maximum compliance) and nonlinear (critical stress and strain and a nonlinear shape factor) regions were revealed. Some correlations were seen with fracture values, but these were not as high as terms related to the nonlinear region of the cheeses. The correlations pointed to strain-weakening behavior being the critical mechanical property. This was associated with higher fat content cheeses breaking down more as strain increased up to fracture. Increased strain weakening associated with an increase in fat content was attributed to fat producing weak points in the protein network, which became initiation sites for fracture within the structure. This suggests that fat replacers need to serve this functional role.

COMPARISON OF COMPOSITION, SENSORY, AND VOLATILE COMPONENTS OF THIRTY-FOUR PERCENT WHEY PROTEIN AND MILK SERUM PROTEIN CONCENTRATES

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The objectives were to identify and compare the composition, flavor, and volatile components of serum protein concentrate (SPC) and whey protein concentrate (WPC) containing about 34% protein made from the same milk to each other and to commercial 34% WPC from 6 different factories. The SPC and WPC were manufactured in triplicate with each pair of serum and traditional

whey protein manufactured from the same lot of milk. At each replication, SPC and WPC were spray dried (SD) and freeze dried (FD) to determine the effect of the heat used in spray drying on sensory properties. A trained sensory panel documented the sensory profiles of rehydrated SD or FD powders. Volatile components were extracted by solid-phase microextraction (SPME) and solvent extraction followed by solvent-assisted flavor evaporation (SAFE) with gas chromatography-mass spectrometry and gas chromatography-olfactometry. Whey protein concentrates had higher fat content, calcium, and glycomacropeptide content than SPC. Color differences (Hunter L, a, b) were not evident between SPC and WPC powders, but when rehydrated, SPC solutions were clear, whereas WPC solutions were cloudy. No consistent differences were documented in sensory profiles of SD and FD SPC and WPC. The SD WPC had low but distinct buttery (diacetyl) and cardboard flavors, whereas the SD SPC did not. Sensory profiles of both rehydrated SD products were bland and lower in overall aroma and cardboard flavor compared with the commercial WPC. Twenty-nine aroma impact compounds were identified in the SPC and WPC. Lipid and protein oxidation products were present in both products. The SPC and WPC manufactured in this study had lower total volatiles and lower concentrations of many lipid oxidation compounds when compared with commercial WPC. Our results suggest that when SPC and WPC are manufactured under controlled conditions in a similar manner from the same milk using the same ultrafiltration equipment, there are few sensory differences but distinct compositional and physical property differences that may influence functionality. Furthermore, flavor (sensory and instrumental) properties of both pilot-scale manufactured protein powders were different from commercial powders suggesting the role of other influencing factors (e.g., milk supply, processing equipment, sanitation).

PROPERTIES OF WHEY PROTEIN-BASED FILMS CONTAINING ORGANIC ACIDS AND NISIN TO CONTROL LISTERIA MONOCYTOGENES

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Whey protein isolate and glycerol were mixed to form a matrix to incorporate antimicrobial agents and produce edible films with antimicrobial activity against *Listeria monocytogenes* strains isolated from cheeses. Various organic acids were used to decrease pH down to approximately 3. In a preliminary assay without nisin, the effect of each organic acid was evaluated with respect to the rheological properties of the film solutions and the inhibitory and mechanical properties of the films. Lactic, malic, and citric acids (3%, wt/vol), which were used in a subsequent study of their combined inhibitory effect with nisin (50 IU/ml), had significantly higher antilisterial activity ($P < 0.05$) compared with the control (2 N HCl, 3% [wt/vol], with nisin). The largest mean zone of inhibition was 4.00 ± 0.92 mm for malic acid with nisin. Under small-amplitude oscillatory stress, the protein-glycerol-acid film solutions exhibited a predominantly viscous behavior or a weak gel behavior, with the storage modulus (G') slightly higher than the loss modulus (G?). The malic acid-based solution was the only one whose viscosity was not influenced by the addition of nisin. The addition of nisin resulted in a nonsignificant ($P > 0.05$) increase in the percentage of elongation at break. Results from tensile and puncture stress were variable, but in general no significant differences were found after the incorporation of nisin. The overall results support the use of malic acid with nisin to produce effective antimicrobial films to control *L. monocytogenes* growth.