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GLYCOSYLATION AND EXPANDED UTILITY OF A MODIFIED WHEY PROTEIN INGREDIENT VIA CARBOHYDRATE CONJUGATION AT LOW pH

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Previously, an acidified, thermally treated whey protein concentrate (mWPC) was developed to produce a cold-set thickening ingredient. Mass spectroscopy revealed an approximate 2.5-fold decrease in the lactosylation of α -lactoglobulin in mWPC starting materials compared with commercial whey protein concentrates, manufactured at a higher pH. Potentially, this should increase the number of reactive sites that remain available for carbohydrate attachment. With this study, the formation of glycoprotein complexes was demonstrated between the mWPC ingredient and lactose, naturally occurring in mWPC powders, or between mWPC protein components with dextran (35 to 45 and 100 to 200 kDa) materials at low pH. In fact, additional dry heating of mWPC powders showed a 3-fold increase in the amount of lactosylated α -lactoglobulin. Evidence of Maillard reactivity was suggested using colorimetry, *o*-phthaldialdehyde assays, and sodium dodecyl sulfate PAGE followed by glycoprotein staining. Resultant glycoprotein dispersions exhibited altered functionality, in which case steady shear and small amplitude oscillatory rheology parameters were shown to be dependent on the specific reducing sugar present. Furthermore, the emulsion stability of mWPC-dextran fractions was 2 to 3 times greater than either mWPC or commercial WPC dispersions based on creaming index values. The water-holding capacity of all test samples decreased with additional heating steps; however, mWPC-dextran powders still retained nearly 6 times their weight of water. Scanning electron microscopy revealed that mWPC-dextran conjugates formed a porous network that differed significantly from the dense network observed with mWPC samples. This porosity likely affected both the rheological and water-binding properties of mWPC-dextran complexes. Taken together, these results suggest that the functionality of mWPC ingredients can be enhanced by conjugation with carbohydrate materials at low pH, especially with regard to improving the emulsifying attributes.

THE ACCELERATED RIPENING OF CHOLESTEROL-REDUCED CHEDDAR CHEESE BY CROSSLINKED α -CYCLODEXTRIN

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This study was carried out to investigate the influence of salt content on cholesterol-reduced Cheddar cheese obtained by a treatment with crosslinked α -cyclodextrin (α -CD) and to find if the ripening process was accelerated in cholesterol-reduced cheese. The crosslinked α -CD used was made by

adipic acid. A primary study indicated that the chemical and rheological properties were not changed by the salt addition and the composition of Cheddar cheese treated with crosslinked α -CD was similar to untreated Cheddar cheese. Approximately 91 to 92% cholesterol reduction was observed in the cheeses that were treated using α -CD. In a subsequent study, we found accelerated ripening by the crosslinked α -CD based on the productions of short-chain free fatty acids and free amino acids. In rheological properties, elasticity, cohesiveness, and gumminess scores in the cholesterol-reduced Cheddar cheese were significantly greater at 5 wk ripening than those in the control at 4 mo ripening. At the early stage of ripening, most flavor properties such as rancidity, bitterness, and off-flavor in the cholesterol-reduced cheese were greater. With ripening, however, those scores changed to similar or lower scores than those in the control. The present study indicated that the crosslinked α -CD treatment for cholesterol removal showed accelerated ripening effect on the properties of Cheddar cheese.

FAT PROPERTIES DURING HOMOGENIZATION, SPRAY-DRYING, AND STORAGE AFFECT THE PHYSICAL PROPERTIES OF DAIRY POWDERS

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Changes in fat properties were studied before, during, and after the drying process (including during storage) to determine the consequences on powder physical properties. Several methods were combined to characterize changes in fat structure and thermal properties as well as the physical properties of powders. Emulsion droplet size and droplet aggregation depended on the homogenizing pressures and were also affected by spray atomization. Aggregation was usually greater after spray atomization, resulting in greater viscosities. These processes did not have the same consequences on the stability of fat in the powders. The quantification of free fat is a pertinent indicator of fat instability in the powders. Confocal laser scanning microscopy permitted the characterization of the structure of fat in situ in the powders. Powders from unhomogenized emulsions showed greater free fat content. Surface fat was always overrepresented, regardless of the composition and process parameters. Differential scanning calorimetry melting experiments showed that fat was partially crystallized in situ in the powders stored at 20°C, and that it was unstable on a molecular scale. Thermal profiles were also related to the supramolecular structure of fat in the powder particle matrix. Powder physical properties depended on both composition and process conditions. The free fat content seemed to have a greater influence than surface fat on powder physical properties, except for wettability. This study clearly showed that an understanding of fat behavior is essential for controlling and improving the physical properties of fat-filled dairy powders and their overall quality.

CHEDDAR CHEESE CLASSIFICATION BASED ON FLAVOR QUALITY USING A NOVEL EXTRACTION METHOD AND FOURIER TRANSFORM INFRARED SPECTROSCOPY

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A rapid and simple solvent extraction procedure in combination with Fourier transform infrared spec-

trospectroscopy was developed for classifying Cheddar cheese based on flavor quality. Fifteen Cheddar cheese samples from 2 commercial production plants were ground into powders using liquid nitrogen. The water-soluble compounds from the cheese powder, without interfering compounds such as fat and protein, were extracted using water, chloroform, and ethanol. Aliquots (10 μL) of the extract were placed on a zinc selenide crystal, vacuum dried, and scanned in the mid-infrared region (4,000 to 700 cm^{-1}). The infrared spectra were analyzed by soft independent modeling of class analogy (SIMCA) for pattern recognition. Sensory flavor quality of these cheeses was determined by trained quality assurance personnel in the production facilities. The SIMCA models provided 3-dimensional classification plots in which all the 15 cheese samples formed well-separated clusters. The orientation of the clusters in 3-dimensional space correlated well with their cheese flavor characteristics (fermented, unclean, low flavor, sour, good Cheddar, and so on). The discrimination of the samples in the SIMCA plot was mainly due to organic acids, fatty acids and their esters, and amino acids (1,450 to 1,350 and 1,200 to 990 cm^{-1}), which are known to contribute significantly to cheese flavor. The total analysis time, including the sample preparation time, was less than 20 min per sample. This technique can be a rapid, inexpensive, and simple tool to the cheese industry for predicting the flavor quality of cheese.

COMPARISON OF PETRIFILM™ STAPH EXPRESS COUNT SYSTEM WITH THE BACTERIOLOGICAL ANALYTICAL MANUAL DIRECT-PLATING METHOD FOR ENUMERATION OF STAPHYLOCOCCUS AUREUS IN ARTIFICIALLY CONTAMINATED HARD CHEESE

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The 3M Petrifilm™ Staph Express Count System was compared with the U.S. Food and Drug Administration's *Bacteriological Analytical Manual* (BAM) direct-plate count method for the enumeration of *Staphylococcus aureus* in 6 types of artificially contaminated hard cheese (Asiago, Cheddar, Gruyère, Parmesan, Romano, and Swiss). Five different samples of each cheese type were inoculated with *S. aureus* (ATCC 25923) to achieve low, medium, and high inoculum levels. *S. aureus* was enumerated by the Petrifilm and BAM methods, and the results were compared. Multivariate analysis of variance revealed no significant differences ($P < 0.05$) between the 2 methods. The Petrifilm method compared favorably with the BAM procedure. The rapid method was more convenient to use, considerably faster, and less expensive to perform than the BAM method.

FUNCTIONAL PROPERTIES OF WHEY PROTEINS AFFECTED BY HEAT TREATMENT AND HYDRODYNAMIC HIGH-PRESSURE SHEARING

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Two batches of native whey proteins (WP) were subjected to microfluidization or heat denaturation accompanied by microfluidization, followed by spray drying. Powders were assessed for their solubility, heat stability, coagulation time, and emulsifying and foaming properties. Effects of denaturation and shearing were examined by particle size analysis, differential scanning calorimetry, reducing and

nonreducing sodium dodecyl sulfate-PAGE, and size exclusion-HPLC. Heat treatment significantly decreased solubility, whereas the number of microfluidization passes markedly improved solubility. The combined effect of heat and pressure significantly increased heat coagulation time. Emulsifying activity index substantially increased upon heat denaturation and was further enhanced by microfluidization. Emulsion stability appeared unaffected by the combined treatment, but the concentration of adsorbed protein on fat droplets was significantly increased. Foaming properties were diminished by heating. Particle size distribution patterns, sodium dodecyl sulfate-PAGE, and size exclusion-HPLC revealed disappearance of major WP and creation of relatively higher, as well as smaller, molecular weight aggregates as a result of the 2 treatments. The use of heat and microfluidization in combination could be used to stabilize WP against heat by producing microparticulated species that have different surface and colloidal properties compared with native WP. These results have implications for the use of WP as an additive in heat-processed foods.

PHOSPHOLIPID ENRICHMENT IN SWEET AND WHEY CREAM BUTTERMILK POWDERS USING SUPERCRITICAL FLUID EXTRACTION

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Milk fat globule membrane contains many complex lipids implicated in an assortment of biological processes. Microfiltration coupled with supercritical fluid extraction (SFE) has been shown to provide a method of concentrating these nutritionally valuable lipids into a novel ingredient. In the dairy industry there are several by-products that are rich in phospholipids (PL) such as buttermilk, whey, and whey cream. However, PL are present at low concentrations. To enrich PL in buttermilk powders, regular buttermilk and whey buttermilk (by-product of whey cream after making butter) were microfiltered and then treated with SFE after drying. The total fat, namely nonpolar lipids, in the powders was reduced by 38 to 55%, and phospholipids were concentrated by a factor of 5-fold. Characterization of the PL demonstrated specific molecular fatty amide combinations on the sphingosine (18:1) backbone of sphingomyelin with the greatest proportion being saturated; the most common were 16:0, 20:0, 21:0, 22:0, 23:0, and 24:0. Two unsaturated fatty amide chains, 23:1 and 24:1, were shown to be elevated in a whey cream buttermilk sample compared with the others. However, most unsaturated species were not as abundant.

EFFECT OF NITROGEN FLUSHING AND STORAGE TEMPERATURE ON FLAVOR AND SHELF-LIFE OF WHOLE MILK POWDER

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This study evaluated the influence of packaging atmosphere, storage temperature, and storage time on WMP shelf-life using sensory and instrumental techniques. Two commercial batches of WMP were repackaged in plastic laminate pouches with air or nitrogen and stored at 2°C or 23°C for 1 yr. De-

scriptive analysis was conducted using a 10-member trained panel; volatile analysis was performed using solid-phase microextraction with gas chromatography-mass spectrometry. Consumer acceptance (n = 75) was conducted every 3 mo with reconstituted WMP and white and milk chocolate made from each treatment. Data were analyzed using ANOVA with Fisher's LSD, Pearson correlation analysis, and principal component analysis. Air-stored WMP had higher peroxide values, lipid oxidation volatiles, and grassy and painty flavors than nitrogen-flushed WMP. Storage temperature did not affect levels of straight chain lipid oxidation volatiles; 23°C storage resulted in higher cooked and milkfat flavors and lower levels of grassy flavor compared with 2°C storage. Consumer acceptance was negatively correlated with lipid oxidation volatiles and painty flavor. Nitrogen flushing prevented the development of painty flavor in WMP stored up to 1 yr at either temperature, resulting in chocolate with high consumer acceptance. Nitrogen flushing can be applied to extend the shelf life of WMP for use in chocolate; storage temperature also plays a role, but to a lesser extent.