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SHORT COMMUNICATION: EFFECT OF STORAGE TEMPERATURE ON THE SOLUBILITY OF MILK PROTEIN CONCENTRATE 80 (MPC80) TREATED WITH NaCl or KCl

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A previous study in our laboratory showed that addition of 150 m*M* NaCl or KCl into diafiltration water improved the solubility of freshly made milk protein concentrate 80 (MPC80). In the present study, the objectives were (1) to evaluate the solubility of NaCl- or KCl-treated MPC80 samples kept at varying temperatures and then stored for extensive periods at room temperature (21°C ± 1°C); and (2) to determine if MPC80 samples stored at different temperatures and protein conformation can be grouped or categorized together. Freshly manufactured MPC80 samples were untreated (control), processed with NaCl, or processed with KCl. One set of sample bags was stored at 4°C; second and third sets of bags were kept at 25°C and 55°C for 1 mo (31 d) and then transferred to room temperature (21°C ± 1°C) storage conditions for 1 yr (365 d). Samples were tested for nitrogen solubility index (NSI) and for protein changes by Fourier-transform infrared (FTIR) spectroscopy. Analysis of variance results for NSI showed 2 significantly different groupings of MPC80 samples. The more soluble group contained samples treated with NaCl or KCl and stored at either 4°C or 25°C. These samples had mean NSI >97.5%. The less soluble groups contained all control samples, regardless of storage temperature, and NaCl- or KCl-treated samples stored at 55°C. These samples had mean NSI from 39.5 to 58%. Within each of these groups (more soluble and less soluble), no significant differences in solubility were detected. Pattern recognition analysis by soft independent modeling of class analogy (SIMCA) was used to assess protein changes during storage by monitoring the amide I and amide II ($1,700^{-1}$ to $1,300\ cm^{-1}$) regions. Dominant bands were observed at $1,385\ cm^{-1}$ for control, $1,551\ cm^{-1}$ for KCl-treated samples, and $1,694\ cm^{-1}$ for NaCl-treated samples. Moreover, SIMCA clustered the MPC80 samples stored at 4°C separately from samples stored at 25°C and 55°C. This study demonstrates that (1) the addition of NaCl or KCl during MPC80 manufacture reduces the deleterious changes in solubility upon prolonged storage at 4°C or 25°C, and (2) the solubility of samples stored at 55°C is poor irrespective of salt treatment.

INCORPORATION OF WHEY PERMEATE, A DAIRY EFFLUENT, IN ETHANOL FERMENTATION TO PROVIDE A ZERO WASTE SOLUTION FOR THE DAIRY INDUSTRY

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This study proposes a novel alternative for utilization of whey permeate, a by-product stream from the dairy industry, in wheat fermentation for ethanol production using *Saccharomyces cerevisiae*. Whey permeates were hydrolyzed using enzymes to release fermentable sugars. Hydrolyzed whey permeates were integrated into wheat fermentation as a co-substrate or to partially replace process water. Cold starch hydrolysis-based simultaneous saccharification and fermentation was done as per the current industrial protocol for commercial wheat-to-ethanol production. Ethanol production was not affected; ethanol yield efficiency did not change when up to 10% of process water was replaced. Lactic acid bacteria in whey permeate did not negatively affect the co-fermentation or reduce ethanol yield. Whey permeate could be effectively stored for up to 4 wk at 4°C with little change in lactose and lactic acid content. Considering the global abundance and nutrient value of whey permeate, the proposed strategy could improve economics of the dairy and biofuel sectors, and reduce environmental pollution. Furthermore, our research may be applied to fermentation strategies designed to produce value-added products other than ethanol.

PHYSICAL AND CHEMICAL CHANGES IN WHEY PROTEIN CONCENTRATE STORED AT ELEVATED TEMPERATURE AND HUMIDITY

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In a case study, we monitored the physical properties of 2 batches of whey protein concentrate (WPC) under adverse storage conditions to provide information on shelf life in hot, humid areas. Whey protein concentrates with 34.9 g of protein/100 g (WPC34) and 76.8 g of protein/100 g (WPC80) were stored for up to 18 mo under ambient conditions and at elevated temperature and relative humidity. The samples became yellower with storage; those stored at 35°C were removed from the study by 12 mo because of their unsatisfactory appearance. Decreases in lysine and increases in water activity, volatile compound formation, and powder caking values were observed in many specimens. Levels of aerobic mesophilic bacteria, coliforms, yeast, and mold were <3.85 $\log_{10}$ cfu/g in all samples. Relative humidity was not a factor in most samples. When stored in sealed bags, these samples of WPC34 and WPC80 had a shelf life of 9 mo at 35°C but at least 18 mo at lower temperatures, which should extend the market for these products.

DEVELOPMENT OF A METHOD TO CHARACTERIZE HIGH-PROTEIN DAIRY POWDERS USING AN ULTRASONIC FLAW DETECTOR

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Dissolution behavior of high-protein dairy powders plays a critical role for achieving functional and nutritional characteristics of a finished food product. Current methods for evaluating powder dissolution properties are time consuming, difficult to reproduce, and subjective. Ultrasound spectroscopy is a rapid and precise method, but requires expensive equipment and skilled technicians to carry out the tests. In the present study, an ultrasonic flaw detector (UFD) was used as an economical alternative to characterize the powder dissolution properties. The objective of study was to develop a method to characterize the dissolution behavior of milk protein concentrate (MPC) using a UFD. The experimental setup included a UFD connected to a 1-MHz immersion transducer that was kept a constant distance from a reflector plate. To validate the method, 2 batches of MPC80 from a commercial manufacturer were procured and stored at 25 and 40°C for 4 wk. Focus beam reflectance measurement and solubility index were used as reference methods. Relative ultrasound velocity and ultrasound attenuation were acquired during the dissolution of MPC samples. To characterize the MPC dissolution, 4 parameters including standard deviation of relative velocity, area under the attenuation curve, and peak attenuation were extracted from ultrasound data. As the storage temperature and time increased, the area under the attenuation curve and peak height decreased, indicating a loss of solubility. The proposed UFD-based method was able to capture the changes in dissolution of MPC during storage at 25 and 40°C. It was observed that a high-quality MPC had a low standard deviation and a larger area under the attenuation curve. As the MPC aged at 40°C, the particle dispersion rate decreased and, consequently, an increase in standard deviation and reduction in area were observed. Overall, the UFD can be a low-cost method to characterize the dissolution behavior of high-protein dairy powders.

EFFECT OF ADJUSTED pH PRIOR TO ULTRAFILTRATION OF SKIM MILK ON MEMBRANE PERFORMANCE AND PHYSICAL FUNCTIONALITY OF MILK PROTEIN CONCENTRATE

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Processing conditions during ultrafiltration of skim milk influence properties of the casein micelle and thereby the physical properties of milk protein concentrate (MPC). The aim of the study was to establish the effects of pH adjustment of skim milk feed to obtain MPC with desired emulsification properties. The ultrafiltration was conducted using commercially pasteurized skim milk with the pH adjusted to 6.7 (control), 6.3, 5.9, or 5.5 at 15°C until a volume concentration factor of 5 was reached. Effects of pH adjustment on selected physico-chemical properties (Ca content, particle size, ζ-potential) and functionalities (solubility, heat stability, emulsification capacity, and stability) of MPC were determined. Lowering the feed pH solubilized colloidal calcium

phosphate that substantially contributed to modifying the properties of casein. This caused a reduction in the particle size while increasing the net negative charge. The structural modifications in proteins were manifested in the Fourier transform infrared spectra. Subsequent concentration did not induce any further protein structural changes. Such modifications to the casein micelles and colloidal calcium phosphate negatively affected the solubility and heat stability of the corresponding MPC powders. However, the emulsion activity index improved only until the pH of the feed was lowered to 5.9 and declined when pH was dropped to 5.5, followed with the loss of stability. Readjusting the pH of MPC powder dispersions to 6.7 restored their surface properties and thereby their functionality. Lowering the feed pH also negatively affected the membrane performance by clogging the membrane pores and lowering the flux, particularly at pH 5.5. Adjusting pH to 5.9 produced MPC with optimum emulsifying properties with minimal influence on membrane performance.

EFFECT OF CERAMIC MEMBRANE CHANNEL DIAMETER ON LIMITING RETENTATE PROTEIN CONCENTRATION DURING SKIM MILK MICROFILTRATION

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The objective was to determine the effect of retentate flow channel diameter (4 or 6 mm) of nongraded permeability 100-nm pore size ceramic membranes operated in nonuniform transmembrane pressure mode on the limiting retentate protein concentration (LRPC) while microfiltering (MF) skim milk at a temperature of 50°C, a flux of 55 $kg \cdot m^{-2} \cdot h^{-1}$, and an average cross-flow velocity of 7 $m \cdot s^{-1}$. At the above conditions, the retentate true protein concentration was incrementally increased from 7 to 11.5%. When temperature, flux, and average cross-flow velocity were controlled, ceramic membrane retentate flow channel diameter did not affect the LRPC. This indicates that LRPC is not a function of the Reynolds number. Computational fluid dynamics data, which indicated that both membranes had similar radial velocity profiles within their retentate flow channels, supported this finding. Membranes with 6-mm flow channels can be operated at a lower pressure decrease from membrane inlet to membrane outlet (ΔP) or at a higher cross-flow velocity, depending on which is controlled, than membranes with 4-mm flow channels. This implies that 6-mm membranes could achieve a higher LRPC than 4-mm membranes at the same ΔP due to an increase in cross-flow velocity. In theory, the higher LRPC of the 6-mm membranes could facilitate 95% serum protein removal in 2 MF stages with diafiltration between stages if no serum protein were rejected by the membrane. At the same flux, retentate protein concentration, and average cross-flow velocity, 4-mm membranes require 21% more energy to remove a given amount of permeate than 6-mm membranes, despite the lower surface area of the 6-mm membranes. Equations to predict skim milk MF retentate viscosity as a function of protein concentration and temperature are provided. Retentate viscosity, retentate recirculation pump frequency required to maintain a given cross-flow velocity at a given retentate viscosity, and retentate protein determination by mid-infrared spectrophotometry were all useful tools for monitoring

the retentate protein concentration to ensure a sustainable MF process. Using 6-mm membranes instead of 4-mm membranes would be advantageous for processors who wish to reduce energy costs or maximize the protein concentration of a MF retentate.

SPORE POPULATIONS AMONG BULK TANK RAW MILK AND DAIRY POWDERS ARE SIGNIFICANTLY DIFFERENT

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To accommodate stringent spore limits mandated for the export of dairy powders, a more thorough understanding of the spore species present will be necessary to develop prospective strategies to identify and reduce sources (i.e., raw materials or in-plant) of contamination. We characterized 1,523 spore isolates obtained from bulk tank raw milk (n = 33 farms) and samples collected from 4 different dairy powder-processing plants producing acid whey, nonfat dry milk, sweet whey, or whey protein concentrate 80. The spores isolated comprised 12 genera, at least 44 species, and 216 *rpoB* allelic types. *Bacillus* and *Geobacillus* represented the most commonly isolated spore genera (approximately 68.9 and 12.1%, respectively, of all spore isolates). Whereas *Bacillus licheniformis* was isolated from samples collected from all plants and farms, *Geobacillus* spp. were isolated from samples from 3 out of 4 plants and just 1 out of 33 farms. We found significant differences between the spore population isolated from bulk tank raw milk and those isolated from dairy powder plant samples, except samples from the plant producing acid whey. A comparison of spore species isolated from raw materials and finished powders showed that although certain species, such as *B. licheniformis*, were found in both raw and finished product samples, other species, such as *Geobacillus* spp. and *Anoxybacillus* spp., were more frequently isolated from finished powders. Importantly, we found that 8 out of 12 genera were isolated from at least 2 different spore count methods, suggesting that some spore count methods may provide redundant information if used in parallel. Together, our results suggest that (1) *Bacillus* and *Geobacillus* are the predominant spore contaminants in a variety of dairy powders, implying that future research efforts targeted at elucidating approaches to reduce levels of spores in dairy powders should focus on controlling levels of spore isolates from these genera; and (2) the spore populations isolated from bulk tank raw milk and some dairy powder products are significantly different, suggesting that targeting in-plant sources of contamination may be important for achieving low spore counts in the finished product. These data provide important insight regarding the diversity of spore populations isolated from dairy powders and bulk tank raw milk, and demonstrate that several spore genera are detected by multiple spore count methods.

MICROSTRUCTURAL CHANGES IN HIGH-PROTEIN NUTRITION BARS FORMULATED WITH EXTRUDED OR TOASTED MILK PROTEIN CONCENTRATE

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Milk protein concentrates with more than 80% protein (that is, MPC80) are underutilized as the primary protein source in high-protein nutrition bars as they impart crumbliness and cause hardening during storage. High-protein nutrition bar texture changes are often associated with internal protein aggregations and macronutrient phase separation. These changes were investigated in model high-protein nutrition bars formulated with MPC80 and physically modified MPC80s. High-protein nutrition bars formulated with extruded MPC80s hardened slower than those formulated with toasted or unmodified MPC80. Extruded MPC80 had reduced free sulfhydryl group exposure, whereas measurable increases were seen in the toasted MPC80. High-protein nutrition bar textural performance may be related to the number of exposed free sulfhydryl groups in MPC80. Protein aggregations resulting from ingredient modification and high-protein nutrition bar storage were studied with sodium dodecyl sulfate polyacrylamide gel electrophoresis. Disulfide-based protein aggregations and changes in free sulfhydryl concentration were not consistently relatable to high-protein nutrition bar texture change. However, the high-protein nutrition bars formulated with extruded MPC80 were less prone to phase separations, as depicted by confocal laser scanning microscopy, and underwent less texture change during storage than those formulated with toasted or unmodified MPC80.