



July 1, 2018

Volume XXXII No. 1

## **INVITED REVIEW: WHEY PROTEINS AS ANTIOXIDANTS AND PROMOTERS OF CELLULAR ANTIOXIDANT PATHWAYS**

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J. of Dairy Sci. 101(6): 4747. 2018.

Oxidative stress contributes to cell injury and aggravates several chronic diseases. Dietary antioxidants help the body to fight against free radicals and, therefore, avoid or reduce oxidative stress. Recently, proteins from milk whey liquid have been described as antioxidants. This review summarizes the evidence that whey products exhibit radical scavenging activity and reducing power. It examines the processing and treatment attempts to increase the antioxidant bioactivity and identifies 1 enzyme, subtilisin, which consistently produces the most potent whey fractions. The review compares whey from different milk sources and puts whey proteins in the context of other known food antioxidants. However, for efficacy, the antioxidant activity of whey proteins must not only survive processing, but also upper gut transit and arrival in the bloodstream, if whey products are to promote antioxidant levels in target organs. Studies reveal that direct cell exposure to whey samples increases intracellular antioxidants such as glutathione. However, the physiological relevance of these in vitro assays is questionable, and evidence is conflicting from dietary intervention trials, with both rats and humans, that whey products can boost cellular antioxidant biomarkers.

## **SHORT COMMUNICATION: EFFECTS OF NANOFILTRATION AND EVAPORATION ON THE GEL PROPERTIES OF MILK PROTEIN CONCENTRATES WITH DIFFERENT PREHEAT TREATMENTS**

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J. of Dairy Sci. 101(6): 4977. 2018.

This study aimed to evaluate the effects of different concentration methods (nanofiltration and evaporation) and heat treatments on the gel properties of milk protein concentrate (MPC). The MPC gels were produced using glucono- $\delta$ -lactone (GDL) as an acidifier with different preheat treatments (30 min at 80°C and 5 min at 92°C). We then evaluated the effect of preheat treatments on MPC gel properties, including storage modulus ( $G'$ ), loss tangent ( $\tan \delta$ ), firmness, whey separation, and microstructure. The results indicated that without preheating, evaporation (EP)-MPC had higher  $G'$  and firmness, and lower  $\tan \delta$  and whey separation than nanofiltration (NF)-MPC. These results suggest that EP-MPC produced a better acid-induced gel than NF-MPC when no preheat treatments were performed. After preheating, however, except for a very small difference in the final  $G'$  (EP-MPC was higher), the 2 MPC did not differ

significantly in firmness, final  $\tan \delta$ , or whey separation. Additionally, compared with the gel of unheated MPC, both preheat-treated gels (NF-MPC and EP-MPC) achieved increased  $G'$  and firmness and decreased  $\tan \delta$  and whey separation. The preheat-treated MPC also displayed a more flexible-stranded network. These findings demonstrate that, given a suitable heating treatment, NF-MPC compares favorably with EP-MPC in achieving desired gel properties.

### **THE EFFECT OF SPRAY DRYING ON THE DIFFERENCE IN FLAVOR AND FUNCTIONAL PROPERTIES OF LIQUID AND DRIED WHEY PROTEINS, MILK PROTEINS, AND MICELLAR CASEIN CONCENTRATES**

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J. of Dairy Sci. 101(5): 3900. 2018.

The objective was to determine the effect of spray drying on the flavor and functionality of high-protein ingredients. Liquid and dried protein ingredients (whey protein concentrate with 80% protein, whey protein isolate, milk protein concentrate with 85% protein, and micellar casein concentrate) were manufactured from the same lot of milk at the North Carolina State University pilot plant. Functional differences were evaluated by measurement of foam stability and heat stability. Heat stability was evaluated by heating at 90°C for 0, 10, 20, and 30 min followed by micro-bicinchoninic acid and turbidity loss measurements. Sensory properties were evaluated by descriptive analysis, and volatile compounds were evaluated by gas chromatography-mass spectrometry. No differences were detected in protein heat stability between liquids and powders when spray dried under these conditions. Whey protein concentrate with 80% protein (liquid or spray dried) did not produce a foam. All powders had higher aroma intensity and cooked flavors compared with liquids. Powder proteins also had low but distinct cardboard flavor concurrent with higher relative abundance of volatile aldehydes compared with liquids. An understanding of how spray drying affects both flavor and functionality may help food processors better use the ingredients they have available to them.

### **SOUTHEAST ASIA'S IMPORT DEMAND FOR SKIM MILK POWDER: IMPLICATIONS FOR US EXPORTERS**

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J. of Dairy Sci. 101(5): 4676. 2018.

Dairy industries in Southeast Asia are small and produce less than the domestic market demands. As expenditure and population grow in Southeast Asia, it is expected that the expenditures on skim milk powder (SMP) will grow. In this study, we examined the competitiveness of US SMP in the Southeast Asian market with respect to other leading dairy exporters, including the European Union (EU-28), New Zealand, and Australia. Using monthly data from 2006 to 2015, Rotterdam models were used to estimate import demands for SMP in 4 Southeast Asian countries. In a scenario using annual averages from 2013 to 2015 as a baseline, our findings suggest that a 10% reduction in the US price of SMP would cause Indonesia, Singapore, Vietnam, and the Philippines SMP imports from the United States to increase by 3.96, 0.44, 2.68, and 1.94 kt, respectively. Under the same scenario, the value of US SMP imports would de-

crease for Indonesia, Vietnam, and the Philippines by \$4.12, \$2.93, and \$2.48 million, respectively; however, the value of US SMP to Singapore would increase by \$0.20 million. Singapore and Indonesia expenditures for the US SMP are elastic, which means that as expenditure and population in Southeast Asia continue to grow, a 1% increase in SMP expenditure in Singapore and Indonesia would result in 1.25 and 1.20% increases in US SMP exports.

### **EFFECT OF MILK PROTEIN COMPOSITION OF A MODEL INFANT FORMULA ON THE PHYSICO-CHEMICAL PROPERTIES OF IN VIVO GASTRIC DIGESTATES**

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J. of Dairy Sci. 101(4): 2851. 2018.

They investigated the effect of protein composition and, in particular, the presence of whey proteins or  $\beta$ -casein on the digestion behavior of a model infant formula using an in vivo piglet model. Three isocaloric diets optimized for piglets were prepared with the same concentrations of protein. For protein source, 1 diet contained only whey proteins and 2 contained a casein:whey protein ratio of 40:60 but differed in the amount of  $\beta$ -casein. To obtain the desired protein compositions, skim milk was microfiltered at 7 or 22°C, and retentates and permeates were combined with whey protein isolate. The diets were optimized to the nutritional needs of the piglets and fed to 24 newborn piglets for 18 d. Eight piglets were also fed ad libitum with sow milk and considered only as reference (not included in the statistical analysis). The study was carried out in 2 blocks, killing the animals 60 and 120 min after the last meal. All gastric contents, regardless of diet, showed a wide range of pH. Postprandial time did not affect the pH or physical properties of the gastric digesta. The digesta from whey protein-casein formulas showed significantly higher viscosity, a higher storage modulus, and a denser microstructure than digesta obtained from piglets fed whey protein formula. The  $\beta$ -casein:total casein ratio at the level used in this study did not significantly affect the physical and chemical properties of the stomach digestate. Although caseins showed extensive gastric hydrolysis, whey proteins remained largely intact at both postprandial times. The results indicate that the presence of different concentrations of milk proteins can be critical to the digestion properties of the food matrix and may affect the nutritional properties of the components.

### **INFLUENCE OF PARTIALLY DEMINERALIZED MILK PROTEINS ON RHEOLOGICAL PROPERTIES AND MICROSTRUCTURE OF ACID GELS**

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J. of Dairy Sci. 101(3): 1864. 2018.

In this study, CO<sub>2</sub>-treated milk protein concentrate powder with 80% protein in dry matter (TMPC80) was mixed with nonfat dry milk (NDM) in different ratios for the manufacture of acid gels. Dispersions of NDM and TMPC80 that provided 100, 90, 70, and 40% of protein from NDM were reconstituted to 4.0% (wt/wt) protein and 12.0% (wt/wt) total solids. Dispersions were adjusted to pH 6.5, followed by heat treatment at 90°C for 10 min. Glucono- $\delta$ -lactone was added and samples were incubated at 30°C, reaching pH 4.5  $\pm$  0.05 after 4 h of incubation. Glucono- $\delta$ -lactone levels were adjusted to compensate for the lower buffering capacity

of samples with higher proportions of TMPC80, which is attributable to the depletion of buffering minerals from both the serum and micellar phase during preparation of TMPC80. Sodium dodecyl sulfate-PAGE analysis indicated a higher amount of caseins in the supernatant of unheated suspensions with increasing proportions of CO<sub>2</sub>-treated TMPC80, attributable to the partial disruption of casein micelles in TMPC80. Heat treatment reduced the level of whey proteins in the supernatant due to the heat-induced association of whey proteins with casein micelles, the extent of which was larger in samples containing more micellar casein (i.e., samples with a lower proportion of TMPC80). Particle size analysis showed only small differences between nonheated and heated dispersions. Gelation pH increased from ~5.1 to ~5.3, and the storage modulus of the gels at pH 4.5 increased from ~300 to ~420 Pa when the proportion of protein contributed by TMPC80 increased from 0 to 60%. Water-holding capacity also increased and gel porosity decreased with increasing proportion of protein contributed by TMPC80. The observed gel properties were in line with microstructural observations by confocal microscopy, wherein sample gels containing increasing levels of TMPC80 exhibited smaller, well-connected aggregates with uniform, homogeneous pore sizes. We concluded that TMPC80 can be used to partially replace NDM as a protein source to improve rheological and water-holding properties in acid gels. The resultant gels also exhibited decreased buffering, which can improve the productive capacity of yogurt manufacturing plants. Overall, the process can be leveraged to reduce the amount of hydrocolloids added to improve yogurt consistency and water-holding capacity, thus providing a path to meet consumer expectations of clean label products.

#### **APPLICATION OF WHEY PROTEIN ISOLATE IN BONE REGENERATION: EFFECTS ON GROWTH AND OSTEOGENIC DIFFERENTIATION OF BONE-FORMING CELLS**

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J. of Dairy Sci. 101(1): 28. 2018.

Recently, milk-derived proteins have attracted attention for applications in the biomedical field such as tissue regeneration. Whey protein isolate (WPI), especially its main component  $\beta$ -lactoglobulin, can modulate immunity and acts as an antioxidant, antitumor, antiviral, and antibacterial agent. There are very few reports of the application of WPI in tissue engineering, especially in bone tissue engineering. In this study, we tested the influence of different concentrations of WPI on behavior of human osteoblast-like Saos-2 cells, human adipose tissue-derived stem cells (ASC), and human neonatal dermal fibroblasts (FIB). The positive effect on growth was apparent for Saos-2 cells and FIB but not for ASC. However, the expression of markers characteristic for early osteogenic cell differentiation [type-I collagen (COL1) and alkaline phosphatase (ALP)] as well as ALP activity, increased dose-dependently in ASC. Importantly, Saos-2 cells were able to deposit calcium in the presence of WPI, even in a proliferation medium without other supplements that support osteogenic cell differentiation. The results indicate that, depending on the cell type, WPI can act as an enhancer of cell proliferation and osteogenic differentiation. Therefore, enrichment of biomaterials for bone regeneration with WPI seems a promising approach, especially due to the low cost of WPI