

## **HARDENING OF WHEY PROTEIN BASED NUTRITIONAL BARS RESULTING FROM PROTEIN-PROTEIN INTERACTIONS**

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The objective was to determine whether protein-protein interactions were one of the causes of this hardening in a whey protein bar model system. The model system contained whey protein isolate and phosphate buffer (PB) at a ratio of 3 to 2. It was stored at various temperatures, and samples were taken at appropriate intervals. The solubility of samples in PB was measured, and the soluble fractions were examined by gel electrophoresis. The insoluble aggregates were further dissolved in various solutions: PB with strong denaturants, PB with reducing reagents, and PB with both strong denaturants and reducing reagents. The dissolved aggregates were also investigated by gel electrophoresis. Insoluble aggregates rapidly formed during the first 3 days of storage with a slower rate afterwards, while, no significant formation of soluble aggregates was observed. Evaluation of the insoluble aggregates showed that intermolecular disulfide bond formation was the main mechanism for protein aggregation, and all major whey proteins participated in the aggregation. The texture of the protein bar model system changed significantly during storage at 45 °C for 3 days, where more than 25% protein became insoluble. The results demonstrate that the formation of intermolecular disulfide bonds between whey proteins may serve as one of the important factors for the hardening of whey protein bars during storage, and the prevention of intermolecular disulfide bonding will provide a possible solution to the bar hardening problem.

## **RAPID VISCO ANALYSIS OF NONFAT DRY MILK. A RAPID METHOD FOR EVALUATION OF EFFECTS OF MANUFACTURER AND STORAGE CONDITIONS ON FUNCTIONAL PROPERTIES**

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Low heat NDM were stored at 4, 22, 35, and 50 °C for 8 weeks, and RVA was used to measure the hydration and thermal stability and properties of yogurts made with these samples. Influence of the manufacturer and storage conditions on the characteristics of NDM was observed. The results suggest that RVA analysis can be used to discriminate the characteristics of NDM and provide valuable information on the influence of processing and storage conditions on the functional properties of NDM.

**APPLICATION OF FLUORESCENCE SPECTROSCOPY FOR MONITORING CHANGES IN NONFAT DRY MILK DURING STORAGE**

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Low heat NDM samples were stored at 4, 22, 35, and 50 °C for 8 weeks, respectively, and principal component analysis was applied to their fluorescence spectra. Maillard reaction, modification of the tryptophan environment and degradation of riboflavin and vitamin A occurred during processing and storage. The results demonstrate that front face fluorescence spectroscopy, coupled with multivariate statistical methods, can be utilized as an analytical technique to monitor variation in the NDM samples from different manufacturers and changes during storage.

**DEVELOPMENT OF FUNCTIONAL BEVERAGES BASED ON WHITE TEA AND SOY OR DAIRY MILK AND THEIR STABILITY DURING STORAGE**

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The objective was to develop a white tea beverage with soy or dairy milk, minimizing the interaction between polyphenols and proteins. Their chemical stability during storage and the effect of protein-polyphenol interaction on antioxidant capacity of these blends were also evaluated. Total polyphenol content (Folin-Ciocalteu assay), antioxidant and antiradical activities (DPPH) were determined in soy and dairy milk-tea beverages. Binding affinity constants between polyphenols in white tea (epigallo catechin gallate, catechin, gallic acid) and proteins in soy and milk (glycinin and beta-casein) were established spectrofluorometrically. Instant powder tea/milk was stored under accelerated conditions (55°C). Stabilities of liquid (4°C) and powder instant milk teas were evaluated every 15 days for 60 days based on pH, color, soluble and insoluble solids, polyphenol content, and antioxidant activity. A ratio of 3:1 (milk:tea, w/w) produced the desired color and sensory characteristics in both teas. Total polyphenol content of soymilk tea ( $1.6 \pm 0.2$  mg eq. gallic acid/ml) and dairy milk-tea ( $1.8 \pm 0.1$  mg/ml) was stable during storage, and their antioxidant capacity only slightly lower than tea ( $70 \pm 5$  vs  $80 \pm 5$  mM Trolox/g). Soy protein had lesser interactions with catechin and gallic acid than milk protein. Overall, quality was unchanged during storage for both soy or dairy milk based teas in both liquid and powder form. The development of a soy or dairy milk-based white tea beverage offers a healthy alternative to consumers.

**FLAVOR DEVELOPMENT OF WHEY PROTEINS THROUGHOUT PROCESSING**

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The objective was to characterize flavor formation of whey proteins throughout processing: from liquid whey to dried powder. Samples were taken at various points (WPI, 8 samples; WPC 80, 6 samples) during the processing of raw whey into both whey protein isolate (WPI) and whey protein concentrate (WPC 80) on three different occasions. Flavor profiles were determined using a trained descriptive sensory panel. Volatile components were extracted by solvent extraction followed by solvent assisted

flavor evaporation (SAFE) and phase separation. Fractions were analyzed and identified using gas chromatography-olfactometry (GC-O) and gas chromatography-mass spectrometry (GC-MS). Selected compounds were quantified using external standard curves. Analysis of variance was applied to identify changes in flavor and flavor volatiles. Fluid whey was characterized by cooked/milky, sweet aromatic, and cardboard flavors and sweet and salty tastes. Reconstituted WPC 80 was characterized by astringency and cooked/milky, sweet aromatic, and cardboard flavors. Reconstituted WPI was characterized by astringency and cardboard and brothy/cabbage flavors. Sour, sweet, and salty tastes decreased, while astringency and brothy or cardboard flavors increased across whey protein processing ( $p < 0.05$ ). Key volatile flavor compounds that changed ( $p < 0.10$ ) from fluid whey to spray dried product were 2-acetyl-1-pyrroline (popcorn), methional (potato/brothy), nonanal (fatty/citrus), dimethyl trisulfide (cabbage), 2-isobutyl-3-methoxypyrazine (bell pepper/burnt), and 2,3 butanedione (buttery). The greatest changes in flavor were observed following storage, filtration, and spray drying. This study identified the changes in volatile components during the processing of whey proteins and the steps in processing with the greatest impact on flavor.

### **OFF-FLAVOR CARRY THROUGH OF WPI AND WPC80 IN PROTEIN SHAKES AND ACIDIC BEVERAGES**

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Off-flavors in whey proteins documented by trained panelists can carry through into ingredient applications and negatively affect consumer acceptability. A trained panel screened ten rehydrated whey proteins (5 WPI and 5 WPC80) using a previously identified lexicon (1). Five whey proteins (3 WPC80, 2WPI) were selected for further testing. Difference tests using rehydrated proteins were conducted to determine if consumers ( $n=50$ ) could detect differences between off-flavored products and the control products. WPC80 were incorporated into meal replacement protein shakes and WPI were incorporated into clear acidic protein beverages. Descriptive sensory analysis was conducted ( $n=8$  trained panelists) and consumer acceptance ( $n = 75$  consumers) was determined. Data were analyzed by analysis of variance with means separation. Descriptive panelists documented sweet aromatic and cardboard flavors in control whey proteins. Cabbage flavors and bitter taste were documented in the off-flavored whey proteins. Consumers could detect differences between the control and off-flavored whey proteins when evaluated alone (no ingredient application) ( $p < 0.05$ ). Descriptive panelists documented off-flavors in the ingredient applications made with the off-flavored WPI. Consumer acceptance scores were lower ( $p < 0.05$ ) for ingredient applications made with 2 of the off-flavored whey proteins, but were not different from the control for one of the off-flavored whey proteins.

### **CONTROL OF NUTTY FLAVOR FORMATION IN CHEDDAR CHEESE**

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The objectives were to determine if application of adjunct starters with elevated production of Strecker aldehydes resulted in Cheddar cheese with increased or accelerated Strecker aldehyde production. Triplicate 227 kg batches of Cheddar cheese were made with different adjunct starter levels (0, 104 cfu/mL

and 105 cfu/mL) and ripened at 5 °C or 13 °C. Cheeses were analyzed after 1 week, 4 mo, and 8 mo by a combination of instrumental and sensory methods to characterize nutty flavor development. Specifically, Strecker aldehydes were quantified by dynamic headspace analysis coupled with gas chromatography-mass spectrometry. Descriptive sensory analysis was conducted using a trained panel (n=10) and a previously identified cheese flavor language. All tests were conducted in triplicate and results were analyzed using analysis of variance with means separation. Cheeses ripened at 13 °C developed aged flavors (brothy, sulfur, and nutty flavors) more rapidly than cheeses held at 5 °C ( $P < 0.05$ ). Additionally, cheeses made with the adjunct culture showed more rapid and more intense nutty flavor development than control cheeses ( $P < 0.05$ ). Cheeses that had higher intensities of nutty flavors also had a higher concentration of 2/3-methyl butanal and 2-methyl propanal ( $P < 0.05$ ) compared to control cheeses, which again confirmed these compounds are a source of nutty flavor in Cheddar cheese. Results from this study provide a simple methodology for cheese manufacturers to obtain consistent nutty flavor in Cheddar cheese.

### **FLAVOR FORMATION IN SKIM MILK POWDER THROUGHOUT PRODUCTION**

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Previous studies have characterized the sensory profiles and chemical compounds responsible for flavors in fresh and stored SMP. Further, studies have documented that flavor variability in SMP can carry-through into ingredient applications and negatively impact consumer acceptance. A better understanding of flavor formation in SMP is needed. In this study, they examined the flavor formation of medium heat SMP throughout the production process, with emphasis on the steps where heat was applied to the product. Samples (3 L or 3 kg) were collected at five sampling points across the SMP production process (raw milk to finished powder) in on three different occasions. Proximate and microbial analyses were conducted. Descriptive sensory analysis was conducted to characterize flavor profiles of products. Volatile compounds were extracted using solvent assisted flavor evaporation (SAFE) followed by GC-MS and gas chromatography-olfactometry (GC-O) to identify and characterize aroma-active compounds. Selected compounds were quantified using external standard curves. All analyses were conducted in triplicate. Where appropriate, analysis of variance with means separation was applied to determine differences between samples. There was not a collection time effect (beginning, middle, end of production) for samples ( $p > 0.05$ ), suggesting that equipment burn-on or soiling throughout the 40 h of facility production does not impact sensory properties of the finished product. The sensory attributes of the milk changed significantly throughout the production process ( $p < 0.05$ ). Specifically, cooked and sweet aromatic flavors increased throughout the process. Concurrently, heat generated compounds, including methional, 2-acetyl-1-pyrroline, and homofuraneol increased with each processing step. The most prominent changes in volatile compounds occurred after HTST pasteurization, evaporation, and spray drying. These results enhance our understanding of SMP flavor development, a crucial property as it becomes more widely used in product applications, nationally and globally.

**FLAVOR COMPOUNDS DEVELOPED DURING BROWNING OF SWEET WHEY POWDER**

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The primary objective is to identify the volatile components associated with browning reactions in sweet whey powder (SWP). SWP was subjected to storage conditions of time and temperature sufficient to yield brown powders as characterized by colorimetry. The powders were extracted with solvents selected on the basis of polarity and acid-base chemistry. Volatiles were concentrated after using high vacuum distillation and then analyzed for identification by gas chromatography-mass spectrometry. In general, chromatograms were complex with over fifty compounds positively identified. Brown SWP samples differentiated from white powders by higher concentrations of compounds typical of non-enzymatic browning, including furanones, furans and pyrazine derivatives. Dimethyl sulfone was also identified in the browned SWP samples. Quantitative assessments show that many of these compounds are present in levels well above threshold concentrations. Browning reactions in SWP generate compounds known to influence aroma. The presence and concentrations of these compounds reflect the extent and mechanisms of brown color development. Understanding pathways to influence the production level of key aroma-active compounds demonstrates potential to improve the flavor quality of food products.

**INFLUENCE OF BUFFER CONTENT, RESIDUAL LACTOSE CONTENT AND SALT-TO-MOISTURE RATIO ON THE VISCO ELASTIC PROPERTIES OF CHEDDAR CHEESE**

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The seasonal variations, stage of lactation, and variations in the concentration methods used in the milk processing affects buffer content, residual lactose content, and salt-moisture ratio of Cheddar cheese. These changes in Cheddar cheese has significant effect on the extent of proteolysis occurring in the cheese during storage and affects the quality. We prepared eight different types of Cheddar cheeses with two levels of buffer content, two levels of residual lactose content, and two levels of salt-to-moisture ratio and the viscoelastic properties were studied at 0,1,2,4,6 and 8 months of storage. Transient stress was applied at a constant strain of 0.005 to 2 mm thick and 28.5 mm diameter cheese samples at 1.0 Hz frequency using ATS rheometer. Storage modulus ( $G'$ ) and loss modulus ( $G''$ ) were measured during heating and cooling from 30°C to 70°C. Low levels of buffer, residual lactose, and salt-to-moisture ratio cheeses had 364.5 %, 291.3 %, 281.8% increases in  $G''$  indicating better flow characteristics compared to 136.2%, 150.8%, 162.9% increases in  $G''$  in the corresponding high levels of treatments during the 8 months of storage. The  $G'$  was significantly higher for low levels of buffer, residual lactose, and salt-to-moisture ratio cheeses at  $P < 0.05$  during initial periods of storage up to 2 months. After 2 months, the  $G'$  was found to be significantly lesser at  $P < 0.05$  compared to the corresponding high levels of the treatments up to 8 months due to the continuous biochemical changes and nature of bonding of protein mycelium. This information is very useful for Cheddar cheese manufacturers for making cheese with better flow characteristics and optimum texture.

**GLYCEROL CONTENT EFFECT ON THE TENSILE PROPERTIES OF WHEY PROTEIN SHEETS FORMED BY TWIN-SCREW EXTRUSION**

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The use of whey protein-based edible films as barrier materials provides the opportunity to reduce packaging waste while controlling oxygen, aroma and lipid migration that affect food quality and shelf-life. Continuous extrusion of whey protein films through a die constitutes a more efficient method with commercial potential compared to solvent-casting and compression molding. Mechanical properties play an important role in the manufacturing and handling of stand-alone films that could be used as food wraps or pouches for dry foods. It was hypothesized that extruding whey protein isolate powder with varying glycerol contents can result in the formation of transparent, flexible sheets with different mechanical properties. The objective was to study the effect of glycerol plasticizer on the tensile properties of extruded whey protein sheets. A Haake-Leistritz co-rotating twin-screw extruder with six independent heating and cooling zones and a length-to-diameter ratio of 30:1 was used to extrude whey protein sheets containing 45.77, 48.79 and 51.86% glycerol (d.b.). Samples were collected at a screw speed of 250 rpm and a barrel temperature profile of 20-20-20-80-110-130°C. Tensile properties in the machine direction were determined using an Instron Universal Testing Machine and a crosshead speed of 50 mm/min. Melt temperature for all three types of sheets was 143-150°C, as recorded by a thermocouple imbedded in the slit die at the time of sheet formation. The average thickness of the sheets was  $1.31 \pm 0.02$  mm. Sheets containing 45.77% glycerol (d.b.) had a significantly higher tensile strength than sheets with higher glycerol contents. Furthermore, as glycerol concentration increased, elastic modulus decreased significantly. No significant differences were found in percent elongation at break among the samples. Extrusion of whey protein sheets constitutes the first step towards extrusion of thinner edible films, which can work together with conventional packaging to improve food quality, while reducing solid waste.

**A MODIFIED BOVINE LACTOFERRIN LF+ – A NEW WHEY-DERIVED NUTRACEUTICAL THAT EXHIBITS INCREASED EFFICACY AGAINST TUMORS**

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Whey is a popular dietary supplement that exhibits antimicrobial and immunomodulatory activity, modulates adiposity, enhances anti-oxidant activity, improves muscle strength and body composition, and is proposed to prevent cardiovascular disease and osteoporosis. The activity of whey can be largely attributed to a small number of major proteins among which is lactoferrin (Lf), a defense protein in secretions exposed to normal flora that displays anti-microbial activity. Lf is an immunomodulator, and is currently being investigated as a nutraceutical to combat cancer, colitis, and asthma. Here we investigated the anti-tumor activity of a modified bovine Lf, designated LF+, which was orally administered alone and in combination with immunotherapy and chemotherapy in mice. LF+ completely inhibited the formation of tumors in up to 14% of mice, whereas natural unmodified Lf only weakly inhibited tumor growth. Large tumors treated by B7-1 immunogene therapy were completely eradicated from mice fed LF+, whereas B7-1 monotherapy had no detectable impact. LF+ enhanced tumor rejection in response to

dendritic cell therapy. Large tumors were completely eradicated from mice fed LF+ and injected intraperitoneally with either paclitaxel or doxorubicin, whereas each monotherapy, and the combination of natural bovine Lf and chemotherapy, only weakly slowed tumor growth. Compared with the tumors of mice fed the control diet, the tumors of mice fed LF+ alone or in combination with chemotherapy exhibited decreased vascularity, and increased apoptosis and leukocyte infiltration. LF+ restored and increased blood cell numbers depleted by chemotherapy. In summary, LF+ renders drug-resistant tumors exquisitely sensitive to immunotherapy and chemotherapy, and hence holds promise as a medical food to augment current cancer regimes.

### **EFFECTS OF WHEY PROTEIN ON BODY WEIGHT, FAT AND HEALTH INDICES IN SUPPLEMENTED OVERWEIGHT AND OBESE ADULTS**

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Much of the information establishing a relationship between dietary protein intake and body composition is based on studies of untrained individuals beginning a weight loss program, typically combining exercise and energy restriction or of trained individuals. Data from these studies suggest that dietary protein, including dairy products, may help improve body composition. Few studies have been conducted using whey protein and few studies have been conducted without weight loss as an intended outcome. In a double-blind, randomized controlled trial of 90 overweight or obese individuals, we investigated the effects of adding to their free-living diet 60 g/d of whey or soy protein compared to carbohydrate on body weight, composition, and other health-related outcomes. After 6 mo of supplementation, body weight and fat of the group consuming the whey protein were lower than the group consuming the carbohydrate treatment, whereas there were no differences between the group consuming the soy treatment and the groups consuming the carbohydrate or whey treatments. Waist circumference was lower in the group consuming the whey protein than the two other groups. These changes were observed without a significant change in energy intake. Concomitant with changes in body composition, the group consuming the whey protein had a significant decrease in blood pressure compared to the group consuming the soy and carbohydrate treatments. Protein intake may alter insulin response; and therefore, may play a role in changes in body composition. In fact, subjects consuming the protein treatments had improved insulin sensitivity compared to the carbohydrate treatment. These results suggest that dietary protein is associated with improving body composition and that whey protein may help improve some risk factors for chronic diseases.

### **DISCRIMINATION OF SWISS CHEESES BASED ON RIPENING AND *PROPIONIBACTERIUM* STRAIN BY FTIR/ATR (FOURIER TRANSFORM INFRARED/ATTENUATED TOTAL REFLECTANCE) SPECTROSCOPY**

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The objective was to develop a rapid method for the classification of Swiss cheese according to ripening time and *Propionibacterium* strain. Three *Propionibacterium freudenreichii* strains with different cold

tolerance were used for Swiss cheese production. Triplicate vats of cheese were produced using each *P. freudenreichii* strain and the same *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains in a commercial facility. Cheeses were precooled at 4°C for 6 days and kept at 21°C for 25 days and stored at 0°C and 4°C for 2 months. Swiss cheese slices (~0.5g) were measured directly using a MIRacle three-reflection diamond ATR accessory at 0, 30, 60, and 90 days. Classification models were developed by using soft independent modeling of class analogies (SIMCA). By selecting the spectral range of 1,800-900 cm<sup>-1</sup>, cheeses of the same age were differentiated by *Propionibacterium* strain and cheeses made with the same strain were differentiated by age. Although the intensity and importance of bands were different among strains and ripening days, the important discriminating bands for classification of cheeses ripened at 0°C were 1715-1750 (carbonyl groups), 1452-1477 (C-H bending), 1172-1182 and 1014-1022 cm<sup>-1</sup> (C-O stretching). The region 1554-1577 (N-H bending) and 1,658-1,685 cm<sup>-1</sup> (probably C=O bands of amides) had also importance for the discrimination based on ripening at 4°C. Discrimination of Swiss cheese samples was due to the presence of specific marker groups formed during the aging process and not the major components in cheese. A simple and rapid ATR-IR protocol was developed by which cheese can be classified, saving time and money.

#### **EFFECT OF SUCROSE ON PHYSICAL PROPERTIES OF SPRAY DRIED WHOLE MILK POWDER**

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The objective was to study the effect of sucrose addition in whole condensed milk prior to spray drying on the milk powder's Tg and other physical properties of interest in chocolate manufacture. Spray Dried Whole Milk Powders (SDWMPs) were prepared from whole condensed milk with five different sucrose concentrations (0-10% w/w), and some physical properties of importance in chocolate manufacture were evaluated. In milk powders, Tg and free-fat content decreased with sucrose addition. Moreover, increasing sucrose concentration reduced the formation of dents on the particle surface. Addition of sucrose in whole condensed milk increased both the apparent density and the particle size of the powders. All samples had a log-normal particle size volume distribution with the exception of the powders with the highest sucrose concentration, due to the formation of large agglomerates. Sucrose addition did not affect either vacuole volume, or the amorphous state of milk powders. These results showed that the physical properties of SDWMPs are significantly affected by sucrose addition. Some of these effects may be important in chocolate manufacture.

#### **EFFECT OF PROTEIN STANDARDIZATION ON HEAT STABILITY OF RECONSTITUTED SKIMMED MILK POWDER**

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This study was undertaken to assess the role of standardizing protein content in milk powders on the heat-stability of reconstituted milk. Protein content in low heat (LH) and medium heat (MH) skim milk powders (SMP) was standardized to 35.5%, 34%, 32% and 30% by adding either edible lactose powder

(ELP) or permeate powder (PP) from skim milk ultrafiltration. The protein-standardized milk powders were then reconstituted with deionized water to 9% total solids. The heat-stability, defined as the heat-coagulation time (HCT) of reconstituted milk (RM), was measured at 140°C in an oil bath. HCT of LH SMP was found to be significantly higher than the HCT of MH SMP ( $p < .001$ ). Protein content of LH SMP correlated with heat stability, as HCT decreased with decrease in protein ( $p < .001$ ). Mean  $\pm$  SD HCT for LH SMP (standardized with PP) containing 35.5% and 30% protein was 24.4( $\pm$ .04) and 17.0( $\pm$ .04) minutes, respectively. Effect of protein content was dependent upon type of standardization in MH SMP ( $p < .001$ ). Mean  $\pm$  SD HCT values for MH SMP standardized with PP with protein content of 35.5%, 34%, 32% and 30% were 9.0( $\pm$ 0.69), 19.4( $\pm$ 1.69), 17.8( $\pm$ 1.65), 14.6( $\pm$ 0.45) minutes while HCT values for SMP standardized with ELP were 9.02( $\pm$ 0.69), 8.6( $\pm$ 0.48), 8.5( $\pm$ 0.15) and 7.7( $\pm$ 0.45) minutes, respectively. These results suggest that degree and method of protein standardization can influence the heat stability of some SMP. This may be important in application of protein standardized milk powder in reconstituted dairy products when high heat treatments are used.

**HIGH HYDROSTATIC PRESSURE MODIFICATION OF WHEY PROTEIN CONCENTRATE FOR IMPROVED BODY AND TEXTURE OF LOW-FAT ICE CREAM**

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Previous research has shown that application of High Hydrostatic Pressure (HHP) can enhance foaming properties of Whey Protein Concentrate (WPC), particularly at 300 MPa for 15 min. The purpose of this research was to determine the practical impact of HHP on the body and texture of lowfat ice cream. WSU-WPC was made by ultrafiltration of separated whey, received from the Washington State University (WSU) Creamery. Commercial WPC 35 powder was reconstituted to the same protein concentration as WSU-WPC. Three batches of low-fat ice cream mix were made: WSU-WPC without HHP (A), WSU-WPC with HHP (300 MPa for 15 min, B), and WPC 35 without HHP (C). All low-fat ice cream mixes contained 10% WSU-WPC or WPC 35. Overrun and foam stability of mixes were measured after whipping for 15 min. Ice creams were made using standard ice cream ingredients and process. The hardness of ice creams was measured with a TA-XT2 texture analyzer. Sensory evaluation by balanced reference duo-trio test was carried out using fifty-two employees and students of WSU. The mix with HHP-treated WSU-WPC had highest overrun and foam stability ( $p < 0.05$ ), confirming the effect of HHP on foaming properties in a complex system. Ice cream with HHP-treated WSU-WPC had significantly higher hardness ( $p < 0.05$ ) than ice cream with untreated WSU-WPC or WPC 35. Panelists were only able to distinguish between ice cream with HHP-treated WSU-WPC and ice cream with untreated WPC 35. These results show that HHP alters WPC significantly enough for differences to be noted in a final product even when the modified ingredient is used at levels as low as 10% in a formulation. In the case of ice cream, impact of HHP on physical properties was more pronounced than impact on sensory properties.

**ROLE OF RESIDUAL SUGARS ON STORAGE BROWNING OF SWEET WHEY POWDER**

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The objective was to analyze the role of monosaccharides on the browning potential of Sweet Whey Powder (SWP). A model whey system was prepared using lactose and lysine in water and divided into four parts: unaltered, with added equimolar lactose, galactose or glucose. The samples were adjusted to represent pHs typical of whey storage, 6.5, 6.0 and 5.5. All samples were then freeze dried. In another experiment, SWP was reconstituted, divided into four parts, sugars were added and pHs were adjusted similar to the model system. The freeze dried samples were analyzed for color development after accelerated browning; lactose, galactose and glucose and lysine contents were determined. Following accelerated browning, the samples containing galactose and glucose had higher browning intensities than the lactose containing samples, irrespective of the pH, confirming the fact that monosaccharides participate in browning reactions more readily than disaccharides. These results suggest that residual sugars play an important role in the browning of SWP with higher concentrations of galactose or glucose yielding a product that browns at much higher rates.

**ANTIHYPERTENSIVE ACTIVITY OF CHEDDAR CHEESES PRODUCED WITH THE ADDITION OF PROBIOTIC *LACTOBACILLUS CASEI* OR *L. PARACASEI***

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Angiotensin-I-converting enzyme (ACE) is responsible for the increase in blood pressure by converting angiotensin-I to the potent vasoconstrictor, angiotensin-II, and by degrading bradykinin, a vasodilatory peptide. ACE-inhibitory peptides have been isolated in various cheeses, released by proteolysis during ripening. The objectives were i) to study the influence of proteolysis on the release of ACE-inhibitory peptides in probiotic Cheddar cheeses during ripening and ii) to isolate, purify and identify the peptides. Cheddar cheeses were made with starter lactococci and *Lactobacillus casei* 279 or *L. paracasei* LAFTI®L26. ACE-inhibitory activities of the water soluble fraction of the cheeses were measured by spectrophotometric assay. Peptide purification was performed using reverse-phase HPLC, and identified by automated Edman degradation protein sequencer and mass spectrophotometer. Probiotic bacteria used survived the long ripening period of nine mo at 4°C, with increased proteolysis and release of soluble peptides in the Cheddar cheeses. Higher ACE-inhibitory activity was detected in cheeses with the higher level of proteolysis. The IC<sub>50</sub> (concentrations of ACE inhibitor needed to inhibit 50% of ACE activity) were highest after six mo of ripening in the probiotic cheeses (0.23- 0.25 mg/mL) compared to nine mo of ripening in the cheese without probiotic (0.28 mg/mL). Ten fractions were collected from water-soluble fraction of each cheese in the first chromatography step. Inhibitory activity was found in all fractions with IC<sub>50</sub> range from 0.1–0.8 mg/mL. Fraction with the highest activity was purified by a second stage chromatography. Various ACE-inhibitory peptides were found, which corresponded to the α<sub>s</sub>-casein N terminal peptides, f(1-9), f(1-7), f(1-6), f(24-32) and β-casein N-terminal peptides, f(193-209). The results suggested that ACE inhibition in Cheddar cheeses was proteolysis dependant. Probiotic strains used in this study can be added successfully in Cheddar cheese while simultaneously producing bioactive peptides.

**PLASMIN ACTIVITY, PLASMINOGEN ACTIVATION, AND B-CASEIN HYDROLYSIS IN RECONSTITUTED NON-FAT DRY MILK**

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Plasmin (PL), an indigenous proteinase in milk, previously was shown to be heat stable. However, different heat treatments (low, medium, and high heat) applied during the production of non-fat dry milk (NDM) may impact the activity of PL and its zymogen, plasminogen (PG), as well as plasminogen activators (PA). Quantifying the activity of plasmin system components in NDM as affected by various conditions is critical in understanding quality parameters that may change as a result of adding NDM to other dairy products. The objective was to investigate the effects of heat treatment, storage time and temperature, and addition of exogenous PA on PL activity, PG-derived PL activity, and b-casein hydrolysis in reconstituted NDM. NDM samples receiving low, medium, and high heat treatments as well as instantized NDM were reconstituted to 10% in 0.02% sodium azide. Samples were dosed with 1 IU/mL urokinase-type PA (uPA) and incubated for 48 h at 21°C or 120 h at 4°C. PL and PG activities were measured using a chromogenic assay. Hydrolysis of b-casein was measured using densitometry with urea-PAGE. In control samples, PL and PG-derived PL activity were significantly higher in instantized NDM. Under both storage conditions, PL activity significantly increased while PG-derived PL activity subsequently decreased in instantized NDM with added uPA, indicating that PG can be activated in reconstituted instantized NDM samples. Furthermore, b-casein proteolysis in reconstituted NDM significantly increased in instantized NDM with added uPA. Proteolysis also was observed in all NDM samples after storage at 4°C, regardless of heat classification, which was likely due to heat stable bacterial proteases that survived NDM production. These results suggest that heat classification, storage conditions, and additional PA affect activity of PL system components in instantized reconstituted NDM, which in turn can have proteolytic effects in other foods.

**MODIFICATION OF WHEY PROTEIN CONCENTRATE WITH HIGH HYDROSTATIC PRESSURE: IMPACT ON LOW-FAT ICE CREAM FLAVOR**

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Whey Protein Concentrate (WPC) is known to increase in flavor binding affinity after high hydrostatic pressure (HHP) treatment of 300 MPa for 15 min in a model system. The purpose of this study was to examine flavor impact of HHP treated WPC in lowfat ice cream. WSU-WPC (TS = 8.23%, Protein = 3.02%) was made by ultrafiltration of separated Cheddar cheese whey, received from the Washington State University (WSU) Creamery, then treated at 300 MPa for 15 min. On two separate days, six batches of lowfat ice cream were made: HHP treated WSU-WPC without diacetyl (A), WSU-WPC with 2µg/L diacetyl added before HHP (B and E), WSU-WPC with 2µg/L diacetyl added after HHP (C), untreated WSU-WPC with 2µg/L diacetyl (D), and untreated reconstituted commercial WPC 35 (TS = 8.23%; Protein = 3.02%) with 2µg/L diacetyl (F). WSU-WPC or reconstituted commercial WPC 35 composed 10% of the ice cream mix. Lowfat ice creams were made using standard ingredients and standard pasteurization, homogenization, aging and freezing methods. Sensory evaluation by balanced reference duo-trio test was carried out using fifty untrained panelists, on two different days. Eighty

percent of panelists were able to distinguish between lowfat ice cream made with or without diacetyl added to WSU-WPC ( $p < 0.05$ ). Panelists were not able to distinguish between lowfat ice creams with diacetyl added to WSU-WPC before or after HHP ( $p > 0.05$ ) or between lowfat ice creams prepared from untreated or HHP treated WSU-WPC with diacetyl ( $p > 0.05$ ). Panelists were able to distinguish between lowfat ice creams with HHP treated WSU-WPC with diacetyl and with untreated commercial WPC 35 with diacetyl ( $p < 0.05$ ). Although HHP treatment modifies WPC, the impact on flavor binding, specifically is not profound enough that is noticeable when WPC is used at low levels (10%) in a complex food system like lowfat ice cream.