International Research Journal of Human Resource and Social Sciences
ISSN(O): (2349-4085) ISSN(P): (2394-4218)
Impact Factor 6.924 Volume 9, Issue 09, September 2022
Website- www.aarf.asia, Email : editoraarf@gmail.com

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

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ABSTRACT

Milk protein concentrates with more than 80% protein (i.e., 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.'

KEYWORDS: Microstructural Changes, High-Protein Nutrition Bars, Milk Protein Concentrate

INTRODUCTION

Powder milk protein concentrates (MPCs), particularly those with more than 80 g protein per 100 g product (i.e., MPC80), possess poor rehydration and solubility characteristics that worsen during storage (Havea 2006; Anema and others 2006; Haque and others 2010). High-protein nutrition (HPN) bars, which contain 20-50% protein (w/w), are intermediate moisture systems

that do not require complete protein solubility and are a potential application for MPCs (Cho 2010). However, when utilized in HPN bars, MPCs present challenges in balancing cohesiveness (e.g., too crumbly), firmness (e.g., too hard), and texture change over the product's shelf life (Baldwin and Pearce 2005; Imtiaz and others 2012; Li and others 2008; Loveday and others 2009). Texture change of HPN bars during storage is likely due to a combination of different phenomena, for example, moisture migration between constituents, macronutrient phase separations, and disulfide bond- and Maillard-induced protein aggregations (Zhou and others 2008a; Loveday and others 2009; McMahon and others 2009; Zhou and others 2013).

In addition to protein, HPN bars are comprised of 10-50 g carbohydrate and 10-15 g fat per 100 g (Zhu and Labuza 2010). Free water is minimized and water activity is kept less than 0.65 to ensure microbial shelf stability (Loveday and others 2009). While other ingredients (e.g., sugar alcohols) and other factors (e.g., storage conditions) can influence HPN bar texture, protein source (e.g., dairy, soy) and type (e.g., concentrate, hydrolysate, crisp) have direct impact (Childs and others 2007; McMahon and others 2009; Imtiaz and others 2012). The physicochemical properties of MPC can be tailored for HPN bars using physical, chemical, or enzymatic modifications (Imtiaz and others 2012). The texture of HPN bars formulated at 30% protein (w/w) with physically modified MPC80 was evaluated over 42 days storage at 22°C, 32°C, and 42°C (Banach and others 2014). HPN bars produced with extruded MPC80 hardened slower than those made with toasted or unmodified MPC80. MPC80 toasted at 75°C or 110°C for 4 h produced HPN bars that had minimal texture change or increased fracture force, respectively, when compared to those formulated with control MPC80. Extruded MPC80s had reduced protein solubility and, based on the rate of free amine reduction during HPN bar storage, were less chemically reactive (Banach and others 2014, 2013).

Free amine reduction was one chemical change that occurred during storage of HPN bars, but it insufficiently explains texture change (Rao and others 2013; McMahon and others 2009; Baier and others 2007; Banach and others 2014). Protein aggregations, including those from disulfide crosslink formations and Maillard reactions, during storage have also been implicated in texture change (Zhou and others 2013; Zhou and others 2008a, 2008b). N-ethylmaleimide prevented disulfide bond formation and extended textural shelf life of a model intermediate moisture food (IMF) 6-times the control (Zhu and Labuza 2010). Free sulfhydryl interactions were texturally relevant in the same IMF, as molecular cysteine slowed or accelerated hardening when added at

low or high levels, respectively (Zhu and Labuza 2010). The objective of the present study was to determine the effect extrusion and toasting had on the free sulfhydryl content of MPC80 and to verify the occurrence of disulfide crosslinking within HPN bars formulated with those modified protein ingredients. Additionally, confocal laser scanning microscopy (CLSM) was used to study macronutrient phase separations in these HPN bars. Instrumental texture properties were presented in detail elsewhere (Banach and others 2014); however, they are related to the microstructural changes presented in this study.

Practical Application

High-protein nutrition bars formulated with extruded MPC80 underwent fewer microstructural changes during storage. Disulfide crosslink formation and free sulfhydryl content changes were not always indicative of texture changes in high-protein nutrition bars. Texture change in high-protein nutrition bars formulated with MPC80 was, thus, only partly due to these aggregations. Pre-extruded MPC80 may produce high-protein nutrition bars with an extended textural shelf life compared to those produced with unmodified MPC80.

Milk Protein Concentrate Modification and High-protein Nutrition Bar Production

MPC80 was modified with extrusion or dry-heat toasting. MPC80 moisture content was adjusted to 38% and extruded at die-temperature of 65°C or 120°C using a low-shear screw profile. The extrudate was dried, milled, and sieved through a 250 μm mesh, as detailed elsewhere (Banach and others 2014, 2013). For dry-heat toasting, MPC80 was put in a laboratory oven at 75°C or 110°C for 4 h and passed through the same screen. These modified proteins are referred to as E65 (78.4% protein, 7.3% moisture), E120 (79.5% protein, 5.8% moisture), T75 (80.6% protein, 4.1% moisture), and T110 (81.7% protein, 3.0% moisture), respectively.

HPN bars, with protein and moisture content indicated, were prepared by Banach and others (2014) using control MPC80 (31.4% protein, 14.4% moisture), E65 (31.7% protein, 14.2% moisture), E120 (31.6% protein, 13.6% moisture), T75 (31.6% protein, 13.4% moisture), and T110 (31.5% protein, 13.5% moisture). After 0, 6, 13, 22, or 42 days storage at 32°C, the HPN bars were frozen in liquid nitrogen, ground with a laboratory blender, and kept at -80°C until free sulfhydryl measurement and SDS-PAGE in the present study.

Free Sulfhydryl Measurement

The free sulfhydryl content of each protein ingredient and HPN bar was determined by Ellman's assay with modifications (Beveridge and others 1974). Free sulfhydryl extraction buffer (pH 8.5) contained 8 mol urea plus 4.1 mmol EDTA per L and was prepared in borate buffer (100 mmol boric acid, 75 mmol sodium chloride, and 25 mmol sodium tetraboratedecahydrate per L). Protein ingredients (0.75 g) were mixed with degassed extraction buffer (11.25 g) for 2 h in 15-mL centrifuge tubes. HPN bars (2.04 g) and degassed extraction buffer (9.96 g) were mixed in 25-mL Erlenmeyer flasks for the same time. For the HPN bars prepared with T110, 2.55 g was mixed with 12.45 g extraction buffer. Protein ingredient and HPN bar dispersions were centrifuged for 15 min at 12,000 g and 15,000 g, respectively.

Sample supernatants (0.5 mL) or cysteine standards (0.5 mL) were vortexed with 50 µL of 10 mmol DTNB/L and 2.5 mL extraction buffer, which was held at room temperature for 15 min and absorbance read at 412 nm. Sample and standard blanks were prepared by substituting DTNB with extraction buffer. Standard net absorbance was plotted against seven free sulfhydryl concentrations (25 to 493 µmole/L) and was fitted with a linear ($R2 \geq 0.995$) curve (not shown) used to determine sample free sulfhydryl concentration. These values were divided by the BCA assay determined soluble protein (g/L) and free sulfhydryl content was reported as µmole per g protein.

Non-reduced and Reduced SDS-PAGE

Sample supernatants from the free sulfhydryl assay (above) were used for nonreduced SDS-PAGE. Reduced extraction followed the same procedures except the extraction buffer contained 50 mL β-mercaptoethanol/L. Soluble protein was diluted to 4 mg/mL and was verified using the appropriate BCA assay. Non-reduced dilutions contained 3.7-4.4 mg protein/mL whereas the reduced dilutions contained 3.8-5.6 mg protein/mL. The non-reduced samples were diluted 1:2 with both reducing and nonreducing 2x Laemmli Sample Buffer. The reduced samples were only diluted 1:2 with reducing 2x Laemmli Sample Buffer. The protein standard and samples were loaded onto the gel at equal volume (10 µL) and were electrophoresed at 150 V for 50 min using standard SDS-PAGE running buffer (250 mmoltris, 1.92 mol glycine, and 10 g SDS per L). The gels were fixed in methanol/acetic acid/Millipore water (40/10/50) for 30 min, stained for 1 h, and de-stained with Millipore water.

Confocal Laser Scanning Microscopy of the High-protein Nutrition Bars

CLSM methodologies were adapted from literature to detect possible macronutrient phase separations within the HPN bars during storage (McMahon and others 2009). A separate 50 g batch of each HPN bar was prepared with the same lot of ingredients. In addition to the protein ingredients described above, each model contained 21.5 g glycerol (99.8% glycerol, 0.1% water), 18.4 g palm kernel stearin, 12.0 g maltitol syrup (Lycasin®80/55, 51.7% D-maltitol, 3.0% D-sorbitol, 24.5% water, Roquette America, Keokuk, IA), 10.0 g high-fructose corn syrup (CornSweet®55, 55% fructose, 41% dextrose, 4% higher saccharides, 23% water, Archer Daniels Midland, Decatur, IL), and distilled water to standardize protein ingredient moisture content per 100 g. A mechanical stand mixer was used to combine the ingredients, according to Banach and others (2014), and a small portion was leveled into a press-to-seal silicone isolator (13 mm diam. × 2 mm depth, Grace™ Bio-Labs, Bend, OR) mounted on a glass microscope slide. One drop of FITC-acetone solution (0.2 g FITC/kg) and one drop of Nile redacetone solution (0.2 g Nile red/kg) were applied to the HPN bar surface with a glass Pasteur pipette. A glass coverslip was placed over the sample and, along with the base of the push-to-seal isolator, was sealed into place with silicone. The freshly prepared slides were kept at room temperature (~22°C) overnight and day 0 images were acquired the following day.

Milk Protein Concentrate Extrusion

MPC80 (80% protein w/w dry-basis, Milk Specialties Global, Eden Prairie, MN) was fed (25 kg/h) into a co-rotating twin-screw extruder (DNDL 44, Bühler AG, Uzwil, Switzerland) at the Joseph J. Warthesen Food Processing Center (University of Minnesota, St. Paul, MN) using systems previously described (Tremaine and Schoenfuss 2014). Screw speed (350 rpm), MPC80 feed rate, and set barrel temperature (50°C) were fixed. Water addition was lowered from 11 kg/h to 10 kg/h to produce extrudates with circular die (3 mm) melt temperature of ~105°C (i.e., E105) and ~116°C (i.e., E116), respectively. Extrudates were pelletized and dried partially on a fluidized bed dryer (OTW 05TRR2, Bühler AG, Braunschweig, Germany). Drying continued at 40°C in a forced draft oven for 26 h. The protein pellets were coarsely ground as described (Banach and others 2014) and the resultant powder was jet-milled.

Protein Powder Particle Size Measurement

Particle size distributions (PSD) were measured (n = 2) by laser diffraction (Mastersizer 2000, Malvern Inc., Worcestershire, United Kingdom) (Gazi and Huppertz 2015). 450 mL isopropanol

(Fisher Scientific, Waltham, MA) in a 600 mL beaker was stirred at 2,000 rpm by the wet dispersion accessory (Hydro 2000MU, Malvern Inc., Worcestershire, United Kingdom). Powder was added to the dispersant such that obscuration was 10-20% and triplicate measurements were taken automatically. Isopropanol's refractive index and sensor threshold were 1.39 and 64, respectively. MPC's refractive index and absorption value were 1.46 and 0.1, respectively (Crowley and others 2015).

High-protein Nutrition Bar Preparation

Protein ingredient moisture content was determined after drying 16 h at 102°C and protein was measured by Dumas nitrogen combustion (AOAC 1998). HPN bars were prepared (n = 2) at 30% protein (w/w) using either control MPC80 (76.8% protein, 5.2% moisture), E105 (74.3% protein, 7.5% moisture), or E116 (74.4% protein, 7.4% moisture). 1.21 kg MPC80, 1.25 kg E105, and 1.25 kg E116 were each dry-blended with 155 g maltodextrin (Maltrin®180, 16.5-19.9 dextrose equivalent, 6% moisture, Grain Processing Corporation, Muscatine, IA). 175 g high-fructose corn syrup (CornSweet®55, 55% fructose, 41% dextrose, 4% higher saccharides, 23% water, Archer Daniels Midland, Decatur, IL), 647 g glycerol (99.7% glycerol, USP Grade, US Glycerin, Jackson, MI), 321 g maltitol syrup (Lycasin®80/55, 51.7% D-maltitol, 3.0% D-sorbitol, 24.5% water, Roquette America, Keokuk, IA), and 111, 69, or 71 g distilled water were combined and heated to 60° for the HPN bars to be prepared with MPC80, E105, or E116, respectively. 465 g non-hydrogenated, trans-free palm oil (SansTrans®39, IOI LodersCroklaan, Channahon, IL) was melted with 15.5 g low-viscosity liquid lecithin (Beakin®LV1, 0.8% moisture, Archer Daniels Midland, Decatur, IL). The wet ingredients were first combined and then the dry ingredient blend was slowly added over the course of 4.5 min mixing with the paddle attachment on speed 1 (A200, Hobart Corporation, Troy, OH).

HPN bar dough was transferred and pressed into two parchment paper-lined cookie sheets (22.9 cm x 33 cm x 1.6 cm). A rolling pin was used to press the HPN bar dough flush with the upper edge of the pan, removing or adding more sample as needed to ensure a uniform height. Each pan was wrapped with lightly oiled plastic wrap and remaining HPN bar dough was pressed into water activity (aw) cups as described previously (Banach and others 2014). Samples were kept at room temperature (~22°C) overnight.

A circular cutter (ID = 1.91 cm) punched samples from each HPN bar sheet. The samples were expelled directly onto heavy-duty waxed plates, which were then heatsealed in metallized bags

(S-16891, Uline, Pleasant Prairie, WI). Samples formulated with E105 and E116 were refrigerated (4°C) for 1 h prior to cutting. Samples were assigned to room temperature (~22°C) or incubated storage (32°C) the following day.

Panellist Recruitment and Training

This study was approved for human subjects by the Office of Responsible Research at Iowa State University (Institutional Review Board # 14-166). Eight female panelists were trained to evaluate the textural attributes of HPN bars for a minimum of 7 h over the course of 8 1-hour training sessions. Panelists measured firmness and crumbliness using their hands and fracturability, hardness, cohesiveness, and mouth coating in their mouths using anchored 15-cm lines (Table 1).

Table 1 High-protein nutrition (HPN) bar texture attributes and sensory panel anchors1

Attribute	Definition	Anchors
Firmness	Force required to compress a sample between thumb and index finger	0 cm - Sara Lee® White Bread 7 cm - DiLusso's Wisconsin American Cheese 15 cm - Baby Carrot
Crumbliness	Extent to which pieces break from a sample after one in-hand compression	0-2 cm - DiLusso's Wisconsin American Cheese 7 cm - HyVee® Chocolate Chip Granola Bar 13-14 cm - Nabisco® Grahams Original
Fracturability	Force required for the sample to break between one's incisors	0-1 cm - Philadelphia® Neufchatel Cheese 6 cm - Nabisco® Grahams Original 14 cm - Old London® Melba Toast
Hardness	Force required to bite through the sample with one's molars	0-1 cm - Philadelphia® Neufchatel Cheese 4-5 cm - DiLusso's Wisconsin American Cheese 12-13 cm - Baby Carrot
Cohesiveness	Degree to which the sample holds together in a mass after three chews	0-2 cm - Baby carrot 7-8 cm - DiLusso's Wisconsin American Cheese 13-14 cm - Little Debbie® Cosmic Brownie

1 Attributes, definitions, and anchors adapted from Childs and others (2007), Imtiaz and others (2012), and Meilgaard and others (2007).

HPN bar texture was evaluated immediately after being cut (i.e., week 0) and then weekly for up to 6 weeks. Since the samples were not placed into storage until day 1 and they were removed from storage 4.5 h prior to each evaluation session for temperature equilibration, the storage time at 32°C was less than each identified week (i.e., 1 wk = 5.7 d, 2 wk = 12.7 d, etc.). With 2 HPN bar preparations, there were two evaluation sessions each week and 6 HPN bars (i.e., 3 proteins × 2 storage temperatures) were evaluated per session. Panelists were randomly presented 3 cut HPN bar samples identified only by a 3 digit code on a white paper plate. One sample was used

for in-hand evaluation and the other two were for in-mouth tests. Panelists were provided water, unsalted crackers, and unscented wet wipes to cleanse their palate and hands between HPN bars.

CONCLUSION

MPC powder particle size was not previously considered to have an effect on HPN bar texture. Reducing the particle size of MPC85 improved its ability to hydrate during HPN bar manufacture. Improved hydration coupled with the attraction that smaller particles naturally have for each other produced more cohesive HPN bars. Jetmilled MPC85 produced HPN bars that were denser, firmer, and more cohesive than the control initially and after 1 year accelerated storage. These HPN bars also exhibited much greater textural stability. Particle size reduced MPC85 did not produce the same level of cohesion as the extrusion-modified MPC80. This showed that protein denaturation by extrusion processing not only slowed hardening, but also decreased HPN bar crumbliness.

MPC processing, including extrusion and particle size reduction, can be used to alter the texture of HPN bars formulated with these proteins. When high-protein MPCs are used in a HPN bar formulation, careful attention must be paid to determine if the particle structure collapses or if it is maintained. If the majority of the powder particles collapse, the HPN bar will be more cohesive and internal chemical reactions will likely proceed at an accelerated rate. These chemical changes (e.g., disulfide bond formation, Maillard-induced aggregations) were not related to texture change in HPN bars formulated with high-protein MPCs. If MPC powder particle structure is maintained, the HPN bar will be crumbly, and textural changes will be influenced by physical interactions between particles in the system. MPC particle size should be considered in future HPN bar studies, as variation exists between sources. Smaller particles likely hydrate better leading to collapse, and if not, the smaller particles will be more fluid in the system and their HPN bars will have higher cohesiveness. On the other hand, the larger particles in coarser MPCs may serve as weak points when formulated into HPN bars and while they won't impart cohesion, these HPN bars will fracture under lower stress.

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