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Instrumental and Sensory Texture Attributes of High-Protein Nutrition Bars Formulated with Extruded Milk Protein Concentrate

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Keywords
crumbliness, extrusion, protein bar, sensory analysis, texture profile analysis

Comments
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Abstract

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Keywords: protein bar, extrusion, texture profile analysis, sensory analysis, hardness, crumbliness

Practical Application

Extruding milk protein concentrate with 80% protein produced a functional ingredient that, when incorporated in high-protein nutrition bars, resulted in favorable texture attributes, e.g., reduced firmness and improved cohesiveness, when compared to the unmodified control. Instrumental texture attributes were correlated with their respective sensory attributes. High-protein nutrition bar crumbliness measurement by sieve analysis promises to be a useful tool for quantifying crumbliness and cohesiveness as results were strongly correlated with the sensory panel.

Introduction

It is well known that high-protein nutrition (HPN) bars (20-50% protein w/w) and other shelf-stable, intermediate moisture foods (IMFs; 10-40% moisture; $0.55 \leq a_w \leq 0.90$) problematically harden to unpalatable levels during storage (Rao and others 2013a; Imtiaz and others 2012; Loveday and others 2009; Banach and others 2014). Reducing the average molecular weight of a protein by hydrolysis can soften HPN bars and slow their hardening by suppressing the system's glass transition temperature (T_g) (Rao and others 2013a; McMahon and others 2009). While enzyme hydrolysates have improved digestibility (Potier and Tome 2008) and reduced allergenicity (Verhoeckx and others 2015), they cost more to produce and taste bitter. Encapsulated casein hydrolysate added at 3% (w/w) to protein bars did not impart bitterness, but encapsulation also increased hydrolysate T_g, and since texture was not measured, it is unknown if the hydrolysate retained its desirable texture-softening functionality (Rocha and others 2009) during storage. While other protein modification techniques, including physical (Osen and others 2015; Banach and others 2013) and chemical (Zhang and others 2015) for improved functionality are available, their focus, for the most part, has been on altering a protein's solubility-dependent properties, such as gelation, emulsification, and foaming, which are unrelated to performance in IMFs or HPN bars that are more solid than fluid.

Milk protein concentrates (MPCs), particularly those with high protein (e.g., ≥ 80%; ≥ MPC80), are not preferentially utilized in HPN bars (Baldwin and Pearce 2005). HPN bars formulated with MPC harden during storage, but hardening alone inadequately characterizes these systems, which also suffer from decreased cohesiveness and increased crumbliness (Imtiaz and others 2012; Loveday and others 2009; Banach and others 2014). Hardness and hardening rate alone cannot fully describe HPN bar texture or its change during storage (Li and others

2008). In our previous study, texture profile analysis (TPA) and shear testing demonstrated that HPN bars formulated with extruded MPC80 remained softer than unmodified controls during storage (Banach and others 2014). TPA is an instrumental texture technique where two successive sample compressions are used to roughly simulate two bites by a consumer with output that has been used to describe the texture of many different foods (Gunasekaran and Ak 2003). TPA has the potential to describe the texture of HPN bars better than the puncture test favored in IMF-based literature, but their correlation with sensory panel perceived attributes remains unknown.

Trained sensory panels can quantitatively describe the texture of HPN bars, but such evaluation is more time-consuming and costly and less utilized when describing these systems. A sensory-based texture study most pertinent to the current work involved two proprietarily modified MPCs and a non-hydrolyzed whey protein concentrate (WPC) (Imtiaz and others 2012). These were blended to make HPN bars with different protein composition at fixed protein content (30% protein w/w) that had altered cohesiveness/crumbliness. The same study found correlation between the results of instrumental puncture with a 5 mm cylindrical probe and select sensory attributes measured by a trained panel. Another sensory-based study found that the predominant protein source (i.e., whey vs. soy) influenced sensory texture in a more realistic HPN bar formulation (Childs and others 2007). Literature has focused on the role of protein in texture change and determined that functionalization prior to HPN bar production can impart textural stability.

Commercially produced HPN bars are complex systems of blended proteins mixed with carbohydrates (e.g., maltodextrins), lipids (e.g., palm oil), plasticizers (e.g., glycerol, sugar alcohols), and other components (e.g., minerals) that can alter the system's stability during

storage. Storage conditions and other added constituents, such as polyols and free sulfhydryl-containing compounds, are also known to affect the rate of hardening (Liu and others 2009; Zhu and Labuza 2010). Simplified models have been used to mechanistically describe texture change, namely hardening, that occurs during storage, but the results might not translate to commercial HPN bars. Multiple factors affect complete HPN bar texture change during storage. Simple models are key for mechanistic-based studies, but their scope is more limited than those using a more realistic HPN bar formulation, like the one used in this study, that have not been reported in abundance (Hogan and others 2012).

The following study was designed to thoroughly characterize the texture attributes of HPN bars formulated with ground extruded MPC80. Commonly reported instrumental TPA attributes were correlated with those measured by the sensory panel. Since increased crumbliness and decreased cohesiveness have been previously reported and observed in MPC-containing HPN bars (Imtiaz and others 2012; Banach and others 2014), a sieve analysis after TPA was employed to better characterize these properties.

Materials and Methods

Milk Protein Concentrate Extrusion

MPC80 (80% protein w/w dry-basis, Milk Specialties Global, Eden Prairie, MN) was fed (25 kg hr^{-1}) 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 hr^{-1} to 10 kg hr^{-1} 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 centrifugally milled in a Retch Mill (Banach and others 2014), followed by jet-milling.

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 (A416, Fisher Scientific, Waltham, MA) in a 600 mL beaker was stirred at 2,000 rpm by the macro wet dispersion accessory (Hydro 2000MU, Malvern Inc., Worcestershire, United Kingdom). Powder was added to the circulating dispersant such that obscuration was 10-20% and triplicate measures were taken. 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 Loders Croklaan, 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 on speed 1 with the paddle attachment (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 (a_w) 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 heat-sealed 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.

Taste Panelist 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).

HPN bar texture was evaluated immediately after preparation (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 at each session. Panelists were randomly presented 3 cut HPN bar samples identified only by a 3 digit code on 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.

Instrumental Texture Evaluation

HPN bars for instrumental texture evaluation were removed from incubated storage concurrent those for sensory evaluation and were evaluated the following day. HPN bar samples (n = 3) were compressed with a flat plate (TA-30) at 2 mm s^{-1} to 60% strain using the TPA test format while force (N) versus time (s) data were recorded (TA-XT2, Texture Technologies, Scarsdale, NY) (Banach and others 2014). Other HPN bar samples (n = 3) were sheared across their circular cross-section with a 45° chisel blade (TA-42) at 1 mm s^{-1} (Banach and others 2014). Max force (N) during the first TPA compression and shear force (N) were used to report HPN bar hardness. Adhesiveness (J) was taken as the absolute area under the force versus time curve during probe withdrawal after the first compression. Cohesiveness (%) was the ratio of area under the second compression curve to the area under first compression curve.

A sieve analysis was used to measure HPN bar crumbliness by modifying a method used to measure the same parameter of Queso Fresco cheese (Hwang and Gunasekaran 2001). After TPA, the sample was transferred to a stack of 3" sieves with descending aperture (i.e., 5.6, 4.0, 2.8, 2.0, 1.4, 1.0, and 0.5 mm). The stack was placed into a custom-made 8" to 3" adapter and was shaken for 30 s on speed 3 (Shaker #18480, CSC Scientific Sieve, Fairfax, VA). Mass percent finer than the top sieve (No. 3.5) was reported as crumbliness.

Color, Water Activity, pH, Moisture, and Protein Measurements

HPN bar color and a_w were measured (n = 3) as described elsewhere (Banach and others 2014). 20% HPN bar dispersions were prepared in Millipore water, mixed for 16 h, and pH was measured (n = 2). HPN bar moisture content was measured (n = 3) by difference after drying 1 g samples at 102°C for 26 h. HPN bars were frozen in liquid nitrogen on the day of manufacture and after 29 weeks storage, kept at -80°C, and were used to determine average HPN bar protein content by Dumas nitrogen combustion (AOAC 1998).

Statistical Analyses

Instrumental measurements were averaged by protein ingredient, storage temperature, storage time, and preparation (n = 2). Sensory panel responses were not averaged prior to statistical analysis. The dependent variables were modeled using the mixed procedure with protein (i.e., MPC80, E105, and E116), time (i.e., weeks), and temperature (i.e., 22°C and 32°C) set as the independent variables. Panelist and preparation of each HPN bar were set as the random effects; only the latter applied to instrumental analysis. Slicing factors were applied to analyze between proteins at fixed time and also within each HPN bar over storage. The Tukey-Kramer adjusted *P*-value ($\alpha = 0.05$) was used to determine differences between the least squares means (LS-means). For correlation analysis, sensory panel responses were averaged by protein

ingredient, storage temperature, storage time, and preparation (n = 2). Pearson correlation coefficients (r) were calculated between sensory and instrumental responses. All statistical analyses were performed with SAS® software (version 9.4, SAS Institute Inc., Cary, NC).

Results and Discussion

Protein Powder Particle Size and its Influence on HPN Bar Production

D_{90}, D_{50}, and D_{10} of spray dried MPC80 were set as the processing targets for the jet-milling such that any HPN bar texture differences were attributable to the extrusion modification rather than a confounded PSD effect. Protein powder volume mean diameters ($D_{4,3}$) were measured (± SD) at 53 (± 0.1), 57 (± 0.8), and 61 (± 0.8) µm for E105, E116, and MPC80, respectively. Although $D_{4,3}$ ranged only 8 µm, particle size span (i.e., $(D_{90}-D_{10})/D_{50}$) for E105 (5.7), E116 (3.3), and MPC80 (2.1) indicated that the jet-milled powders had broader PSD than the more uniform spray-dried MPC80 . On average (± SD), 1162 (± 7), 1372 (± 9), and 1365 g (± 5) of HPN bar dough prepared with MPC80, E105, and E116, respectively, was required to fill each production pan (1209 cm^3). The control HPN bar (0.96 g cm^{-3}) was less dense than those prepared with E105 (1.13 g cm^{-3}) and E116 (1.13 g cm^{-3}). The finer protein particles, which were more common in the milled extrudates, positioned themselves between the larger powder particles. E105 had the largest span, smallest $D_{4,3}$, and produced the densest HPN bar. The control protein powder, with more uniform PSD, could not accomplish this level of particle packing due to volume constraints within the HPN bar. Excess pressure did not add more mass to the control HPN bar, and when applied, would cause textural differences from production rather than protein modification. Uniform sample geometry was important for texture analysis and, despite density differences, the HPN bar dough was pressed to a uniform height.

HPN bar doughs prepared with extruded MPC80 had higher fluidity than the control during manufacture and were pourable whereas the control required force to take shape. This fluidity made it difficult to remove cut samples from the sheeted HPN bars prepared with extruded MPC80 and prompted chilling prior to cutting. The control HPN bar was rigid and samples were easily cut at room temperature. The samples prepared with extruded MPC80 (14.3 mm ± 0.5) were about 1.5 mm shorter than those prepared with control MPC80 (15.8 mm ± 0.0), but all samples maintained their cylindrical shape during storage. Height differences were attributed to the incompressibility of unmodified MPC80 and potential settling within the HPN bars formulated with extruded MPC80. The less viscous HPN bar dough formulated with extruded MPC80 may be more difficult to process into and hold bar form.

Particle size parameters of protein powder, including diameter, uniformity, span, and PSD (Figure 1) should not be ignored while discussing HPN bar texture. These parameters will affect the volume fraction required to obtain HPN bar solidity and texture change during storage (Hogan and others 2016; Thomar and others 2012). Protein powders that form a suspension in a particular HPN bar formulation rather than a jammed or solid product are not expected to change texturally during storage (Hogan and others 2016). In our previous study involving extruded MPC80 in HPN bars, E65, E120, and the control had $D_{4,3}$ (± SD) of 119 (± 12), 88 (± 15), and 73 µm $D_{4,3}$ (± 2), respectively (unpublished data). These extruded MPC80s had greater average particle size than the control and produced HPN bars that were softer and less prone to hardening (Banach and others 2014). This result aligned with the work of Cho (2010), who found that coarsely ground (~84% < 150 µm) soy protein crisps, or extruded and milled soy protein concentrate, produced HPN bars that were softer and less prone to hardening than those produced using the finely ground (~100% < 150 µm) fraction. In the present study, E105 and

E116 were milled slightly finer than the control MPC80, and if similar textural results are obtained, it can be partially attributed to the extrusion modification, despite there being an incompletely accounted for PSD effect. More in-depth particle size and density will provide a topic of interest in future studies, but its effect on texture change are beyond the scope of this study.

HPN Bar Protein, Moisture, Water Activity, Color, and pH

HPN bar protein (% ± SD) was 32.2 ± 0.9, 32.6 ± 0.5, and 32.5 ± 0.7 when formulated with MPC80, E105, and E116, respectively. Changes in as-is protein during storage were not expected, but might have occurred from measurable moisture content change ($P < 0.05$) (Table 2). Initial HPN bar moisture (% ± SD) was 17.9 ± 0.9, 14.7 ± 0.2, and 14.9 ± 0.1 when formulated with MPC80, E105, and E116, respectively, with any increase during storage due to more free water in the bulk phase as verified by increased a_w. HPN bar a_w increased slightly during storage ($P < 0.05$), but after day 3 no significant change was detected. HPN bars prepared with extruded MPC80 maintained lower a_w than the control when stored at 22°C (Table 3). Increasing HPN bar a_w during storage was observed in other samples formulated with extruded MPC80 and was explained on a microstructural basis (Banach and others 2016; Banach and others 2014). HPN bar color (Figure 2) change during storage was dependent on protein, time, and temperature ($P < 0.05$). The HPN bars formulated with extruded MPC80 did not undergo significant total color change (ΔE) while stored at 22°C for 6 weeks ($P > 0.05$) (Table 3). Extrusion can destroy lysine, which limits its ability to participate in Maillard browning during HPN bar storage (Banach and others 2014). Sample pH was measured to determine if browning was possibly affected by differences in initial pH. However, protein ingredient did not have an

effect on pH ($P > 0.05$), and although it decreased slightly during storage and was influenced by storage temperature, no trend with ΔE was observed (Table 2).

HPN Bar Instrumental Texture

Select HPN bar instrumental attributes are reported based on convention in the field (i.e., max force, shear force), their relatability to the sensory panel measured attributes, and those TPA-generated attributes where differences between the samples were easily discerned. Protein ingredient, storage temperature, and storage time each had a significant effect ($P < 0.05$) on max force, shear force, adhesiveness, cohesiveness, and crumbliness (Figure 3). Instrumental attribute correlation with the sensory responses are discussed in the following section.

HPN bar and IMF literature has focused heavily on storage time-dependent hardening. Hardness is commonly measured using non-MPC formulated, hand-pressed samples in a_w sample cups and has been expressed as the peak force obtained while puncturing with a small diameter (3 to 5 mm) cylindrical probe to a predefined strain (35 to 50%) (Hogan and others 2012; Rao and others 2013b; Zhou and others 2008). Many other important texture attributes are overlooked using this methodology. Max force and force at maximum strain (i.e., 60%) convey important textural information. An elevated max force just prior to sample fracture followed by a weak force at 60% strain, a particularly common trait of the control HPN bar, indicated that a structural collapse occurred after initial fracture. A HPN bar of this nature would require a great deal of force to bite through, but would not contain much body or bar-like structure after the initial fracture. HPN bars prepared with extruded MPC80 rarely underwent this type of structural collapse during the early stages of the study. The degree to which a HPN bar holds together without being too fluid has been referred to as “bar integrity” (Li and others 2008), but its quantification or that of related attributes such as cohesiveness or crumbliness, has been

ignored by many HPN bar studies. Most studies have focused on whey protein utilization and since these proteins typically produce a more cohesive HPN bar than MPC, it is likely the main reason why "bar integrity" has been neglected and only hardening parameters have been reported.

Max force for MPC-formulated HPN bars was determined as the best instrumental output to represent sample firmness as perceived by a trained panel (Imtiaz and others 2012). HPN bar shearing was predicted to be more comparable to biting than puncture and TPA, and was used previously to describe hardness (McMahon and others 2009; Banach and others 2014). Max force and shear force showed that those samples formulated with extruded MPC80 remained softer than those formulated with unmodified MPC80 (Figures 3A and 3B). At time 0, max force of the HPN bars formulated with extruded MPC80 was significantly lower than those formulated with control MPC80 ($P < 0.05$). Max force increased with storage time and the increase was more pronounced at 32°C ($P < 0.05$). Increasing shear force mirrored that of the max force, except that on day 0 there was no difference between the samples. The control always required more force to shear than the HPN bars prepared with extruded MPC80, of which the one made with E116 required less force to shear than the one formulated with E105. Significant differences in shear force between the control and extruded MPC80-formulated HPN bars were not observed until after 12 and 4 weeks at 22°C and 32°C, respectively. Max and shear force measurement data showed that the HPN bars prepared with extruded MPC80 continued to remain softer than the control even as storage was extended to 7 months, which was much longer than, but in alignment with previous results (Banach and others 2014).

Instrumental probe withdrawal force and cohesiveness/crumbliness measured by a trained sensory panel were strongly correlated for MPC-formulated HPN bars (Imtiaz and others 2012).

TPA withdrawal characteristics are related to adhesiveness (J) or the work necessary to overcome internal and external HPN bar attractive forces. A HPN bar that adheres to the probe also adheres to itself and forms a cohesive mass that holds its bar form. These three texture attributes, adhesiveness, cohesiveness, and crumbliness, are not always related and are reported separately in this study. Initial adhesiveness of the control was significantly lower than those HPN bars formulated with extruded MPC80 for which E105 produced a more adhesive system than E116 ($P < 0.05$) (Figure 3C). Adhesiveness of the HPN bars formulated with extruded MPC80 decreased quickly when stored at 32°C while at 22°C it slowly plateaued towards the same final value. At the end of storage, there were no differences between sample adhesiveness at 32°C ($P > 0.05$), but at 22°C the HPN bar made with E105 was still the most adhesive ($P < 0.05$). The HPN bars prepared with control MPC80 felt powdery to the touch and their adhesiveness values, which were near baseline, did not change significantly during storage ($P > 0.05$). Excessive stickiness is not a favorable HPN bar attribute, but neither is powdery and dry. If increased adhesiveness translates to cohesiveness, extrusion would produce an improved MPC80 ingredient since much criticism has focused on producing unwanted crumbliness in HPN bars.

TPA cohesiveness, or strength of internal interactions, measurements initially showed that extruded MPC80 produced HPN bars that were more cohesive than the control ($P < 0.05$) (Figure 3D). Unlike adhesiveness, TPA cohesiveness values decreased sharply after 1 week at both storage temperatures and were not differentiable for the remainder of storage. Around week 10 at 32°C and week 18 at 22°C, the control HPN bar became numerically less cohesive, based on TPA measurement, but the values were not significantly different from the other HPN bars.

After one compression during the 2-bite test, the HPN bars were either permanently deformed or so crumbly that the area ratio was not well suited to differentiate cohesiveness.

Instead of relying on TPA cohesiveness or withdrawal force as cohesiveness measurement, it was assumed that crumbliness and cohesiveness have an inverse relationship. As HPN bar mass percentage passing the top sieve increased, crumbliness increased and in turn cohesiveness decreased. A large sieve aperture was selected since any crumb generation during a first or second bite would be undesirable and uncharacteristic of soft-textured HPN bars. Furthermore each HPN bar formulated with extruded MPC80 was completely retained on the top sieve until the sixth week at 22°C when underpass increased from essentially 0% to 1.2%. Sieved sample mass did not have normal distribution, therefore, geometric mean diameter was not calculated.

These crumbliness measurements (Figure 3E) and its affiliated cohesiveness was better equipped to differentiate the HPN bars than TPA. Crumbliness of the HPN bars formulated with extruded MPC80 increased slowly while kept at 22°C whereas the increase was more pronounced at 32°C ($P < 0.05$). At 22°C, the HPN bars formulated with extruded MPC80 were always less crumbly than the control, but significance varied by time point when stored at 32°C. After 2 weeks at 32°C, which roughly simulated 17.3 weeks at ambient (Li and others 2008), crumbliness of the extruded MPC80 containing HPN bars increased to 6.5%. After 18 weeks at 22°C, average crumbliness of the HPN bars formulated with extruded MPC80 was 9.0%, and was quite similar to the value obtained at the simulated 17.3 weeks storage. Other texture attributes changed faster at elevated temperature storage and at many equivalent storage time points they were not differentiable from the control. After 29 weeks (~7 months) at room

temperature, the HPN bars formulated with extruded MPC80 were less crumbly than the control and imparting cohesiveness makes extruded MPC80 more usable in these applications.

HPN Bar Evaluation by the Trained Sensory Panel

The least square-means for sensory panel measured firmness, crumbliness, fracturability, hardness, and cohesiveness (Table 3) were significantly influenced by protein, temperature, and time ($P < 0.05$). Panelists also measured mouth coating, or the powdery/chalky feeling left in one's mouth, but they were unable to distinguish any difference between the HPN bars ($P > 0.05$). Commercial anchors for most texture attributes evaluated were readily available (Table 1). However, the in-mouth residual after swallowing or expectorating our HPN bars was not scalable using previously identified anchors (Meilgaard and others 2010), and our attempt to make anchors by varying the ratio of WPC80 to MPC80 in different HPN bars was not helpful for differentiating the samples. Similar properties (e.g., powderiness) were reported in other HPN bar sensory studies, as it cannot be measured by instrumental analysis (Childs and others 2007; Imtiaz and others 2012). Smoothness, stickiness, chewiness, dissolvability, tooth packing, denseness, adhesiveness, and visual appeal were not measured by the sensory panel, partly because they were not stressed during training and partly to avoid too many evaluation criteria.

HPN bar firmness and crumbliness were evaluated as in-hand parameters. Since HPN bars have a difficult-to-chew reputation, it would not be uncommon for a consumer to press on a HPN bar before purchase or consumption. An excessively firm sample or one that easily crumbles would not be appealing. At equivalent temperature and time stored, the HPN bars formulated with extruded MPC80 were softer and more cohesive than those prepared with control MPC80 ($P < 0.05$). The HPN bars, especially those formulated with control MPC80, firmed quicker at 32°C ($P < 0.05$). At this temperature, firmness did not change significantly

after the second and third weeks for the HPN bars made with E116 and E105, respectively. The control HPN bar became firmer after 1 week storage at 22°C ($P < 0.05$), after which its firmness did not change. Firmness of the extruded MPC80-containing HPN bars continued to increase after week 1 while kept at 22°C. Firmness was strongly correlated with instrumental max force ($r = 0.87$) and shear force ($r = 0.87$), and thus both instrumental techniques are representative of in-hand firmness (Table 4). The HPN bars formulated with extruded MPC80 maintained lower firmness than the control, even after 1 year of simulated storage.

The panelists easily distinguished that the control was more crumbly than those HPN bars formulated with extruded MPC80 at fixed storage time and the same storage temperature ($P < 0.05$). Panelists were not able to detect any significant change in the control's crumbliness during storage ($P > 0.05$). HPN bar crumbliness increased from 0.6 to 5.9 cm and from 0.3 to 6.7 cm after 1 week at 32°C when formulated with E105 and E116, respectively, after which no further changes in crumbliness were detected. At 22°C, in-hand crumbliness slowly increased for these two HPN bars and values at week 6 approached those obtained after 1 week at 32°C, which was similar to the previous estimate of 1 week at 32°C being equivalent to 8.7 weeks at room temperature (Li and others 2008). Sensory panel crumbliness data were strongly correlated ($r = 0.85$) with the instrumental crumbliness data (Table 4). Similar to Imtiaz and others (2012), instrumental withdrawal energy, in the present study it is labeled adhesiveness, was inversely correlated ($r = -0.85$) with crumbliness. Pieces or crumbs were unlikely generated during analysis of a more adhesive HPN bar. These data support that sieve analysis and mass percent finer than a specified sieve can be used in lieu of panelists to measure HPN bar crumbliness.

The panelists measured fracturability, hardness, and cohesiveness as in-mouth attributes. Compared with the in-hand measurements, less of each attribute-specific 15-cm line scale was

used to differentiate the samples, which indicated that the HPN bars had greater textural similarity when evaluated in one's mouth. HPN bars formulated with extruded MPC80 fractured with less force between the panelists' incisors than the control each week at 22°C ($P < 0.05$), but significance varied by time point at 32°C. Instrumental shearing with a 45° chisel blade was predicted to mimic one's incisors. However, fracturability had the strongest correlation with max force (r = 0.85), which was slightly stronger than its correlation with shear force (r = 0.83). Other correlations with fracturability were also strong, but they were inherent to the HPN bars used in this study. For example, fracturability was correlated with instrumental crumbliness (r = 0.84), but only because the HPN bars with higher fracture force, mainly those formulated with control MPC80, also tended to be more crumbly. By no means would a HPN bar with high crumbliness be implicated with a high fracture force. This happened in our study, but it is not a global property of the instrumental crumbliness test. Snapping, breaking, and fracturing are not typical texture attributes found in soft textured HPN bars, and extruded MPC80 helped reduce their presence.

Hardness, which was evaluated between each panelist's molars, of the control was greater than the HPN bars formulated with extruded MPC80 at each time point ($P < 0.05$). Each HPN bar hardened significantly during storage ($P < 0.05$) except for the sample formulated with E116 and stored at 32°C, where hardness did not change significantly between week 1 and week 6. At 22°C, the panelists did not detect significant hardening of the control HPN bar until week 6 and magnitude of change (2.1 cm) was just slightly greater than those formulated with E105 (1.7 cm) and E116 (2.0 cm). Sensory hardness measurements correlated strongly with max force (r = 0.84) and shear force (r = 0.84) (Table 4). Strong correlations with hardness were observed with other instrumental parameters. While those relationships in these particular HPN bars make

sense, they do not translate to all HPN bars. When evaluated in-mouth, the HPN bars formulated with extruded MPC80 were softer than the control.

Cohesiveness of mass was the attribute measured before swallowing or expectorating and it decreased during storage at both temperatures ($P < 0.05$). Initially, the HPN bars formulated with extruded MPC80 were more cohesive than the control, but cohesiveness quickly decreased at 32°C. Extruded MPC80 produced HPN bars that maintained their structure more so than the control after three chews while stored at 22°C. Sensory measured cohesiveness was inversely correlated with instrumental crumbliness ($r = -0.84$), but it had the weakest correlation with TPA-measured cohesiveness ($r = 0.43$). TPA cohesiveness values were not representative of HPN bar cohesiveness and the newly proposed instrumental crumbliness assay better approximated in-mouth perceived cohesiveness. Although sieve analysis required timely weighing and reweighing sieves and was more involved than TPA alone, it is advantageous in the sense that it does not require panelists, which eliminates training, panelist commitment, and allows for non-food-grade modifications or ingredients to be thoroughly evaluated in HPN bars.

Conclusions

Extruded MPC80 performed more favorably in a model HPN bar when compared to the control. Instrumentally-measured max force and shear force and sensory-measured firmness and hardness showed that the HPN bars hardened during storage. HPN bar adhesiveness, cohesiveness, and crumbliness also changed during storage and their change may negatively impact HPN bar quality just as much as hardening. Sensory-measured hardness parameters, including firmness, fracturability, and hardness were correlated with instrumentally-measured max force and shear force. Sensory-measured crumbliness and cohesiveness were strongly correlated with the instrumental results from the newly-implemented HPN bar crumbliness assay

and it may be used to measure these two attributes in future HPN bar studies. Instrumental TPA was able to measure most of the reported texture attributes as perceived by humans. Extruded MPC80 produced HPN bars that were softer, more stable, and more cohesive than those made with spray dried control MPC80.

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Tables

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

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

Attributes, definitions, and anchors adapted from Childs and others (2007), Imtiaz and others (2011), and Meilgaard and others (2007).

Table 2. Moisture Content (%) and pH of the high-protein nutrition (HPN) bars after 0, 6, and 29 weeks at 22°C or 32°C

			Week		
Property	**°C**	**Protein**[+]	0	6	29
Moisture (%)	22	MPC80	$17.9^{a,y}$	$20.2^{a,z}$	$21.6^{a,z}$
		E105	$14.7^{b,y}$	$18.4^{a,z}$	$20.3^{a,z}$
		E116	$14.9^{b,y}$	$19.1^{a,z}$	$19.9^{a,z}$
	32	MPC80	-	$19.3^{a,z}$	$21.6^{a,z}$
		E105	-	$18.7^{a,z}$	$19.5^{b,z}$
		E116	-	$19.0^{a,z}$	$19.5^{b,z}$
pH	22	MPC80	$6.77^{a,z}$	$6.53^{a,y}$	$6.47^{a,y}$
		E105	$6.78^{a,z}$	$6.49^{a,y}$	$6.42^{a,y}$
		E116	$6.72^{a,z}$	$6.52^{a,y}$	$6.24^{b,x}$
	32	MPC80	-	$6.53^{a,z}$	$6.09^{a,y}$
		E105	-	$6.46^{a,z}$	$6.10^{a,y}$
		E116	-	$6.50^{a,z}$	$6.08^{a,y}$

+ MPC80, unmodified milk protein concentrate with 80% protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively.

$^{a-b}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column for each property at fixed temperature.

$^{x-z}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same row for each property at fixed temperature.

Table 3. Water activity (a_w) and total color change (ΔE) of the high-protein nutrition (HPN) bars during 6 weeks storage at 22°C or 32°C

			Week				
Property	**°C**	**Protein+**	0	$^3/_7$*	1	3	6
a_w	22	MPC80	$0.48^{a,y}$	$0.51^{a,z}$	$0.51^{a,z}$	$0.50^{a,z}$	$0.50^{a,z}$
		E105	$0.44^{b,y}$	$0.47^{b,z}$	$0.48^{b,z}$	$0.48^{b,z}$	$0.48^{b,z}$
		E116	$0.44^{b,y}$	$0.47^{b,z}$	$0.48^{b,z}$	$0.48^{b,z}$	$0.49^{ab,z}$
	32	MPC80	-	-	$0.51^{a,z}$	$0.50^{a,z}$	$0.50^{a,z}$
		E105	-	-	$0.49^{b,z}$	$0.50^{a,z}$	$0.50^{a,z}$
		E116	-	-	$0.49^{b,z}$	$0.50^{a,z}$	$0.49^{a,z}$
ΔE	22	MPC80	$0.0^{a,y}$	$2.8^{a,z}$	$3.5^{a,z}$	$3.4^{a,z}$	$4.8^{a,z}$
		E105	$0.0^{a,z}$	$1.4^{a,z}$	$2.3^{a,z}$	$2.1^{a,z}$	$2.0^{b,z}$
		E116	$0.0^{a,z}$	$0.7^{a,z}$	$1.1^{a,z}$	$1.3^{a,z}$	$1.1^{b,z}$
	32	MPC80	-	-	$4.9^{a,x}$	$12.6^{a,y}$	$21.7^{a,z}$
		E105	-	-	$3.6^{a,x}$	$8.6^{b,y}$	$15.7^{b,z}$
		E116	-	-	$3.4^{a,x}$	$7.2^{b,y}$	$12.9^{c,z}$

+ MPC80, unmodified milk protein concentrate with 80% protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively.

* Measurements taken after 3 day storage.

$^{a\text{-}c}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column for each property at fixed temperature.

$^{x\text{-}z}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same row for each property at fixed temperature.

Table 3. Sensory attributes (cm) of the high-protein nutrition (HPN) bars during 6 weeks storage at 22°C or 32°C

			Week					
Attribute	**°C**	**Protein[+]**	0	1	2	3	4	6
Firmness	22	MPC80	$6.7^{a,y}$	$9.4^{a,z}$	$10.2^{a,z}$	$9.0^{b,z}$	$9.5^{a,z}$	$9.3^{a,z}$
		E105	$2.0^{b,x}$	$3.6^{b,xy}$	$3.4^{b,xy}$	$4.8^{b,yz}$	$5.3^{b,z}$	$5.8^{b,z}$
		E116	$0.9^{b,x}$	$2.6^{b,y}$	$2.9^{b,y}$	$3.6^{b,yz}$	$3.6^{c,yz}$	$5.2^{b,z}$
	32	MPC80	-	$10.2^{a,x}$	$10.9^{a,xy}$	$12.0^{a,yz}$	$11.9^{a,y}$	$13.6^{a,z}$
		E105	-	$6.7^{b,x}$	$8.2^{b,xy}$	$9.9^{b,z}$	$9.3^{b,yz}$	$10.2^{b,z}$
		E116	-	$6.3^{b,y}$	$8.5^{b,z}$	$8.6^{b,z}$	$8.5^{b,z}$	$9.5^{b,z}$
Crumbliness	22	MPC80	$8.2^{a,y}$	$9.4^{a,yz}$	$10.7^{a,z}$	$10.0^{a,yz}$	$9.0^{a,yz}$	$9.7^{a,yz}$
		E105	$0.6^{b,x}$	$1.2^{b,x}$	$1.4^{b,xy}$	$2.1^{b,xy}$	$3.1^{b,yz}$	$4.2^{b,z}$
		E116	$0.3^{b,y}$	$0.9^{b,xy}$	$1.5^{b,xy}$	$2.1^{b,xyz}$	$2.4^{b,yz}$	$3.5^{b,z}$
	32	MPC80	-	$9.9^{a,z}$	$11.4^{a,z}$	$10.6^{a,z}$	$9.9^{a,z}$	$10.8^{a,z}$
		E105	-	$5.9^{b,y}$	$8.4^{b,z}$	$8.1^{b,z}$	$7.8^{b,z}$	$8.7^{b,z}$
		E116	-	$6.7^{b,z}$	$8.0^{b,z}$	$7.2^{b,z}$	$7.8^{b,z}$	$8.3^{b,z}$
Fracturability	22	MPC80	$5.2^{a,z}$	$6.9^{a,z}$	$6.7^{a,z}$	$5.9^{a,z}$	$6.1^{a,z}$	$6.9^{a,z}$
		E105	$1.8^{b,y}$	$1.9^{b,y}$	$2.2^{b,yz}$	$3.6^{b,yz}$	$3.8^{b,yz}$	$4.1^{b,z}$
		E116	$1.0^{b,y}$	$1.6^{b,y}$	$2.0^{b,yz}$	$2.8^{b,yz}$	$2.7^{b,yz}$	$3.8^{b,z}$
	32	MPC80	-	$6.2^{a,y}$	$8.0^{a,yz}$	$8.1^{a,yz}$	$7.7^{a,yz}$	$9.1^{a,z}$
		E105	-	$4.9^{a,y}$	$5.9^{b,yz}$	$6.6^{a,yz}$	$6.8^{a,yz}$	$7.7^{ab,z}$
		E116	-	$5.5^{a,z}$	$5.3^{b,z}$	$6.4^{a,z}$	$6.5^{a,z}$	$6.6^{b,z}$
Hardness	22	MPC80	$4.2^{a,y}$	$5.1^{a,yz}$	$5.1^{a,yz}$	$5.6^{a,yz}$	$5.2^{a,yz}$	$6.3^{a,z}$
		E105	$0.9^{b,y}$	$1.7^{b,yz}$	$1.7^{b,yz}$	$2.6^{b,z}$	$2.2^{b,yz}$	$2.6^{b,z}$
		E116	$0.5^{b,y}$	$1.5^{b,yz}$	$1.7^{b,yz}$	$2.0^{b,yz}$	$1.8^{b,yz}$	$2.5^{b,z}$
	32	MPC80	-	$4.9^{a,x}$	$6.1^{a,xy}$	$6.8^{a,y}$	$6.5^{a,y}$	$10.0^{a,z}$
		E105	-	$2.7^{b,y}$	$3.2^{b,yz}$	$3.8^{b,yz}$	$4.1^{b,yz}$	$4.4^{b,z}$
		E116	-	$3.1^{b,z}$	$3.0^{b,z}$	$3.8^{b,z}$	$3.8^{b,z}$	$4.5^{b,z}$
Cohesiveness	22	MPC80	$8.4^{b,z}$	$7.0^{b,z}$	$6.3^{b,z}$	$6.3^{b,z}$	$6.5^{b,z}$	$5.8^{b,z}$
		E105	$12.2^{a,z}$	$11.3^{a,yz}$	$11.2^{a,yz}$	$11.0^{a,yz}$	$10.9^{a,yz}$	$9.4^{a,y}$
		E116	$11.6^{a,z}$	$11.1^{a,z}$	$11.4^{a,z}$	$11.3^{a,z}$	$10.5^{a,z}$	$10.1^{a,z}$
	32	MPC80	-	$7.8^{a,z}$	$5.7^{b,yz}$	$5.4^{a,yz}$	$5.3^{a,yz}$	$5.1^{a,y}$
		E105	-	$8.1^{a,z}$	$8.4^{a,z}$	$7.1^{a,z}$	$7.0^{a,z}$	$6.3^{a,z}$
		E116	-	$9.0^{a,z}$	$7.8^{ab,yz}$	$7.1^{a,yz}$	$6.7^{a,yz}$	$5.6^{a,y}$

+ MPC80, unmodified milk protein concentrate with 80% protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively.

$^{a\text{-}c}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column for each attribute at fixed temperature.

30 [x-z] Least squares means are significantly different ($P < 0.05$) if they do not share a common

31 superscript within the same row for each attribute at fixed temperature.

Table 4. Pearson correlation coefficients (r) for the instrumental and sensory panel measured high-protein nutrition (HPN) bar texture attributes

Instrumental Attribute	Sensory Attribute				
	Firmness	Crumbliness	Fracturability	Hardness	Cohesiveness
Max Force	0.87***	0.76***	0.85***	0.84***	-0.84***
Cohesiveness	-0.48***	-0.40**	-0.48***	-0.39*	0.43**
Adhesiveness	-0.82***	-0.85***	-0.84***	-0.79***	0.83***
Crumbliness	0.86***	0.85***	0.84***	0.89***	-0.84***
Shear Force	0.87***	0.76***	0.83***	0.84***	-0.84***

*** $P < 0.0001$; ** $P < 0.001$; * $P < 0.05$

Figures

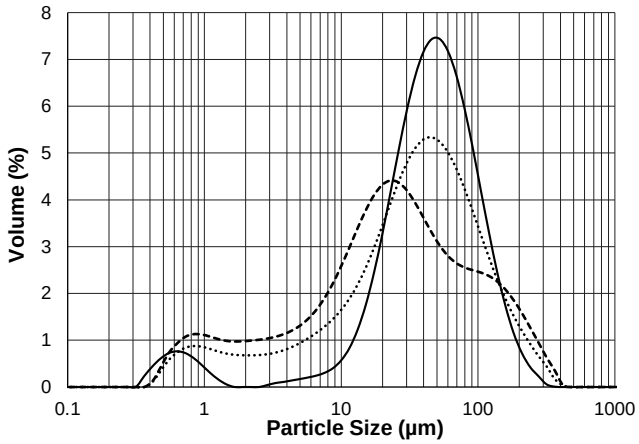


Figure 1 – Particle size distributions (PSD) for control and extruded MPC80. MPC80 (solid line), spray dried control milk protein concentrate with 80% protein. E105 (long dashed line**)** and E116 (dotted line), jet-milled MPC80 that was extruded at die-end melt temperature of 105°C and 116°C, respectively.

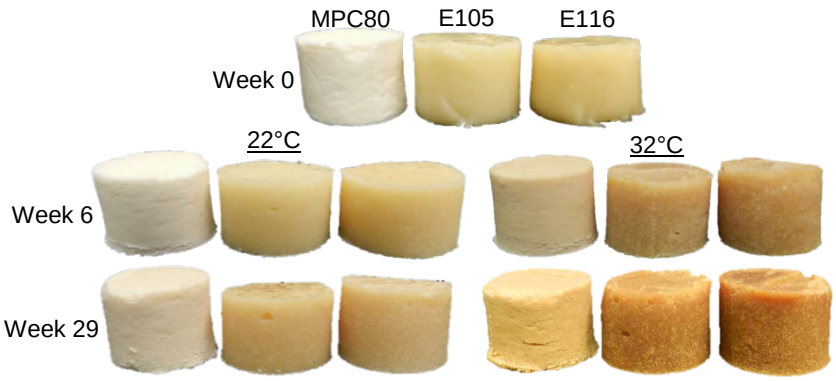
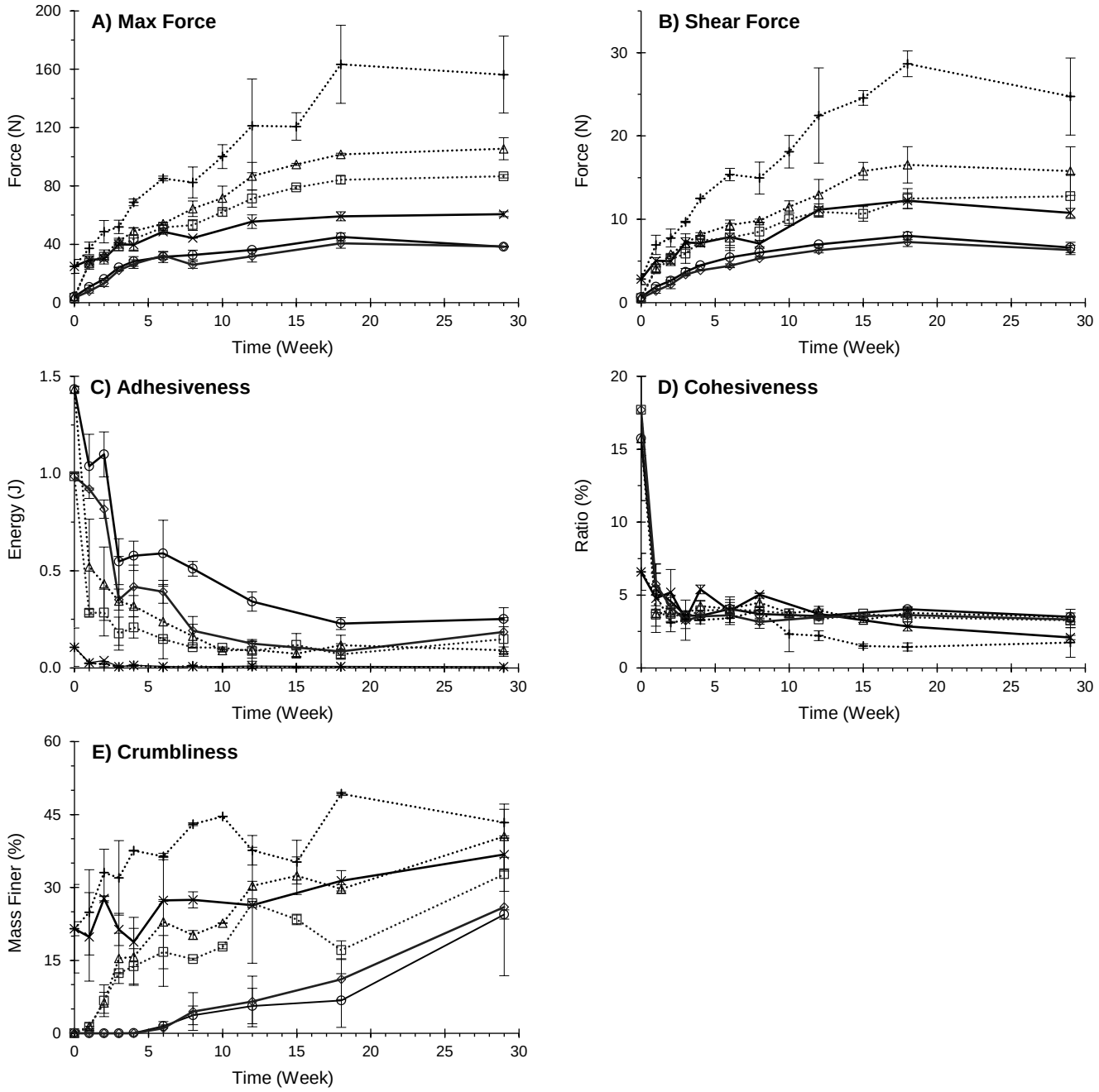


Figure 2 – Images of the model high-protein nutrition (HPN) bars on week 0, and on week 6 and week 29 after storage at 22°C or 32°C. MPC80, unmodified control milk protein concentrate with 80% protein was used to make the control HPN bar. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively, was used as the protein source in their respective HPN bars.



49 **Figure 3 – Instrumental max force (A), shear force (B), adhesiveness (C), cohesiveness (D),**

50 **and crumbliness (E) of the high-protein nutrition (HPN) bars during storage.** HPN bars

51 formulated with MPC80 (×), E105 (○), and E116 (◊) were stored at 22°C (solid lined). HPN bars

52 formulated with MPC80 (+), E105 (Δ), and E116 (□) were stored at 32°C (dotted lines).

MPC80, unmodified control milk protein concentrate with 80% protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively.