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Textural performance of crosslinked or reduced-calcium milk protein ingredients in model high-protein nutrition bars

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ABSTRACT

Transglutaminase (Tgase) crosslinking and calcium reduction were investigated as ways to improve the texture and storage stability of high-protein nutrition (HPN) bars formulated with milk protein concentrate (MPC) and micellar casein concentrate (MCC). The MPC and MCC crosslinked at none, low, and high levels, and a reduced-calcium MPC (RCMPC) were each formulated into model HPN bars. Hardness, crumbliness, moisture content, pH, color, and water activity of the HPN bars were measured during accelerated storage. The HPN bars prepared with MPC were harder and more cohesive than those prepared with MCC. Higher levels of Tgase crosslinking improved HPN bar cohesiveness and decreased hardening during storage. The RCMPC produced softer, yet crumblier HPN bars. Small textural differences were observed for the HPN bars formulated with the transglutaminase crosslinked proteins or RCMPC when compared with their respective controls. However, modification only slightly improved protein ingredient ability to slow hardening while balancing cohesion and likely requires further improvement for increased applicability in soft-texture HPN bars.

Key words: micellar casein concentrate, milk protein concentrate, transglutaminase, protein bar

INTRODUCTION

High-protein foods are popular among consumers seeking satiety, increased muscle mass, or decreased risk of sarcopenia (Sloan, 2012). Consumers are turning to high-protein nutrition (**HPN**) bars to conveniently add more protein to their diet. High-protein nutrition bars have used new, trendy protein sources (e.g., insects), but have traditionally relied on dairy and soy ingredients such as concentrates, isolates, and hydrolysates. Protein content typically ranges from 20 to 50% (wt/wt), whereas carbohydrates (e.g., high-fructose corn syrup), polyols (e.g., glycerol), sugar alcohols (e.g., sorbitol), and lipids (e.g., palm oil) comprise the rest of the formulation (McMahon et al., 2009; Imtiaz et al., 2012).

It is well known that HPN bars, especially those prepared with high-protein milk protein concentrates (**MPC**; ≥80% protein wt/wt), are texturally unstable during storage (Loveday et al., 2009; Imtiaz et al., 2012). Specifically, HPN bars formulated at 30% protein (wt/wt) using MPC that contained 80% protein rapidly hardened and lost cohesion during storage (Banach et al., 2014, 2016a). Nutritionally, MPC maintain the casein-to-whey protein ratio (80:20) of typical bovine skim milk and are a complete protein with higher digestible indispensable AA score (1.18) than whey protein isolate (1.09), whey protein concentrate (0.97), soy protein isolate (0.90), and pea protein concentrate (0.82; Rutherfurd et al., 2015). The nutritional aspects of MPC and their ability to be ultra-filtered directly from skim milk independent of other processes make HPN bars a primary target application.

Micellar casein concentrates (**MCC**) are produced by micro-filtering skim milk such that the final spray dried powder has an elevated casein-to-whey protein ratio (92:8; Dairy Management Inc., 2015). Micellar casein concentrates, which are undefined by the global trade atlas and the US Food and Drug Administration, are less studied than MPC (Lagrange et al., 2015). Model HPN bars (45% protein wt/wt) prepared with MCC remained softer than those formulated whey protein hydrolysate, β-lactoglobulin, α-lactalbumin, whey protein isolate, or sodium caseinate after 10 d at 37°C (Hogan et al., 2012). Agglomerated MCC produced HPN bars (40–50% MCC powder wt/wt) that were less dough-like and less prone to hardening than those prepared with nonagglomerated MCC over 7 d storage at 37°C (Hogan et al., 2012). Further validation of MCC in HPN bars is needed because, based on protein com-

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position, similar textural performance as MPC would be expected in these applications.

The HPN bar texture changes during storage cannot be attributed to a single mechanistic cause, and although it is a multicomponent system (e.g., protein, carbohydrate, fats, minerals, vitamins), most work has focused on the protein source and ingredient type while the system hardens. Suggested HPN bar hardening mechanisms include moisture migration between constituents, limited free water for complete protein plasticization, entropy-driven macronutrient phase separations, internal disulfide bond formations, and Maillard-induced protein aggregations (Zhou et al., 2008; Loveday et al., 2009; McMahon et al., 2009; Zhou et al., 2013). Mineral (e.g., Na^+, K^+, Mg^{2+}) addition or removal, including those natively associated with the protein (e.g., Ca^{2+}), may alter the protein's structure, increase internal moisture migration, and subsequently accelerate HPN bar texture change (Book, 2008). Protein hydrolysis has been the main modification technique to impart textural stability during HPN bar storage (McMahon et al., 2009; Rao et al., 2016a). Proprietarily modified (Imtiaz et al., 2012) and extruded MPC (Banach et al., 2014) also improved textural stability when incorporated into model HPN bars. Further, MPC and MCC modification techniques must be explored to not only slow HPN bar hardening during storage, but also to improve cohesion in order to be a preferred protein source for intermediate moisture food applications.

Most protein powders, especially MPC, are modified to improve solubility (Mao et al., 2012; Sikand et al., 2013) as well as dependent functional properties (e.g., emulsification, foaming). However, no clear relationship is apparent between these properties and performance in intermediate-moisture foods such as HPN bars. Transglutaminase (**Tgase**), an enzyme produced by *Streptoverticillium mobaraense*, was used to improve the texture of solid foods such as restructured meats, fish pastes, yogurts, breads, and confectionaries (Kieliszek and Misiewicz, 2014; Gaspar and de Góes-Favoni, 2015). Transglutaminase builds texture by crosslinking glutamine residues with intra- or inter-protein lysine residues, which occurs faster and with greater specificity than its acyl transfer and deamidation processes (DeJong and Koppelman, 2002; Gaspar and de Góes-Favoni, 2015). Transglutaminase treatment has historically been applied to processed foods seeking textural improvement, but is not commonly used to functionalize protein ingredients for multiple applications (DeJong and Koppelman, 2002). Previously, MPC and MCC were crosslinked by Tgase and functionality was evaluated in processed cheese and yogurt (Salunke, 2013; Salunke et al., 2013a,b), but they were not evaluated in HPN bars.

Transglutaminase crosslinked proteins typically have increased water-holding capacity (Gaspar and de Góes-Favoni, 2015). The effect of increased water-holding capacity on HPN bar texture is unknown as water may move toward the protein as driven by water activity ($\mathbf{a_w}$) gradient (Gautam et al., 2006; Book, 2008; Li et al., 2008; Hazen, 2010) or toward the low molecular weight, poly-hydroxyl compounds by osmotic pull (Loveday et al., 2009). Reduced-calcium MPC (**RCMPC**) was manufactured by carbon dioxide acidification of milk protein retentate during ultra-filtration, which solubilized micellar calcium and phosphate (Marella et al., 2015). The RCMPC had improved solubility, which may allow for more rapid hydration during HPN bar production that along with its lower calcium, ash, and net negative charge may limit moisture migration and slow moisture-induced hardening during HPN bar storage.

This study was designed to compare relative textural performance of Tgase crosslinked MPC and MCC, and RCMPC, in a previously used model HPN bar formulation (Banach et al., 2014). Crosslinked protein ingredients will have fewer amine groups available for participation in the Maillard browning reaction (Gerrard, 2002), which may limit formation of protein aggregates that have been associated with HPN bar texture change (Zhou et al., 2013; Banach et al., 2016b). Model HPN bars (30% protein wt/wt) were prepared with MPC and MCC previously Tgase crosslinked at none, low, and high levels and RCMPC, and hardness, crumbliness, moisture content, pH, color, and a_w were measured during storage.

MATERIALS AND METHODS

Materials

The MPC and MCC powders with none (**N**), low (**L**), and high (**H**) Tgase crosslink levels, including MPC-N (74.4% protein, 3.7% moisture, 8.9% lactose), MPC-L (74.4% protein, 3.9% moisture, 8.7% lactose), MPC-H (74.3% protein, 2.7% moisture, 8.6% lactose), MCC-N (77.6% protein, 3.2% moisture, 4.4% lactose), MCC-L (77.6% protein, 3.6% moisture, 4.5% lactose), and MCC-H (76.9% protein, 3.2% moisture, 4.5% lactose), and the RCMPC (71.9% protein, 3.4% moisture, 14.4% lactose) were previously produced (Salunke, 2013; Marella et al., 2015) at South Dakota State University. Urea, SDS, β-mercaptoethanol, bromophenol blue, and glycerol (99.8% glycerol, 0.1% water) were obtained from Fisher Scientific (Waltham, MA). Supplies for SDS-PAGE, including Tris, Precision Plus Protein Standard, Any kD TGX precast gels, Bio-Safe Coomassie Stain, and 10× Tris/glycine/SDS running buffer, were obtained