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Hardness of high protein nutrition bars based on milk protein concentrates:a review

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ABSTRACT
High protein nutrition (HPN) bars are gaining increased global popularity as convenient and high nutritious food products. Proteins of different origins have been used singly or in combinations in HPN but milk proteins (whey protein products, casein and caseinates) remain the favorable proteins used in HPN formulations. Milk protein concentrates (MPC) of different protein contents are new milk protein ingredients of multifunctional properties for diversified food applications. They contain both casein and whey protein in their native form and ratio found in milk. The use of high protein MPCs in nutrition bars is one of the promising applications for these products. HPN based on different protein sources develops hardness during storage particularly at high temperatures but more pronounced hardness develops on the use of MPCs. Several approaches have been suggested to overcome this problem through modification of MPCs. This review presents an overview ofthe HPN bar hardness mechanisms and MPC modifications to combat this problem.
Keywords:*High protein nutrition bars, milk protein concentrates, polyols, hardness.*

1. INTRODUCTION
The high protein nutrition (HPN)bars, are bar-shaped dense nutrient products based on balanced combinations of proteins, carbohydrates and fats. Vitamins minerals and/or fiber are usually added to enhance the nutritional value of HPN. They are regarded as intermediate moisture foods developed for sports nutrition, muscle building, health supplement, and weight reduction markets [1]. They provide healthy substitutes to conventional snacks because they contain 15% to 35% (wt/wt) of protein. HPN isformulated to have water activity (αw) less than 0.65 in order to inhibit the growth of spoilage microorganisms. Nutritional bars are generally classified as high-protein (HPN bars), balanced nutrition (40%/30%/30% carbohydrates/ non-trans fat/protein caloric-basis), carb conscious, and carbohydrate-rich. In USA, the number of commercial nutrition bars increased from 226 products in 2005 to 1012 product in 2015 whereas the high protein bars shows better marketability than other categories of nutrition bars [2].

High protein nutrition bars were first commercially released in 1986 under the name of "power bars", as a new segment of energy supplements for athletes to provide them with a source of high level and quality proteins. Soon protein bars have been formulated to appeal to a wide range of health-conscious consumers and became a regular replacement of many protein diets. Globally, sales of protein and nutrition bars in 2016 exceeded 4 billion US $ and areexpected to register a CAGR of 4.23 %, during the forecast period (2019 – 2024) [3].

Single or mixed proteins of animal and plant sources have been used in the formulations of HPN bars. There is no official standard for the amount of protein to be added in HPN bars formulations but ratios that range between 15 and 35% are usually added [4]. Proteins are added in HPN bars formulations to provide texture, flavour, consumer acceptability and stability of the product. Milk proteins including whey protein products, casein, caseinates and milk protein concentrate vegetable proteins such as soy protein, rice proteins and pea proteins and protein hydrolysates have been used in the formulations of HPN bars. However, Casein, caseinates and whey protein products are heavily used in HPN due to their unique functional and nutritional properties.

With the advent of membrane processing and ion exchange technologies, several milk protein products of diversified functional products became available in the market [5]. Traditionally casein and caseinates have been the main industrially produced milk protein products but growth in the production of whey protein concentrates and milk protein concentrate became evident in recent years.

Ultrafiltration/diafiltration of skim milk followed by concentration and spray drying of the concentrate results in a total milk protein concentrate (MPC) with casein/whey protein ratio (80/20) similar to that of the original milk. MPCs are produced with different protein contents and identified by the number directly following MPC (i.e., MPC80 has almost 80% protein content). MPCs with protein content ≥ 90% are referred to as milk protein isolates (MPIs).The use of membrane filtration in the manufacture of MPCs allows the casein micelles to retain its structure offering emulsion and heat stabilities, opacity, flavour, and protein fortification for different food applications. Compared to other protein sources, MPC has the highest digestible indispensable amino acid score (DIAAS; 1.18) while the scores for soy protein isolate and whey protein concentrate are 0.91 and 1.10 respectively [6]. Also, MPC can be considered as a natural and rich source for calcium which adds to the nutritional quality of these products [7]. Also, MPCs have unique multifunctional properties such as water holding, gelling, emulsification, foaming and heat stability that diversify its potential uses as food ingredients [7]. Significant ($P<0.05$) differences in the sensory flavor profile between MPC, caseins and caseinates have been reported [8] whereas MPC showed better bland flavor. Lagrange *et al.* [9] stated that data on the production of MPC was only

available from USA where 50,000-55,000 metric tons (MT) from MPC42 and MPC56 and 17,000-18,000 MT from MPC70, MPC80 and MPI were produced in 2013. Nowadays several MPCs of variable composition and functional properties are available in the market.

Although MPCs contain the two protein fractions (casein and whey proteins) commonly used in the formulation of HPN bars, bars formulated with MPCs tend to develop hardness, to become unpalatable, and to have reduced shelf-life. Also, HPN bars containing high MPC content tend to lack cohesiveness and are too crumbly [1]. During the last decades, studies have been directed to investigate changes in the texture of HPN during storage, the mechanism of hardening of high protein bars particularly with the use of MPCs in formulation, ways to overcome this problem through MPC modification and to design suitable approaches to successful incorporation of MPC in HPN bars. This paper presents a comprehensive overview ofthis subject.

2. COMPOSITION AND INGREDIENTS OF HIGH PROTEIN NUTRITION BARS

High protein nutrition (HPN) bars are composed ofblends of proteins, sugar and /or polyol-based syrups and fats/oils as the main ingredients. In addition, vitamins, minerals and/or fibers are usually added to enhance the nutritional value of the products. HPN bars have typically moisture content that range from 10% to 15% and a low water activity (< 0.65) to avoid microbial growth and to ensure consumer safety as they are usually made with no heat treatment applied. Low molecular weight humectants such as glycerol and sorbitol are added to control the water activity of the product.

3. HARDENING OF HIGH PROTEIN BARS

Hardening is the main defect ofconsumer acceptability of HPN bars. Understanding the mechanism of hardening is a key element for developing HPN bars of acceptable quality and storage stability. The number and complexity of the ingredients used, and the low moisture and low water activity of the different formulations are the main difficulties in understanding and solving the problem of hardening. Changes in proteins and their interaction with small molecules polyol and sugars play the main roles in hardening of HPN and they should be taken into consideration. Hogan *et al.* [10] showed that the hardness of bars was dependent on protein type, concentration and co-solvents and that hardening arises from solvent-induced plasticisation and re-conformation of protein secondary structures.

4. CHANGES IN MILK PROTEIN CONCENTRATES DURING STORAGE

Briefly, MPC ismade by ultrafiltration (UF)/diafiltration (DF) of skim milk where milk proteins, colloidal calcium phosphate and residual fat are retained in the UF retentate while the lactose and soluble minerals are removed in the permeate [11]. In order to produce MPC of protein content higher than 65% DF must be used. The retentate is then spray dried directly or after further concentration by evaporation under reduced pressure. The American Dairy Products Institute set standard specification for MPC and MPI of different protein contents (Table1). Only a single study [13] gave data on the mineral composition of commercial MPC samples (Table 1) which shows marked differences between MPC of different protein contents. This study [13] showed a high negative correlation between solubility and Ca content of the MPC.

MPCs of high protein content usually exhibit poor dissolution, even after long rehydration time. This has been explained by the difficulty for water to transport into powder particles, and the particle surface activity which may decrease the powder dispersion [14]. High-protein MPC powders (≥ 80% protein) exhibited the poor wettability, which negatively affected rehydration. This behavior was attributed to the combined effect of the high calcium ion activity and poor solvent quality, which promote the formation of aggregates between casein micelles [15].

The changes in the texture and structure of milk protein concentrates (MPC) have been attributed to decreases in the interparticle distances, their spatial correlations and diffusivity between casein micelles during concentration [16]. Both solubility and flavors of MPC change during storage but only solubility that received much attention [8]. Solubility of MPC varies depending on the method and conditions used for measurement [17]. For example the differences in the solubility of aged MPC were more pronounced in the presence of sugars than in water [18]. The changes in the solubility of MPCs during storage are affected by their protein contents whereas high protein MPCs can be severely affected by storage at high temperature and humidity [13]. During storage the percentage of the insoluble materials increased in MPC which has been attributed to non-covalent interactions between α-casein and β-casein [19]. Although, β-LG interact with κ-casein and some αs2-casein through disulphide bonds the formed aggregates have no significant role in the formation of the insoluble materials [19]. Anema *et al.* [20] showed that the solubility of MPC 85 decreased with time, the insoluble proteins were caseins and caseins underwent lactolysation. They [20] postulated that the insolubility of MPC 85 aroused from cross-linking of proteins at the MPC surfaces. Slight decrease in rigidity of molecular domains in MPC during storage brought by the plasticizing effect of moisture was observed by FTIR and NMR analysis which could facilitate protein interaction and denaturation [21, 22]. A crust was observed on the surface of the stored powders by scanning electron microscopy [23]. This crust consisted of a thin layer of fused casein micelles. Also, the hydrophobicity at the surface of the particles increased as evident by X-ray photoelectron spectroscopy analysis and atomic force microscopy measurements [23]. Mimouni*et al.,* [14, 24] attributed the decreased solubility of MPC during storage to changed rehydration kinetics. The release of casein micelles from MPC particles was suggested to be the rate-limiting step of the rehydration process which was inhibited with storage. Also, interactions between and within casein micelles were increased during storage of MPC leading to compaction of micelles and formation of a tightly packed surface layer of casein micelles. These changes may be also responsible for the slow solubilization

of stored MPC powders [14]. The high calcium ion activity and its gradual transfer from the aqueous phase to the non-aqueous phase surrounding the casein micelles were considered to contribute to insolubility of MPC [25]. The effect of storage temperature on MPC 80 flavor was more pronounced than that of MPC 40 whereas animal and burnet sugar flavors developed in both stored samples [8].

Table 1. Composition of milk protein concentrates

Component	MPC40	MPC80	MPI	Reference
Protein % min	39.5	79.5	89.5	[12]
Fat % max	1.25	2.5	2.5	
Lactose % max	52.0	9.0	5.0	
Ash % max	10.0	8.0	8.0	
Moisture % max	5.0	6.0	6.0	
Ca mg/100 g	903-956	1423-1496	1436-1506	Modified from [13]
Mg mg/100 g	83-89	68-80	66-69	
K mg/100 g	1134-1177	217-336	199-266	
Na mg/100 g	284-303	45-100	52-64	
P mg/100 g	816-882	375-1109	1083-1118	
Cl mg/100 g	1049-1059	70-213	155-183	
Total minerals	4279-4456	2614-3339	3022-3173	

5. CHANGES IN WHEY PROTEIN PRODUCTS DURING STORAGE

The changes in the solubility of whey protein concentrate during storage were less pronounced compared to MPC. A slight decrease in the solubility of WPC (34% protein after storage at 37ºC and 75% humidity for 42 days but pronounced losses in free lactose and available lysine and formation of large aggregates were evident [26]. Hsu &Fennema [27] reported that browning was the most important change in WPC during storage. The storage temperature and time were the most important factors in the developed changes in the WPC and αw wereless important. High protein WPC (80% protein) exhibited less solubility than WPC of lower protein content (34%) and in both the solubility decreased slightly during storage at high temperature [28]. The loss in solubility was attributed to thiol-disulphide bonding and Maillard reaction.

6. POLYOLS/PROTEIN INTERACTIONS

Polyols have the capacity to protect proteins from thermal unfolding depending mainly on their molecular volume [29] but the mechanism of these effects still debatable. The stabilizing effect of a polyol originates mainly from its preferential exclusion effect from the protein surface whereas a thin hydration shell is formed on the protein surface and a cluster of many polyol molecules at a distance of 4 Å around the protein indicating no direct contact between the polyol and the protein. Vagenende*et al.* [30] gave evidence for significant electrostatic and hydrophobic interactions between glycerol and specific side chains on the surface of the protein. This formed an amphiphilic surface which prevented the protein from undergoing conformational changes and intermolecular aggregations. Also, electrodynamic force was suggested to have a role in the protein stability due to the polyol cosolvents including glycerol [31].The type of polyol and protein determine the changes observed in the intermediate moisture high protein model system. Glycerol, sorbitol and maltitol stabilized the native structure of whey proteins, provided thedesired texture, and slowed the hardening of the model systems but glycerol was the most effective in this respect [32]. Differences were observed in protein stability with the increase in glycerol concentration depending on the protein nature [33]. Thus the stability of β-LG increased with the increase of glycerol concentration but the stability of α-LA first increased and then decreased beyond 50% glycerol which may be attributed to penetration of glycerol the protein hydration layer [34].

7. MECHANISM OF HPN BARS HARDENING

Several mechanisms based on moisture migration, phase separation, protein aggregation and Maillard reaction have been proposed to explain the developed hardening of HPN but no single mechanism has agreed upon. Table 2 summarizes the different proposed mechanisms for HPN hardening. More than one of these mechanisms may be responsible for the developed hardening of HPN.

Differences in the moisture content of the HPN ingredients were responsible for the hardening of HPN bars [10]. During storage, particularly at high temperature, it was hypothized that the moisture migrates from high αw components to low αw components in order to attain equilibrium [10]. Also, simple sugars in the formulations lose their ability to hold water on crystallization or glass transition and the released water migrates to the protein phase [1]. Consequently, changes in moisture distribution result in the development of hard texture. Also, the differences in the osmotic pressure between phases were considered to be responsible for the observed changes HPN microstructure as it results in segregation of proteins from small polyhydroxy compounds [38]. On the other hand, migration of water and small molecules (such as glycerol) into the protein phase was reported to be responsible for the observed changes in the microstructure of HPN [35].

Aggregation of proteins during storage is another factor for the hardening of HPN. In a model system of HPN based on WPI, addition of cysteine and *N*-ethylmaleimide (NEM) reduced hardening [36]. Formation of thiol-disulphide bonds was suggested to dominate for the hardening at the early stages of storage while the non-covalent interactions were responsible for the hardening in later stages of storage.

Phase separation into protein-rich and carbohydrate-rich phases has been proposed as a mechanism for the hardening in HPN bars due to the preferential exclusion of sugar/polyols surrounding the protein molecules [37]. The decreased protective effect of polyols on protein structure and closer proximity of protein molecules would increase the protein-protein and protein-moisture interaction resulting in protein aggregation. Hassan & McMahon [41] proposed that the hardening of HPN bars aroused from the interactions between the co-solvents and the protein surface and not because of phase separation. The orientation of glycerol on the protein surfaces whereas its carbon backbone masks the hydrophobic regions thus avoiding a decrease in entropy of water molecules.

Zhou *et al.* [40] showed that the early stages of the Maillard reaction caused little changes in the hardness of HPN. On further storage, dramatic modifications were developed in proteins with the formation of high-molecular-weight polymers and significant hardening of HPN bars.

Table 2. Proposed mechanisms for hardening of high milk protein bars.

Mechanism	Basis	Supporting evidences	Suggested steps to control	Reference
Moisture migration	1-Non-equilibrium αw distribution between bar constituents leading to of moisture migration from high to low αw constituents	Changes in microstructure and conformation of proteins.	1-Minimizing the osmotic differences between bar components	[10]
	2- Loss of the ability of sugars to hold water either by crystallization or glass transition which results in migration of moisture to the component proteins.	Marked decrease in mobility of solid-like domains of water and low molecular weight polyhydroxy components, which may indicate glucose crystallization.	2-Selection of type and concentration of used protein concentrate.	[1] [35]
	3- Migration of small molecules into protein particles.	Reduced mobility of small molecules and changes in microstructure		
Protein aggregation	Formation of intermolecular disulphide bonds and non-covalent interactions	Reduced aggregation of WPI via thiol-disulphide was positively correlated with bar softening	Minimizing protein aggregation	[36]
Phase separation	Separation of the protein from the sugars into two distinct phases High osmotic pressure of sugar/glycerol phase.	Phase separation between proteins and carbohydrates. Low molecular mobility of proteins	Selection of polyol/sugars to be preferentially excluded from the salvation layer surrounding proteins.	[37] [38]
Maillard reaction	Glycation of proteins	Replacement of reducing sugars with non-reducing polyol minimized changes in texture	Use of non-reducing polyol in formulation	[39,40]

8. MODIFICATION OF MPCS IN ORDER TO REDUCE THE HARDNESS OF HPN BARS

Industrial production of MPCs utilizes basically ultrafiltration (UF) and diafiltration (DF) of skim milk without pH adjustments with or without further removal of water by evaporation under vacuum followed by spray drying for the obtained retentate [42]. The protein content of the obtained MPC can be manipulated by changing the number of DF stages. Solubility is considered as the most important functional properties of MPC that determine the expression of other MPC properties [17]. Reduction of solubility of the high protein MPC during storage particularly at high temperature is a major problem that limits its use in many food applications including HPN [17]. Therefore, modifications in MPC should target decreased hardening and improved cohesion being the two important quality attributes for HPN acceptability [43]. Specifically modified MPC (PowerProtein™ 4857 andPowerProtein™ 4861) produced by Fonterra™ have been reported suitable for inclusion in HPN formulation with minimal hardness development but increased crumbliness during storage [43]. Several approaches have been developed to modify the conformational structure, functional properties and storage stability of high protein MPCs (>80% protein).

8.1.Reducing calcium content of MPCs.

Partial removal of Ca was reported to improve the stability of MPCs during storage as apparent from its improved solubility. Acidification of skim milk before ultrafiltration, addition of monovalent cations or calcium chelators, and injection with CO2 have been used to reduce the Ca content of MPC. A decrease in the pH of skim milk during UF was reported to affect the integrity and supramolecular structure of casein micelles due to the partial removal of Ca [44, 45] and the internal structure of the non-dissociated micelles became more homogenous. Replacement ~ 30% of Ca with Na in MPC powder was carried by contacting an aqueous solution of MPC with a strong cation ion exchanger in the sodium form [46]. Partial removal of Ca (0-38.7%) using ion exchange treatment was accompanied byincreased dissociation of casein from casein micelles and the size of casein micelles

decreased markedly [47]. Injection of CO_2 in skim milk before and during UF significantly decreased the zeta-potential and increased casein micelle size and 28 and 34% decrease in ash and Ca contents respectively of the resultant MPC [48]. The modified MPC80 had significantly higher solubility after storage both at room temperature and at elevated temperatures. Adjusting the pH of skim milk to pH 5.9 before UF exhibited minimal effect on membrane performance and resulted in MPC of optimum emulsifying properties [49]. Addition of 150 mMNaCl or KCl during diafiltration reduced the Ca content and changed the partition of minerals and proteins between the colloidal and soluble phases of the resulting MPCs [50, 51]. The retained high solubility of NaCl or KCl treated MPCs during storage was attributed to the modification in powder hydrophobicity and sulfhydryl-disulfide interchange reactions and/or increased electrostatic repulsion between casein micelles. Addition of 20-30 mM calcium chelators (EDTA or citrate) to skim milk prior to UF resulted in MPC with enhanced solubility and heat stability [52]. The enhanced solubility was explained by the partial removal of Ca and modification of casein micelle structure. Reducing the Ca content of MPC altered its emulsifying properties [53]. Compared with emulsions formed with higher calcium MPCs emulsions formed with low calcium MPCs were finer with low total surface protein concentration and altered protein composition [53].

8.2.Modifying the manufacturing steps of MPC.

Treatments of concentrate prior to spray drying affected the solubility of the obtained MPC. High shear treatment (high pressure homogenization, microfluidization and ultrasonication) increased the nitrogen solubility of MPC powders but microfluidization was most effective in improving the long term solubility of MPC during storage [54]. Application of combined high hydrostatic pressure (HHP) and heat (200 MPa and 40ºC) for concentrate before spray drying improved the solubility of the obtained MPC [55]. This treatment increased the solubility of MPC from 66% to 85% and the powder retained the initial solubility for 6 weeks at 20ºC and 85% of the initial solubility after 12 mo of storage. The HHP assisted improvement in MPC solubility was attributed to an increase in the non-micellar casein content. Preparation of concentrate by combined UF and evaporation steps resulted in MPC80 of higher ash, total calcium, and bound calcium contents compared to concentration with only membrane filtration but with no marked influence on powder solubility [56]. Hydrodynamic cavtation(HC) , a process of vaporisation, bubble generation and bubble implosion which occurs in a flowing liquid as a result of a decrease and subsequent increase in local pressure, decreased markedly the viscosity of UF skim milk retentate and improved the performance of the spray dryer [57]. The HC treatment improved the bulk density and tapped density of the obtained MPC powder indicating smaller particle size but had no effect on the solubility of the product. The effect of HC treatment on the changes in the solubility of the stored MPC was not studied Spray drying conditions affect the morphological properties and distribution of constituents within the MPC particles and intern the solubility of the powder. The lipids and proteins were reported to be preferentially located at the particle surface whereas lactose was found in the core irrespective of the drying temperature. However, surface enrichment with lipids and proteins increased in powders spray dried at low outlet pressure [58]. At low inlet air temperature MPC particles had spherical shape while those obtained at high inlet air temperature appeared deflated and exhibited low solubility due to protein denaturation [59]. Increasing the solids in the retentate reduced the shrinkage, increase the rate oftemperature rise during drying and slowdown the rehydration of the obtained MPC [60]. Protein cross linking was carried out by treatment of concentrate with trasglutaminase (0.3 unit/g protein) before spray drying but small textural differences were found between the cross linked MPC and the control [61].

8.3.Mechanical treatments of MPC powder.

Extrusion of MPC induces the formation of disulphide bonds and may create non-reducible protein association in the texturized product and in turn modifying its functional properties [62]. The extruded MPC80 had reduced solubility, surface hydrophobicity and water holding capacity suggesting its suitability for use in HPN bars [62]. This has been proved by incorporating extruded MPC80 in HPN formulation as the obtained product was less prone to phase separation and low textural changes during storage but these changes were not related to disulphide bonds formation [63]. MPC powder (85% protein) of fine (22 μm) and coarse (49 μm) particle sizes were prepared using jet-milling of control (86 μm). Reducing the particle size improved the ability of MPC to plasticize within HPN and in turn the cohesiveness and textural stability of the HPN bar [64]. Extrusion-porosification increased the number and size of pores in powder particles and improved significantly the rehydration properties of MPC [65].

8.4.Enzymatic hydrolysis.

Limited hydrolysis of MPC 80 with chymotrypsin, trypsin, pepsin and papain improved its solubility within the pH range of 4.7-7.0, reduced its surface hydrophobicity and gel strength [66]. Also, the emulsifying properties of MPC were improved by hydrolysis with trypsin and chymotrypsin. However, these hydrolysates were not tested in the formulation of HPN bars.

9. EFFECT OF USING MODIFIED MPC ON THE HARDNESS OF HPN BARS

Few studies have been done on the effect of useing modified MPC on the textural properties of HPN bars.Using MPC as the only protein source to provide 30% protein in the formulations [61, 67].TGase cross linked, calcium reduced, toasted and texturized MPCs were tested for their effects on HPN textural properties (Table 3). These results concluded the TGase cross linking and calcium reduction and toasting did not improve the storage stability of HPN bars compared to the unmodified MPC. However, the use of extruded MPC improved the storage stability of HPN and MPC modified by extrusion can be considered as promising candidates in the formulation of HPN bars.

Table 3. Effect of using modified MPC on the hardness of HPN bars during storage.

Modification	Formulation (g/100 g)	Storage conditions	Findings	Reference
TGase crosslinking	MPC (251-271), lactose (0-28), glycerol (50.6), maltitol syrup (26.9),palm kernel stearin (18.4) water (1.0-2.2)	32ºC/42 days	Hardness and crumbliness decreased initially with the increase of TGase treatment but differences almost disappeared during storage	[61]
Reduced calcium	Same as above	Same as above	Produce softer and crumblier bars but did not improve the bar stability during storage	[61]
Extruded MPC at 65ºC and 120ºC	E65(38.11) or E120 (37.41), glycerol (21.50), maltitol syrup (12.0), high fructose syrup (10.0), palm kernel stearin (18.45) water (0.0-0.65)	22 ºC, 32ºC, 42 ºC / 42 days	Decreased hardness, fracturability and shear stresss compared to unmodified MPC. E65 gave softer bars than E120 when stored at 22 ºC, 32ºC but no difference when stored at 42 ºC	[67]
Toasted MPC at 75 ºC and 110 ºC	Same as above but with the use of T75 and T110 instead of E65 and E120	22 ºC, 32ºC, 42 ºC / 42 days	No differences in textural properties compared with unmodified MPC	[67

10. CONCLUSION

High protein nutrition bars produced with MPC are prone to develop textrural changes during storage, particularly at high storage temperatures. Modification of MPC by reducing its calcium content, cross linking using and toasting had no practical advantage over the unmodified MPC with respect to textural changes in HPN bars during storage while extrusion gave promising results. Blending MPC with WPC was reported to improve the texture of HPN bars during storage [43]. This trend needs further studies using other protein sources. Formulation and the ratio of the used ingredient have received much less attention in the studies on the use of MPC in HPN bars. These factors were reported to be of notable impact on the rheological properties of HPN bars [68]. Several additives can reduce the hardness of HPN bars during storage and needs to be investigated in HPN based on the use of MPC. Addition of a low concentration of xenthan gum was reported to soften the high protein intermediate-moisture model system containing sodium caseinate [69]. Also, the addition of SiO2 and $Ca_3(PO_4)_2$ as anticaking agents slowed down the increase in hardness and inhibited the Millard reaction in HPN based on whey protein concentrate [70].

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