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Effects of Polyols on the Stability of Whey Proteins in Intermediate-Moisture Food Model Systems

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The objective of this study was to investigate the influence of polyols on the stability of whey proteins in an intermediate-moisture food model system and to elucidate the effect of polyols on the hardening of whey protein-based bars during storage. Four major polyols, glycerol, propylene glycol, maltitol, and sorbitol, were evaluated in model systems, which contained whey protein isolate, polyols, and water. The results showed that glycerol was the most effective polyol in lowering water activity and provided the soft texture of intermediate-moisture foods, followed by sorbitol and maltitol. These three polyols stabilized the native structure of whey proteins, provided a desired texture, and slowed the hardening of the model systems. Propylene glycol should not be used in whey protein-based high-protein intermediate-moisture foods because it caused changes in protein conformation and stability as observed by differential scanning calorimeter and Fourier transform infrared spectroscopy and resulted in aggregation of whey proteins and hardening of the bar texture during storage, causing loss in product quality.

KEYWORDS: Whey protein; polyols; nutritional bars; intermediate -moisture foods

INTRODUCTION

Intermediate-moisture foods (IMF) have often been described as food products with a moderate moisture content and a reduced water activity ($a_w = 0.5-0.9$) that were created to be shelf stable without refrigeration (*1, 2*). There is no precise definition based on water content or water activity for IMF, but their moisture content is generally in the range of 10−40%, and their a_w is from 0.5 to 0.9 (*2, 3*). Nutrition bars, a rapidly growing segment of the sports nutrition, muscle building, health supplement, and weight reduction markets of the food industry, fit into the IMF category. They are generally composed of protein, various carbohydrates, and other humectants/plasticizers (glycerol, sorbitol, etc.). During manufacturing, the dry ingredients are mixed with water and humectants/plasticizers and then formed into a bar shape with no heat applied except for a possible chocolate coating.

Currently, a wide variety of protein sources are commonly used in nutritional bar manufacture including whey proteins, soy proteins, caseinates, egg proteins, and gelatins. Whey protein isolates and concentrates are typically smooth in flavor and contribute to a chewy and caramel-like texture (*4*); they are widely used in the formulation of nutritional bars. In addition, humectants/plasticizers are usually added in IMF to control the appropriate water activity and texture. They include a group of polyols such as glycerol, sorbitol, maltitol, and propylene glycol (*2*).

Studies of dilute solutions of protein showed that polyols could influence the functionality and stability of proteins in various ways. Glycerol and sorbitol stabilized the native state of globular proteins as measured by circular dichroism (CD) and differential scanning calorimetry (DSC) (*5*−*8*). Compared with water, glycerol and sorbitol are preferentially excluded from the surface of proteins, so the protein−water−polyol system requires more free energy for protein unfolding in solution in the presence of these polyols (*9*−*13*). However, propylene glycol destabilizes the native state of globular proteins by its hydrophobic interaction-rupturing capacity, thereby disorganizing the hydrophobic interior of globular proteins (*14*−*16*). The weakening of the native tertiary structure of globular proteins by propylene glycol has often been observed together with the simultaneous or subsequent promotion of helical structures as determined by CD (*17*).

One of the major concerns for loss of quality of nutritional bars during storage is that the bars may become undesirably hard during storage with no moisture loss, leading to the loss of consumer acceptability. Previous research has shown that moisture-induced protein aggregation was possibly one of the main reasons for the hardening of whey protein-based bars during long-term storage (*18, 19*). The objective of this study was to investigate the influence of polyols on the stability of whey proteins in a nutritional bar model system and to elucidate

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Table 1. Molecular Weight and Glass Transition Temperature of Water and Polyols

compound	formula	mol wt	dry glass transition temperature (°C)
water	H_2O	18	−137[a]
propylene glycol	$C_3H_8O_2$	76	−101[b]
glycerol	$C_3H_8O_3$	99	−93[c]
sorbitol	$C_6H_{14}O_6$	182	−2[d]
maltitol	$C_{12}H_{24}O_{11}$	344	44[d]

[a] Data from ref *20*. [b] Data from ref *21*. [c] Data from ref *22*. [d] Data from ref *23*.

Table 2. Formula of Protein/Buffer/Polyol IMF Model Systems

sample	formulas of model systems			ratio of polyol in polyol + buffer (%, wt basis)
	WPI (g)	buffer (g)	polyols[a] (g)	
control	6	4.5	0	0
I	6	3	1.5	33
II	6	2.25	2.25	50
III	6	1.5	3	67

[a] Polyols include glycerol, maltitol, sorbitol, and propylene glycol.

the effects of polyols on the hardening of whey protein-based high-protein nutritional bars under abusive storage conditions.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI, BioPRO) was obtained from Davisco Foods International, Inc. (Eden Prairie, MN). The WPI powder contains <0.1% lactose (dry basis) and <0.3% fat (dry basis). It was placed in desiccators containing dry anhydrous calcium sulfate (W. A. Hammond Drierite Co. Ltd., Xenia, OH) before use. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Preparation of Protein/Buffer/Polyol IMF Model System. The whey protein/buffer/polyol model system contained WPI, sodium phosphate buffer (10 mM, pH 7), and polyols, with sodium azide (0.05%, wet weight basis) being added to control microbial growth. The basic physical properties of the polyols used in this study are shown in **Table 1**. The protein/buffer/polyol IMF model systems were prepared on the basis of the formula in **Table 2**. The polyol was first mixed with buffer to get a solution, then the protein powder was added, and the matrix was then mixed by hand with a spatula until a uniform dough texture was achieved. The premixed model system was placed in a plastic water activity sample cup (Decagon Device, Inc., Pullman, WA) that was then tightly covered with the lid and further double sealed with Parafilm laboratory film (Pechiney Plastic Packaging, Chicago, IL) completely around the cup/lid junction to avoid moisture loss. The sample cups were then placed into a sealed glass jar and equilibrated at room temperature for 2 h before being placed into an incubator at 45 °C. The water activity of samples was determined using an AquaLab 3TE Water Activity Meter (Decagon Devices, Inc., Pullman, WA).

Determination of Insoluble Proteins. The formation of insoluble protein aggregates was determined by the solubility of the sample in phosphate buffer (10 mM, pH 7) as described before (*18*). A 60 mg sample was added into 10 mL of phosphate buffer. The suspension was stirred at room temperature at a speed of 400 rpm with a magnetic stirrer (IKA Works, Inc., Wilmington, NC) for 60 min and then centrifuged at 20000*g* for 15 min. The concentration of soluble proteins in the supernatant was then determined using the bicinchoninic acid (BCA) Protein Assay Kit (Pierce Chemical Co., Rockford, IL). Any decrease in the amount of soluble fraction would suggest the formation of insoluble proteins.

The molecular mechanisms of protein aggregation were determined by procedures as described in the previous study (*18*). A 180 mg sample (after storage for 7 days at 45 °C) was dissolved in 10 mL of buffer, followed by stirring and centrifugation. The insoluble aggregates were dissolved in various solutions: phosphate buffer solution alone (control), buffer solution with strong denaturants, that is, 0.1% SDS, 6 M guanidine−HCl, or 8 M urea. The latter determines the non-covalent interactions including hydrophobic interaction and hydrogen bonding. In addition, a buffer solution with an added reducing reagent [10 mM dithiothrietol (DTT)] was used to determine intermolecular disulfide bond formation. Lastly, a buffer solution with both of the strong denaturants plus the reducing reagent (0.1% SDS, 8 M urea, and 10 mM DTT) was also used in the present study. The suspension was stirred at a speed of 400 rpm for 60 min and then centrifuged at 20000*g* for 15 min. The protein concentration dissolved in the different solutions was determined using the BCA Protein Assay Kit, and the BCA assay in the presence of DTT was conducted according to the modified method of Hill and Straka (*24*) to minimize the influence of the reducing reagent.

Texture Measurement. Changes in texture were measured using a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) with a cylindrical plunger (1/8 in. diameter) for 50% of deformation. After storage at 45 °C, the samples containing different polyols were first equilibrated in an incubator at 23 °C for 2 h before the measurements. The samples were stable and did not exhibit any obvious change during the examination period between sampling and subsequent incubation and texture measurements at 23 °C. The crosshead speed for the deformation was 1 mm/s, and the trigger force was 0.15 N. The maximum force (N) during deformation was recorded as the hardness. The relative changes in texture after storage were calculated as

$$\text{relative change (\%)} = [(\text{hardness}_{7\text{days}} - \text{hardness}_{0\text{days}})/\text{hardness}_{0\text{days}}] \times 100 \quad (1)$$

where $\text{hardness}_{0\text{days}}$ represents the initial hardness of freshly prepared systems and $\text{hardness}_{7\text{days}}$ represents the hardness of systems after storage at 45 °C for 7 days.

Differential Scanning Calorimetry (DSC). The measurements were done on duplicate samples using a Perkin-Elmer model DSC 7 (Perkin-Elmer Life and Analytical Sciences, Inc., Norwalk, CT) that was calibrated using distilled water and indium for temperature and indium for energy. Samples were heated from 25 to 105 °C at a heating rate of 5 °C/min. The denaturation temperature of proteins was recorded as the peak temperature of the endothermic peak, and the denaturation enthalpy (J/g of sample) was measured using Pyris software (Perkin-Elmer Life and Analytical Sciences, Inc.). Given the total protein content in the samples, the endothermic enthalpy of denaturation peaks was further calculated to give a value as joules per gram of protein:

$$\Delta H(\text{J/g of protein}) = \Delta H(\text{J/g of sample})/\text{protein content(g of protein/g of sample)} \quad (2)$$

All sample pans were weighed before and after experiments to confirm that there was no loss of water.

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were collected from 1600 to 1700 cm^{-1} at a resolution of 2 cm^{-1} using a Nicolet 560 FTIR spectrometer (Nicolet Instrument Corp., Madison, WI), with a ZnSe ATR crystal accessory (Thermo Spectra-Tech, Madison, WI). Samples were equilibrated at 23 °C for 2 h before the measurements. The samples were stable and did not exhibit any obvious change during the examination period between sampling and subsequent incubation and FTIR measurements at 23 °C. A background spectrum of the ZnSe crystal was recorded before the sample measurement, and a total of 400 scans were accumulated to define each spectrum. The collected spectra were smoothed using a 9-point Savitsky−Golay method, and deconvolution of the spectra was then performed using Nicolet FTIR OMNIC software (OMNIC E.S.P., Nicolet Instrument Corp.). The bandwidth used for deconvolution was 18 cm^{-1}, with a narrowing factor of 3. All FTIR experiments were done in triplicate.

Statistical Analysis. The analysis of variance (ANOVA) using the General Linear Model procedure and the difference between means using the Duncan test were determined using SAS (SAS Institute Inc., Cary, NC), and all statistics were applied at an α level of 0.05.

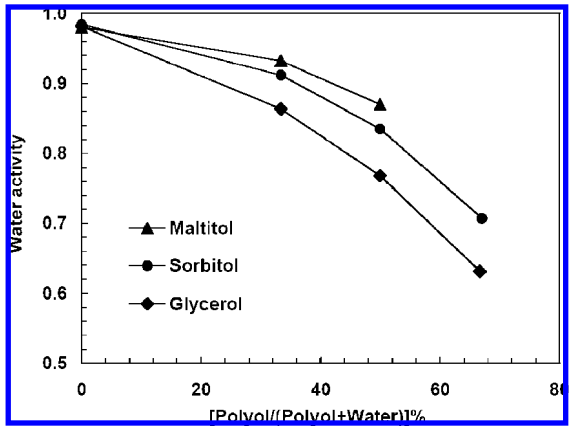

Figure 1. Water activity of various protein/buffer/polyol IMF model systems.

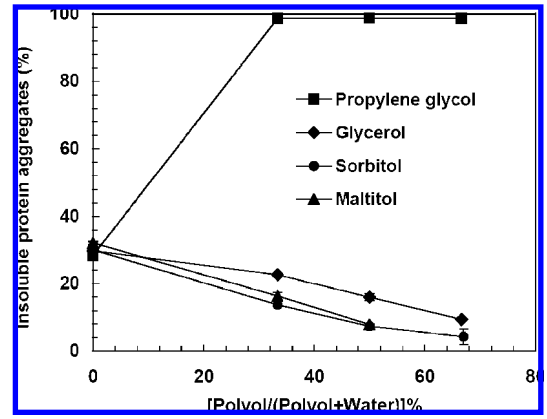

Figure 2. Effect of the type and relative content of polyols on the formation of insoluble whey protein aggregates after the storage of the model systems at 45 °C for 7 days.

RESULTS AND DISCUSSION

Decreases in the Water Activity of Model Systems by Replacing Water with Polyols. One of the characteristic aspects in making IMF such as nutritional bars is to reduce the water activity of products to a certain level that could prevent the growth of microorganisms and provide a relatively shelf-stable food in a convenient form. Microbes will not grow below an a_w of 0.6−0.65 in IMF foods (*25*). This reduction of water activity, in many cases, cannot be successfully achieved by simply drying the foods, because the resulting texture would be too dry and too hard for direct consumption (*2*). Thus, humectants/plasticizers such as polyols are often used in IMF. **Figure 1** shows the change in water activity in the model systems with different replacement ratios of polyols. It should be noted that the water activity of systems containing propylene glycol cannot be measured accurately with the AquaLab water activity meter, because propylene glycol co-condenses with water on the surface of the chilled mirror during the measurement, thereby causing an error in the water activity value. The results suggested that glycerol is the most effective polyol to lower water activity, followed by sorbitol, whereas maltitol is the least effective one, as expected from Raoult's law. Raoult's law predicts water activity based on the ratio of the moles of water divided by the sum of moles of water and moles of solute. Thus, the molecular weight of a solute at a given w/w fraction with water dominates the ability to lower water activity (*26*). If the weight fraction of solute to water in the different systems is the same (% by weight), the lower the molecular weight of a solute, the lower the water activity of the system. Because propylene glycol has a lower molecular weight than the others, it would be expected to be on a curve lower than glycerol, that is, greater water activity lowering ability. As seen in **Figure 1**, to get the a_w below 0.65 as would be needed if no antimicrobials are used to prevent mold growth, it would require that the polyol level needs to be ≥65% of the water−polyol mixture, which might affect the sensory properties negatively, so many manufacturers lower the polyol content and add in an antimicrobial agent.

Formation of Insoluble Protein Aggregates in Model Systems. The formation of moisture-induced protein aggregation in concentrated protein systems has been documented in previous studies (*18, 19, 27−29*). Water, a compound with a low molecular weight and a low glass transition temperature, increases the molecular mobility of protein macromolecules by working as a plasticizer and affects the occurrence and progress of protein aggregation. The replacement of water with polyols could influence the moisture-induced protein aggregation at least in two ways. First, the addition of polyols may stabilize or destabilize the native state of globular proteins, depending on their type and concentration (*5−8, 14−17*). Second, compared with water, polyols have a higher molecular weight and a higher glass transition temperature (**Table 1**), so they are less effective in acting as plasticizers. As a result, the replacement of water with polyols would decrease the molecular mobility of whey protein in the model systems.

As seen in **Figure 2**, model systems with glycerol, sorbitol, and maltitol showed a decreased formation of insoluble protein aggregates during storage as compared to water alone, with the latter two polyols having the least amount of aggregation. However, propylene glycol (PG) in the model systems caused a significant increase in protein aggregation to almost 100% at the three levels tested, which may be similar to its interaction with hemoglobin when fed to cats, causing a syndrome known as Heinz body formation (*30, 31*). This causes the cats to have reduced respiratory functionality, leading to anemia. Therefore, the FDA declared PG as unsafe for use in intermediate-moisture cat food (*32*). It should be noted, however, it was not banned from use in human foods. To illustrate the variation in the action of the different polyols on whey protein aggregation, the effect of each polyol on the stability of whey protein structure was further investigated in the IMF model systems.

Effect of Polyols on Stability of Whey Protein Structure. **Figure 3** shows changes in the thermal stability of whey proteins as water was replaced with polyols. This can provide information on the effect of polyols on the native structure of these globular proteins. There are two thermal transition peaks due to the denaturation of whey proteins. Peak 1, at the lower temperature, has been identified as the thermal transition of bovine serum albumin (BSA) and α-lactalbumin, whereas peak 2, at the higher temperature, has been identified as the thermal transition of β-lactoglobulin. The transition temperature for each denaturation peak and the total transition enthalpy are shown in **Table 3**. For the freshly prepared model systems, the replacement of water with glycerol, sorbitol, or maltitol increased the denaturation temperature of whey proteins (**Figure 3A**; **Table 3**), especially in the systems containing sorbitol or maltitol. All thee of these polyols slightly decreased the denaturation enthalpy, but no specific pattern for the decreases was observed (**Table 3**), which was consistent with the results found in dilute protein solutions (*5*). After storage at 45 °C for 7 days, peak 1 of the control system (containing only whey protein and buffer at 45% moisture content) almost disappeared (WB in **Figure 3B**), and the total thermal transition enthalpy also decreased significantly

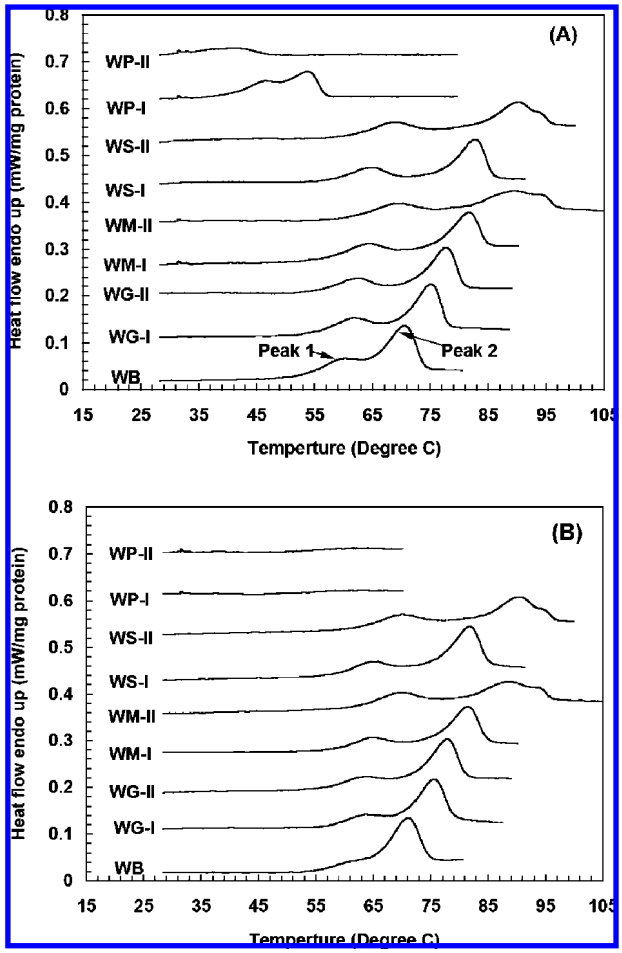

Figure 3. DSC scans of protein denaturation on protein/buffer/polyol IMF model systems: (**A**) freshly prepared protein/buffer/polyol systems; (**B**) systems stored at 45 °C for 7 days. WB refers to the control system containing only 6 g of whey protein and 4.5 g of buffer, WG represents the protein/buffer/glycerol system, WM represents the protein/buffer/maltitol system, WS represents the protein/buffer/sorbitol system, and WP represents the protein/buffer/propylene glycol system. The symbol "I" represents systems containing 6 g of whey protein, 3 g of buffer, and 1.5 g of poyol; "II" represents systems containing 6 g of whey protein, 2.25 g of buffer, and 2.25 g of polyol.

(**Table 3**), which suggests a significant change of protein native structure in the control system during storage. In model systems containing sorbitol or maltitol, the thermal transition enthalpy did not change significantly during storage ($P > 0.05$), indicating the capability of these two polyols to stabilize the native structure of the whey proteins. Compared with sorbitol and maltitol, glycerol was less effective in preventing changes in protein structures, showing a decrease in thermal transition enthalpy after 7 days of storage (WG in **Table 3**).

The stabilizing effect of polyols on protein structures has been extensively investigated in many previous studies in dilute protein solutions as noted earlier (*5*−*11*, *14*−*17*). In aqueous medium, glycerol and sorbitol were preferentially excluded from the surface of proteins. Because this exclusion was nonspecific and thermodynamically unfavorable, proteins would tend to minimize this unfavorable situation by reducing the entire protein solvent interface. Any changes in the native structure of globular proteins, such as denaturation or unfolding, would possibly expose more protein surface and increase the protein solvent interface, which was thermodynamically unfavorable for solutions containing glycerol or sorbitol. Therefore, in the presence of glycerol or sorbitol, it would require more free energy for protein unfolding/denaturation, and the native structure of globular proteins would be more thermodynamically favorable and stabilized (*6*−*11*). This mechanism should also work for maltitol.

Table 3. Changes of Whey Protein Conformation in Protein/Buffer/Polyol IMF Model Systems by DSC Measurements ($n = 2$)

	0 days			7 days at 45 °C		
sample[a]	temp of peak 1[b] (°C)	temp of peak 2[b] (°C)	ΔH[b,c] (J/g of protein)	temp of peak 1 (°C)	temp of peak 2 (°C)	ΔH (J/g of protein)
WB	59.9E	70.2G	10.4A		70.9F	7.9G
WG-I	61.8D	75.0F	9.7C	63.1C	75.3E	8.6D
WG-II	62.6C	77.7E	9.0D	63.2C	77.7D	8.1F
WM-I	64.5B	81.8D	8.3E	65.0B	81.8C	8.3E
WM-II	69.3A	89.5B	10.0B	69.8A	88.5B	9.8A
WS-I	64.7B	82.7C	9.3D	64.9B	81.9C	9.0C
WS-II	69.0A	90.8A	9.0D	70.0A	90.5A	9.3B
WP-I	46.3F	53.7H	6.3F			
WP-II		41.3I	1.9G			

[a] WB refers to the control system containing only 6 g of whey protein and 4.5 g of buffer, WG represents the protein/buffer/glycerol system, WM represents the protein/buffer/maltitol system, WS represents the protein/buffer/sorbitol system, and WP represents the protein/buffer/propylene glycol system. The symbol "I" represents systems containing 6 g of whey protein, 3 g of buffer, and 1.5 g of polyol; "II" represents systems containing 6 g of whey protein, 2.25 g of buffer, and 2.25 g of polyol. [b] Means in each column followed by different letters are significantly different at $p < 0.05$. [c] ΔH includes peaks 1 and 2.

In dilute protein solutions, protein molecules are soluble in water and fully hydrated. In a study using aqueous solutions containing 10% β-lactoglobulin, when it was fully hydrated, it was shown that when the concentration of polyol was added at 50% of the solution, the thermal transition temperature of β-lactoglobulin increased by 2 and 12 °C for glycerol and sorbitol, respectively, as determined by DSC (*8*). In the semimoist concentrated systems such as the model systems in the present research, the limitation in the availability of water molecules indicated that whey protein molecules were only partly hydrated. Moreover, our previous study on low- and intermediate-moisture whey proteins systems suggested that lowering moisture content itself could also contribute to the increase in the thermal transition temperature because water works as plasticizer in such a macromolecular system. Similarly, the partial replacement of water with polyol in the protein/buffer/polyol IMF system also decreases the solution plasticizing capability due to the increases in molecular weight (molecular volume) of plasticizers (*33*). Thus, the molecular mobility of proteins decreased with the increasing ratio of polyols in the systems, which also contributes to the increase in thermal transition temperature in these concentrated systems. **Table 3** shows that in the present study, a 50% replacement of water with polyol increased the thermal transition temperature of β-lactoglobulin (peak 2), and increases of 7.5, 20.6, and 19.3 °C in the thermal transition temperature were observed for glycerol (WG-II), sorbitol (WS-II), and maltitol (WM-II), respectively.

On the other hand, the presence of propylene glycol in IMF model systems made whey proteins unstable and led to the loss of their native structure as shown in **Figure 3**. A 33% replacement of water with propylene glycol (WP-I) significantly decreased ($P < 0.05$) the thermal transition temperature of BSA/α-lactalbumin and β-lactoglobulin, whereas a 50% replacement ratio caused a loss of most of the tertiary structures of the proteins. Moreover, after 7 days of storage at 45 °C, none of

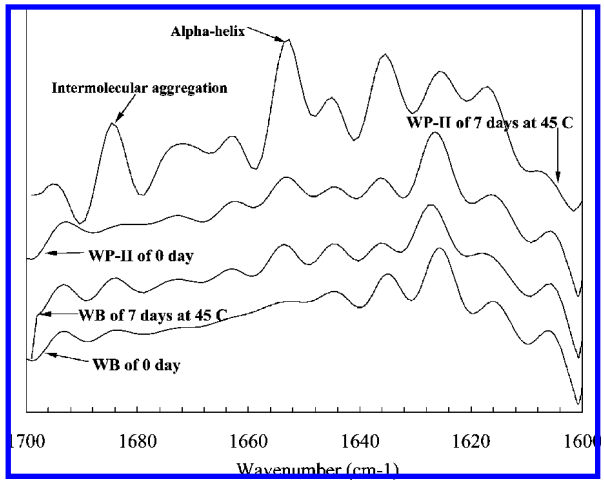

Figure 4. Deconvoluted spectra of IMF model systems by FTIR. WB refers to the control system containing only 6 g of whey protein and 4.5 g of buffer; WP-II is the model system containing 6 g of whey protein, 2.25 g of buffer, and 2.25 g of propylene glycol. "0 day" represents freshly prepared sample, whereas "7 days at 45 C" represents sample after storage at 45 °C for 7 days.

Table 4. Solubility of Protein Aggregates[a] in Various Solutions with Denaturing or Reducing Chemicals

	solubility of protein aggregates of WB[b] (%)	solubility of protein aggregates of WP-II[b] (%)
buffer (10 mM, pH 7)	2.5 ±0.4	1.7 ±0.8
buffer with 0.1% SDS	6.8 ±0.7	1.7 ±0.2
buffer with 6 M guanidine−HCl	8.7 ±0.9	6.2 ±1.1
buffer with 8 M urea	11.3 ±0.5	10.3 ±0.3
buffer with 10 mM DTT	83.1 ±3.3	29.1 ±1.7
buffer with 0.1% SDS, 8 M urea and 10 mM DTT	99.1 ±0.4	97.9 ±4.0

[a] The insoluble protein aggregates refer to those formed after storage at 45 °C for 7 days. [b] WB is the control system containing only 6 g of whey protein and 4.5 g of buffer, WP-II is the model system containing 6 g of whey protein, 2.25 g of buffer, and 2.25 g of propylene glycol.

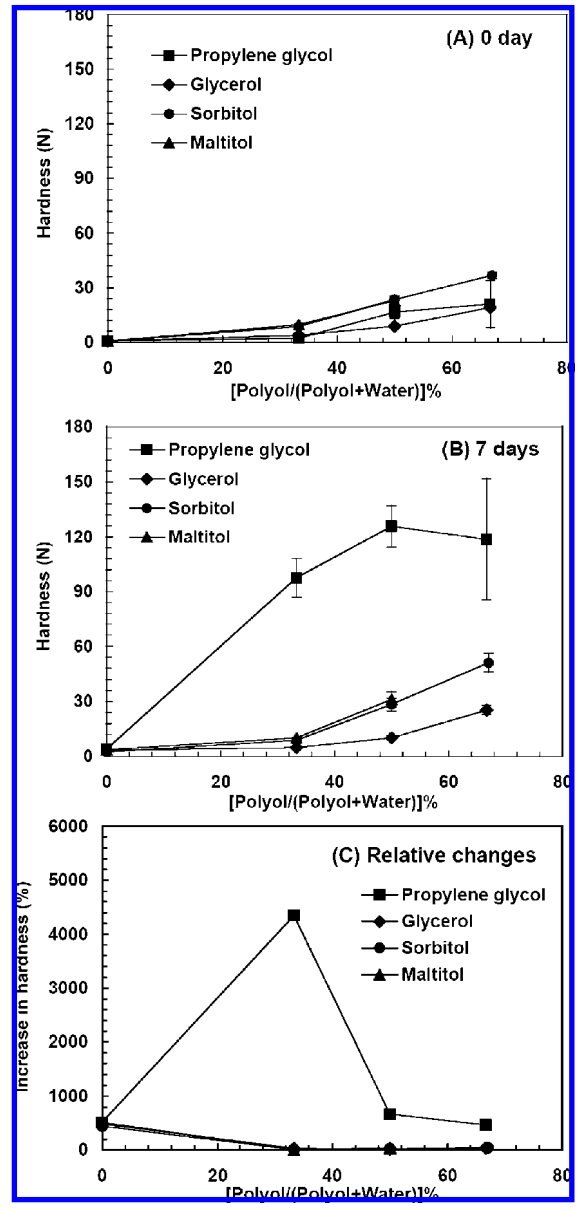

Figure 5. Effect of the type and relative content of polyols on the texture of IMF model systems during storage at 45 °C: (**A**) freshly prepared protein/buffer/polyols systems; (**B**) systems that were stored at 45 °C for 7 days; (**C**) relative changes in the texture after storage at 45 °C for 7 days, which is equal to the relative hardness change; (■) propylene glycol; (◆) glycerol; (●) sorbitol; (▲) maltitol.

the thermal transition peaks were observed. Previous studies suggested that propylene glycol could induce two processes in the structure of globular proteins: the disruption of the native tertiary structure and the promotion of α-helical structure (*14*−*17*). Hydrophobic interactions between propylene glycol and hydrophobic side chains in proteins were involved in the disruption of tertiary structure, whereas the induction of helical structures was mainly through the enhancement of local hydrogen bonds within the peptide chain (*17*).

FTIR was applied to further investigate the changes in the α-helical structure of whey proteins after the addition of propylene glycol. The FTIR spectra in the 1700−1600 cm^{-1} region for the model systems with and without propylene glycol during storage at 45 °C are shown in **Figure 4**. The band at 1650 cm^{-1} is attributed to the α-helix (*34*, *35*), and its intensity increased slightly right after the addition of propylene glycol (WP-II of 0 days, freshly prepared model system with a 50% replacement of water with propylene glycol). After storage at 45 °C for 7 days, the peak at 1650 cm^{-1} increased sharply, suggesting a significant induction of α-helical structure. Moreover, a peak at 1681 cm^{-1} was observed for model systems after 7 days of storage at 45 °C, which corresponds to the formation of intermolecular aggregates between whey proteins (*36*). The size of this peak at 1681 cm^{-1} was larger in the system with propylene glycol than the one without, suggesting that the presence of propylene glycol induced the formation of more protein aggregates.

Moisture-induced protein aggregation has been suggested to occur through at least two possible mechanisms: (1) formation of intermolecular covalent bonds and/or (2) formation of noncovalent interactions (*29*). In our previous study on the protein/buffer system (*18*), we reported that the formation of intermolecular disulfide bonds played the most important role in whey protein aggregation, and most of the aggregates formed during storage were soluble in a solution containing 10 mM DTT as the reducing reagent. However, in the presence of propylene glycol, <30% of the aggregates could be dissolved in a solution with 10 mM DTT (**Table 4**), but nearly all of the aggregates were dissolved in the solution containing 0.1% SDS and 8 M

urea along with 10 mM DTT. Therefore, the mechanisms for aggregates formed in systems containing propylene glycol seem to be more complex, and both covalent bonding and noncovalent interaction (especially intermolecular hydrogen bonding) likely play important roles in promoting the formation of aggregates.

Effect of Polyols on the Texture of Model Systems. For the freshly prepared model systems, the texture of IMF systems was initially harder with an increase in the percentage of polyol (**Figure 5A**). At the same replacement ratio, systems with sorbitol or maltitol are harder than those with glycerol or propylene glycol, most likely due to the difference in the glass transition temperature of the specific polyol. That is to say, at the same replacement ratio, the lower the glass transition temperature of the specific polyol, the more effectively it will work as a plasticizer to soften the texture of the macromolecular systems. After storage at 45 °C for 7 days, the texture of all model systems became harder (**Figure 5B**), particularly for the systems containing propylene glycol. To evaluate the storage stability of the nutritional bar model system, especially for changes in texture, it would be more important to compare the changes with the initial hardness of each individual system to get a relative value (**Figure 5C**). For the control system with only protein and buffer, the texture after 7 days of storage at 45 °C became 4 times harder than the initial one, mostly due to the formation of protein aggregates. Because the replacement of water with glycerol, sorbitol, or maltitol slowed protein aggregation, it also slowed the changes in texture (the relative changes were lower than 50% for all the model systems containing these three polyols). On the contrary, for model systems containing propylene glycol, the texture after storage became from 5 to 40 times as hard as the initial state (**Figure 5C**), given the fact that almost all of the proteins in the systems were involved in forming aggregates (**Figure 2**).

In conclusion, among the polyols that were studied in the present research, glycerol is the most effective one in lowering water activity and providing the softest texture in model systems, followed by sorbitol and maltitol. A good combination of these polyols will help to stabilize whey proteins and maintain a desired soft texture of IMF through decreased protein aggregation. Propylene glycol should be avoided in whey protein-based high-protein IMF, because it causes adverse changes in protein stability, resulting in the loss of product quality during storage.

ACKNOWLEDGMENT

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