Effects of Sugars on Whey Protein Isolate Gelation

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Whey protein isolate (WPI) gels were prepared from solutions containing ribose or lactose at pH values ranging from 6 to 9. The gels with added lactose had no color development, whereas the gels with added ribose were orange/brown. Lactose stabilized the WPI to denaturation, which increased the time and temperature required for gelation, thus decreasing the fracture modulus of the gel compared to the gels with added ribose and the gels with no sugar added. Ribose, however, favored the Maillard reaction and covalent cross-linking of proteins, which increased gel fracture modulus. The decreased pH caused by the Maillard reaction in the gels containing ribose occurred after protein denaturation and gelation, thus having little if any effect on the gelation process.

Keywords: *Whey protein isolate (WPI); protein gelation; sugars; Maillard reaction; protein stabilization*

INTRODUCTION

Whey protein ingredients are used in a variety of food applications including dairy products, baked goods, confectionery products, and convenience foods (Giese, 1994). Heat-induced gelation is one of the more common mechanisms utilizing whey proteins to create or modify texture (Ziegler and Foegeding, 1990; Errington and Foegeding, 1998; Foegeding et al., 1998). Whey protein ingredients have the distinct advantage to allow processors to use an "all-dairy" food ingredient label, as well as the potential to be a cost-effective alternative to other gelation products, including egg whites, soy protein, and gelatin in foods such as surimi, meat products, and dairy-based foods (Broihier, 1999).

There are two main processes involved in globular protein gelation. The first is a change in the protein system, which increases intermolecular interactions. This step can be considered the initiation phase of the overall reaction. The second step is aggregation of protein molecules into a gel network (Foegeding et al., 1998). Clark and Ross-Murphy (1985) point out that heat-induced gelation requires a critical minimum concentration of molecules to form a gel network, a critical minimum time for the reaction to occur, and a minimal temperature required to cause structural changes that ultimately produce a gel. Ingredients added to the native protein solution can change the critical requirements for gelation.

Sugars can have two different effects on protein gelation. First, sugars can act to stabilize proteins to heat denaturation (Ball et al., 1943; Back et al., 1979; Arakawa and Timasheff, 1982; Garrett et al., 1988; Jou and Harper, 1996). This mechanism would affect gel strength by increasing the onset temperature of heat denaturation and altering bond formation during gelation. The second effect of sugars on proteins is the Maillard reaction. Reducing sugars, when heated, can react with available amines on the protein and form covalent cross-links within the protein network (Hill et al., 1992). These covalent cross-links then change the rheological properties of the protein gel. The Maillard reaction can also cause a pH reduction due to the production of acidic side products (Hill et al., 1992). Decreased pH can cause changes in the gelation process, thus causing changes in the rheological properties of gels.

The Maillard reaction is a carbonyl−amine reaction between reducing sugars and amino acids and/or proteins (Yaylayan and Huyghues-Despointes, 1994). The initial phase of the reaction proceeds by the reaction between amines with reducing sugars to form an imine (Schiff base), which partially isomerizes to a somewhat more stable amino ketone (Amadori compound). Further reactions of the Schiff base and Amadori compound are strongly affected by pH. Under alkaline conditions, Schiff bases and Amadori compounds mainly undergo chain fragmentation to form two- and three-carbon fragments, which then react further to form melanoidins (Rizzi, 1994). This stage of the reaction is well documented, but the subsequent reactions of the Amadori rearrangement product are still mainly unknown (Yaylayan and Huyghues-Despointes, 1994).

For the food scientist, the most significant and obvious effects of the Maillard reaction are the development of color, flavor, and aroma (Ames, 1990). The type of sugar used in the system affects the rate of the Maillard reaction. Pentose sugars (ribose) react more readily than hexoses (glucose), which, in turn, are more reactive than disaccharides (lactose). The reaction rate with these sugars depends on the amount of open-chain aldehyde or ketone present as a reactant (Ames, 1990). Lysine tends to be the amino acid that results in the most color development with the Maillard reaction. Therefore, foods containing proteins that are rich in lysine residues (milk proteins) are likely to brown readily.

Researchers have concentrated on the effects of the Maillard reaction in protein solution or during protein gelation (Hill et al., 1992; Armstrong et al., 1994;

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Cabodevila et al., 1994; Mat Easa et al., 1996). Hill et al. (1992) concluded that there is a correlation between the extent of the Maillard reaction (evidenced by color) and the strength of bovine serum albumin gels. The effectiveness of the reducing sugars on promoting gelation followed the reactivity of reducing sugars (lactose < fructose < mannose < xylose). Armstrong et al. (1994) found that there was a decrease in pH for gels in which the Maillard reaction occurs. They also observed that gels in which the Maillard reaction had occurred were less soluble than gels without the Maillard reaction, suggesting the Maillard reaction caused additional nondisulfide covalent cross-links in the gels.

The purpose of the following research was to investigate the effects of lactose and ribose on the large- and small-strain rheological properties of whey protein isolate (WPI) gels. It is still unknown if the pH decrease caused by the Maillard reaction or the addition of covalent cross-links to the gel by the Maillard reaction is causing the changes in the rheological properties of the gels. By understanding the mechanisms of whey protein gelation under high-sugar conditions, greater utilization of whey protein ingredients in foods with elevated sugar levels can be obtained.

MATERIALS AND METHODS

Materials. The protein contents of two lots of WPI powders (Davisco Foods International, LeSueur, MN) were 92.7 and 91.0% (w/w), determined by combustion method with a conversion factor of 6.38 × N = % protein (AOAC, 1995). Differing levels of ribose (Aldrich Chemical Co., Inc., Milwaukee, WI) and lactose (Sigma Chemical Co., St. Louis, MO) were added to the WPI solutions. All other chemicals were of reagent grade.

Gel Formation. Stock solutions of 0.5 M lactose and 0.5 M ribose were prepared. In experiment I, WPI suspensions of 13.5, 14.0, 14.5, and 15.0% (w/v) protein were prepared by hydrating WPI in lactose or ribose solutions (0, 100, 300, and 500 mM) under constant stirring for 2 h. The pH of each solution was adjusted to 6.0, 7.0, 8.0, or 9.0 using 1 N HCl or 1 N NaOH. The solutions were then brought to volume with the appropriate sugar solution, degassed for 1 h, and poured into glass tubes (19 mm internal diameter) that had been coated with Sigmacote (Sigma Chemical Co.) or Pam (American Home Foods, Madison, NJ). Gels were formed in a water bath at 80 °C for 30 min.

In experiment II, gels were made as stated above except the protein concentration was fixed at 14.0% (w/v) and the pH was fixed at 9.0. Lactose and ribose levels were varied between 0 and 500 mM at increments of 50 mM.

Large-Strain (Fracture) Rheology. Fracture stress (strength) and fracture strain (deformability) of the gels were determined by twisting gel samples until fracture (Diehl et al., 1979). Gels were cut into cylinders (29 mm in height), and each end was glued to plastic disks using cyanoacrylate glue. The cylinders were ground into capstan shapes with a center diameter of 10 mm using a precision milling machine (model GC-TG92 US, Gel Consultants, Raleigh, NC). Gels were vertically mounted and twisted to fracture at 2.5 rpm on a Hamann Torsion Gelometer (Gel Consultants). At least five gels were tested for each replication of each treatment. Fracture stress and fracture strain were calculated from the torque and angular displacements according to the method of Diehl et al. (1979). A fracture modulus (G_f) was calculated by dividing fracture stress by fracture strain.

Small-Strain Rheology. Rheological transitions during gelation and rheological properties of the gels were obtained using a controlled stress rheometer (Stresstech Rheometer, ATS Rheosystems, Bordontown, NJ/Reologica Instruments AB, Lund Sweden) equipped with a cup (diameter = 27.5 mm) and a bob (diameter = 25 mm) testing fixture. Solutions (14% protein w/v, 0 or 500 mM lactose or ribose, pH 9) were prepared as stated above. The solution covered the bob, and mineral oil was layered over the solution to minimize drying. Stress sweeps of 100.0−2000.0 Pa were conducted in oscillation at 0.1 Hz to establish the linear viscoelastic region (LVR) of the gels at 80 °C. On the basis of those results, a stress of 200 Pa was used unless otherwise specified.

A temperature ramp from 25 to 80 °C at 1 °C min^{-1}, followed by holding at 80 °C for 30 min, was used to gel the protein solutions. Oscillation measurements were taken during heating and holding at a frequency of 0.1 Hz.

Mechanical spectra were determined over a range of 0.0015−10.0 Hz on gels at 80 °C. Creep testing was conducted on gels at 80 °C at a stress of 200 Pa. Creep testing was run for 30 min, followed by a 30 min recovery. Each treatment was replicated twice, and the range of values is shown in the graphs and tables.

Water-Holding. Held-water (HW; grams of water per gram of protein) of the gels was determined using the microcentrifuge-based method developed by Kocher and Foegeding (1993). Cylinders were cut with a sharpened metal cylindrical tube. Samples were 1 cm in height and 0.48 cm in diameter. The samples were placed into microcentrifuge filtration units (Lida Corp., Kenosha, WI) and spun in a Beckman Microfuge 11 horizontal rotor microcentrifuge (Beckman Instruments, Inc., Palo Alto, CA) at 38.3*g* for 10 min. HW was then calculated from the following equation:

$$\mathrm{HW} = \frac{\text{total g of water in sample} - \text{g of water released}}{\text{total g of protein in sample}}$$

Assays of Available Amine. Available amine was determined for heated and unheated WPI (2 mg/mL) and WPI−sugar (2 mg/mL and 100 or 500 mM ribose or lactose) solutions at pH 9.0. WPI solutions were heated in a water bath at 80 °C for 30 min and then removed and cooled to room temperature (23 ± 2 °C).

The amount of available amine was measured on the basis of the reaction of primary amines with *o*-phthaldialdehyde (OPA) (Fisher Scientific, Fair Lawn, NJ). The spectrophotometric assay of Church et al. (1983), as applied to carbohydrate-rich products by Vigo et al. (1992), was used. The procedure was modified such that 50 μL of test solution and 1 mL of OPA reagent were added directly into a cuvette and allowed to react for 2 min, and absorbance at 340 nm was then measured (UV160U UV−visible recording spectrophotometer, Shimadzu Corp., Columbia, MD). Percent available amine was calculated by dividing the sample absorbance values by the absorbance values of the unheated no-sugar-added solution and multiplying by 100. Each treatment was replicated three times.

Differential Scanning Calorimetry (DSC). Suspensions of 14.0% protein (w/v) were hydrated in solutions of 0, 300, or 500 mM ribose or lactose. The pH of the suspensions was adjusted to 9.0 using 1 N NaOH. Each suspension was tested using a Perkin-Elmer DSC 7 equipped with an intracooler II refrigeration unit and drybox (Perkin-Elmer Corp., Norwalk, CT). Nitrogen gas at a flow rate of 40 mL/min was used to flush the sample holder, and nitrogen at 25 psig was used to flush the drybox. The differential scanning calorimeter was calibrated using indium (temperature and enthalpy) and dodecane (temperature). Samples (50 μL) in stainless steel pans were heated from 20 to 100 °C at a rate of 5 °C min^{-1} using an empty pan as a reference. The onset temperature (T_o) and temperature at peak maximum (T_{max}) of denaturation were recorded for each protein suspension. Each solution was replicated three times.

Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis (SDS—PAGE). Gels of 14% protein (w/v), 500 mM ribose or 500 mM lactose, at pH 9 were prepared as described above. Gels were solubilized using a modified version of the procedure used by Kamath et al. (1992). Gels were cut into small pieces, and 0.8 g of these pieces was suspended in 15.0 mL of 2% SDS, 8 M urea, 2% mercaptoethanol, and 20 mM Tris-HCl (pH 8.0) buffer. The suspensions were then heated at 100 °C for 2 min, brought to room temperature,

covered, and stirred continuously for 24 h at room temperature. The suspension was centrifuged at 10000*g* for 20 min. Protein concentration of the supernatant was measured with a Coomassie blue dye binding assay (Bio-Rad protein assay instruction manual, Bulletin 88-0843, Bio-Rad Chemical Division, Richmond, CA).

SDS−PAGE was conducted on the protein from the solubilized gels, unheated WPI in deionized water, and unheated WPI in the SDS, urea, mercaptoethanol, and Tris-HCl buffer solution. The stacking gels were 2% (w/v) acrylamide (Bio-Rad Chemical Division) and the separating gels were 10% (w/v) acrylamide. The molecular weight standard contained proteins of molecular weights of 205000, 116000, 97400, 66000, 45000, and 29000 (Sigma Chemical Co., St. Louis, MO). There was 10 μg of protein per lane analyzed for all samples. Gels were fixed and stained overnight with Novex Colloidal Blue staining kit (Novel Experimental Technology, San Diego, CA), which contains a Colloidal Coomassie G-250 stain. The stained gels were destained overnight with deionized water. Gels were then dried using The Dry Ease gel drying system (Novex-Novel Experimental Technology).

pH Changes during Gelation. Solutions for gelation (no sugar, 500 mM lactose, or 500 mM ribose) were made as stated above, and the pH was monitored during heating to 80 °C using a Ross combination flat surface electrode, model 8135 (Orion Research, Inc., Beverly, MA) attached to an Orion EA 940 pH/ISE instrument (Orion Research, Inc.). Temperature compensation was controlled with an automatic temperature compensation probe (Orion Research, Inc.). The pH and temperature were recorded every minute for 30 min. Each type of solution/gel was replicated three times.

Experimental Design. The statistical design used in experiment I was a fractionated factorial design of 32 treatment combinations including two lots of WPI, two types of sugar (lactose and ribose), four sugar concentrations (0, 100, 300, and 500 mM), four protein concentrations (13.5, 14.0, 14.5, and 15.0%), and four pH levels (6.0, 7.0, 8.0, and 9.0). On the basis of the results from experiment I, a full factorial experimental design consisting of 66 treatment combinations was developed for experiment II. Treatment combinations for experiment II included one lot of WPI, two types of sugar (lactose and ribose), eleven sugar concentrations (0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 mM), one protein concentration (14.0%), and one pH level (9.0).

All statistical analyses used the SAS System 6.12 for Windows (SAS Institute Inc., Cary, NC). The data were analyzed using the analysis of variance, and PROC GLM in SAS was used to perform the arithmetic.

RESULTS AND DISCUSSION

Experiment I—Effect of pH, Protein Concentration, and Sugar Type. *Gel Color.* As expected, the gels with no sugar added showed no color development. The gels containing lactose also showed no color development at any pH (6−9) or any lactose concentration (100, 300, or 500 mM). However, color development was seen in the gels containing ribose. The color ranged from light orange to dark brown depending on the pH and concentration of ribose. As the pH was increased from 6 to 9, the color of the gels containing ribose went from lighter orange to darker brown at all concentrations of ribose (100, 300, or 500 mM). Similarly, as the ribose concentration was increased from 100 to 500 mM, the color of the gels ranged from lighter orange to darker brown at any pH (6−9) of the gels. If both higher pH and higher ribose concentrations were paired, the gels were dark brown.

Large-Strain Rheological Properties. Gels with no sugar added had an average fracture stress of 18.6 kPa. Ribose caused an increase in fracture stress with a maximum of 37.6 kPa at a concentration of 500 mM ribose. In contrast, fracture stress increased and then decreased with increased lactose concentration. Increased protein concentration from 13.5 to 15.0% increased fracture stress values from 16.6 to 28.9 kPa. This trend is seen throughout the literature (Modler and Emmons, 1977; Foegeding, 1992; Li et al., 1999). Sugar type and sugar concentration did not alter fracture strain.

Table 1. Color of Gels and Percent Available Amine in Solutions after Heating at 80 °C for 30 min[a]

sugar (mM)	available amine (%)				color	
	lactose		ribose		lactose	ribose
	control	heated	control	heated		
0	100 ± 4[b]	104 ± 4	100 ± 4	104 ± 4	none	none
100	97 ± 4	101 ± 4	97 ± 4	91 ± 4	none	light orange
200					none	orange
300					none	dark orange
400					none	brown
500	90 ± 4	98 ± 4	87 ± 4	68 ± 4	none	dark brown

[a] Solutions were pH 9 and 14% protein for gels and pH 9 and 2 mg/mL for available amine determination. [b] Average values ± standard error.

Water-Holding. HW was increased from pH 6 to 7, and little or no changes were seen from pH 7 to 9. At pH 6 the gels are much closer to the isoelectric point of whey protein and thus more particulate than the gels at pH 7 (Ziegler and Foegeding, 1990). Gels close to a protein's isoelectric point synerese more than gels further away from the isoelectric point. HW was not affected by either sugar type or sugar concentration.

Experiment II—Effect of Lactose and Ribose at pH 9. Analysis of the results from experiment I indicated that sugar type and sugar concentration affected the fracture properties of the gels at all pH levels and all protein concentrations. A full factorial experiment was created to examine specific effects of increasing sugar concentration on the large-strain rheological properties of the gels. Sugar concentration was varied from 0 to 500 mM in increments of 50 mM. Protein concentration was held constant 14.0% because this concentration produced gels that were easily handled for torsion analysis, and pH 9.0 was used to increase the Maillard reaction.

Gel Color. The gels containing ribose showed orange to brown color development with increased concentration of ribose (50 mM ribose = light orange; 500 mM ribose = dark brown). As in experiment I, color development was not observed in the gels containing lactose at any concentration (Table 1).

Large-Strain Rheological Properties. A clear picture of the texture of the gels can be seen when the fracture modulus (G_f) is observed (Figure 1). Fracture modulus increased with increasing ribose concentration but slightly decreased with increasing lactose concentration ($P < 0.05$). At 50 mM sugar, the gels containing ribose had a fracture modulus of 9.8 kPa, whereas the gels containing lactose had values of 8.2 kPa. At 450 mM sugar, the fracture modulus of the gels containing ribose had increased to 13.1 kPa, but the values for the lactose-containing gels had decreased to 6.4 kPa. Increased fracture modulus indicated that the gels containing ribose were more rigid than the gels containing lactose. Increased rigidity of the gels containing ribose was caused by decreases in fracture strain (data not shown). Similarly, decreased rigidity of the gels containing lactose was caused by increases in fracture strain (data not shown).

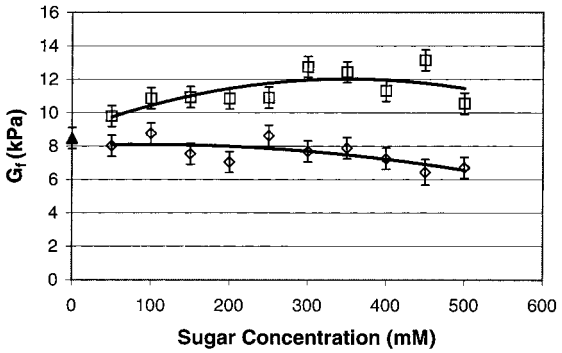

Figure 1. Influence of sugar type on fracture modulus as a function of sugar concentration: (◇) lactose; (□) ribose; (▲) no sugar added. Error bars indicate standard errors. The trend line was drawn using a second-order polynomial equation.

The effect of the Maillard reaction on the fracture properties of protein gels is complicated. Hypotheses have been developed to explain the increased gel strength and decreased gel deformability brought about by the Maillard reaction. Hill et al. (1992) theorized that the Maillard reaction could alter the charge of the protein by modifying the lysine residues, thus increasing the potential for cross-linking of proteins. Hill's observations, as ours, implied that the cross-linking of the proteins had a profound effect on the rheology of the gels. Hill et al. (1992) also found that there was a correlation between the extent of the Maillard reaction, evidenced by the color of the gels, and the strength of the gel. They found that there was a general phenomenon with proteins and reducing sugars. The effectiveness of the reducing sugars in increasing gel strength and gel color followed the series of reactivity of reducing sugars (lactose < fructose < ribose < xylose). Armstrong et al. (1994) found that the force required to rupture bovine serum albumin gels containing xylose was higher than for the gel formed at comparable pH with no reducing sugar added. Hill et al. (1992) also hypothesized that the mechanism of gelation relied greatly on a pH reduction, which is the result of acidic products from the Maillard reaction. From the explanations above, it is difficult to determine if cross-linking of the proteins, pH reduction, or both are involved in the mechanism affecting the large-strain rheological properties of the gels. The following series of experiments will explain what is taking place in each gel studied.

Small-Strain Analysis of the Gels. The phase angle, also called the phase lag, represents differences between stress and strain during oscillation (Steffe, 1996). The phase angle is directly related to the energy loss per cycle divided by the energy stored per cycle. It is calculated by dividing G'' (viscous component) by G' (elastic component). A value for the gel point can be approximated at a phase angle of 45°; this is when G'' is equal to G'.

During heating from 25 to 80 °C, the phase angle reached 45° for the three solutions at different times (Figure 2). The WPI solution gelled at an average temperature of 66 °C. The ribose-containing solution gelled at an average temperature of 67 °C, whereas the lactose-containing solution gelled at an average temperature of 79 °C. The lactose delayed gelation, which might explain the decreased gel rigidity and increased gel deformability seen in large-strain analysis.

During holding at 80 °C for 30 min, the phase angle of the gel without sugar stayed constant while the phase

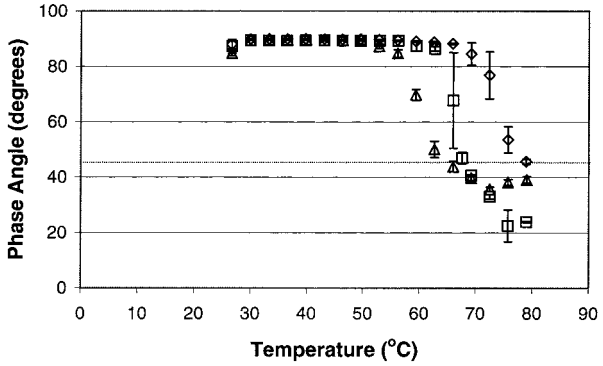

Figure 2. Influence of sugar type on phase angle as a function of temperature during heating: (◇) lactose; (□) ribose; (△) no sugar added. Dashed line indicates a 45° phase angle. Error bars indicate ranges of values.

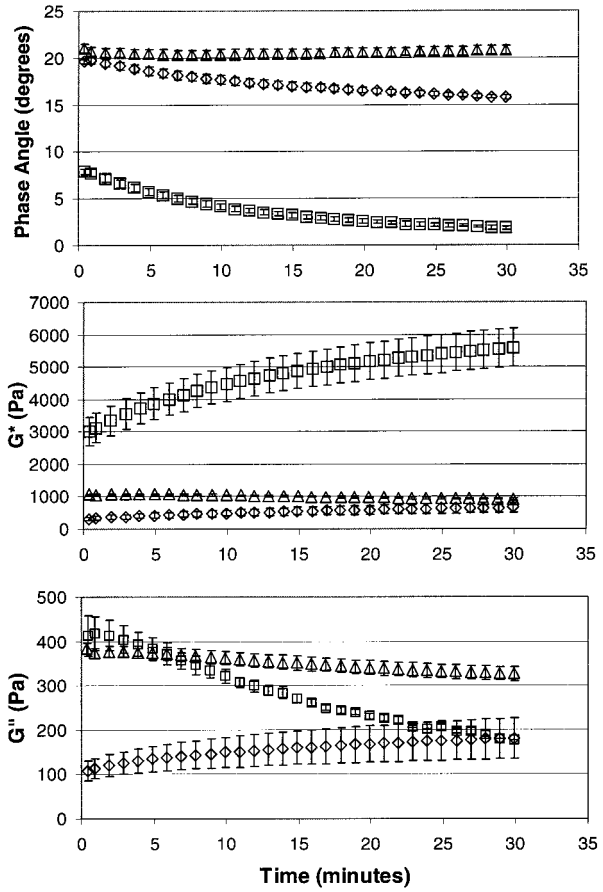

Figure 3. Influence of sugar type on phase angle, complex modulus, and viscous modulus as a function of time during holding at 80 °C: (△) lactose; (□) ribose; (△) no sugar added. Error bars indicate ranges of values.

angles for the gels containing lactose or ribose decreased (Figure 3). The gels containing ribose showed a more dramatic decrease, from 7.9° to 1.8°, and the gels containing lactose decreased from 19.6° to 15.7°. These values show that the gels containing ribose were more elastic than the gels containing lactose and the gels with no sugar added. It is important to note that the gels containing lactose were not less rigid because of a kinetic limitation on the gelation process. The complex modulus, the sum of elastic and viscous components, was much higher for the gels containing ribose than for the gels containing lactose and the gels with no sugar added (Figure 3). The complex modulus increased from 3000 to 5600 Pa in the gels containing ribose and

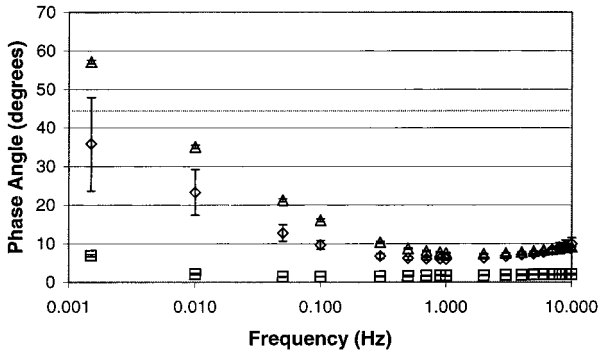

Figure 4. Influence of sugar type on phase angle as a function of frequency at 80 °C: (◇) lactose; (□) ribose; (△) no sugar added. Dashed line indicates a 45° phase angle. Error bars indicate range of values.

remained fairly constant for the gels containing lactose and the gels with no sugar added. Increases of the complex modulus in the gels containing ribose are probably due to continuation of the Maillard reaction during holding. Continuation of the Maillard reaction would create more covalent linkages and possibly decrease the pH. Another finding was the dramatic decrease of G'' during holding for the gels containing ribose (Figure 3). This showed that the gels containing ribose were losing their viscous component, whereas the other two gels had fairly constant viscous components during holding. The phase angle was decreasing in the gels containing ribose because G' (elastic component) was increasing and G'' (viscous component) was decreasing.

Mechanical spectra of the three gels at 80 °C showed major differences in the viscoelastic properties of the gels. The phase angle of the gels containing ribose was much lower than that of either the gels containing lactose or the gels with no sugar added (Figure 4). Frequency increases had less effect on the phase angle of the gels containing ribose than the other two gels. Once again this showed that the gels containing ribose formed more elastic networks than the gels containing lactose or the gels with no sugar added.

A creep recovery measurement is divided into two parts, creep and recovery. J_{max} is the maximum compliance observed during creep testing. J_f is the final compliance observed during recovery testing. J_0 is the initial compliance reading at time = 0 of creep testing. The values reported for this test are percent recovery. Percent recovery is calculated

$$\frac{J_{max} - J_f}{J_{max} - J_0} \times 100$$

The creep recovery data at 80 °C showed that the gels containing ribose recovered 80% of initial compliance, whereas the gels containing lactose and those with no sugar added recovered 16 and 12%, respectively (Table 2). In other words, the gels containing ribose were by far the most elastic. Creep compliance becomes much more ideal as the amount of cross-linking is increased (Clark and Ross-Murphy, 1987). From the creep recovery and the mechanical spectra at 80 °C, it is apparent that there was more cross-linking in the gels containing ribose.

Assays of Available Amine. Maillard browning starts with a reaction between a reducing sugar and an available amine. The available amines in WPI are mostly the ϵ-amino group of lysine. Therefore, it would be expected that percent available amine would decrease with increasing progress of the Maillard reaction.

Table 2. Rheological Properties[a] Determined from Creep Recovery Data

gel type	J_0	J_{max}	J_f	recovery
no sugar	2.47×10^{-3} (1.33×10^{-5})	3.84×10^{-2} (3.07×10^{-4})	3.42×10^{-2} (3.84×10^{-4})	11.6
500 mM lactose	2.48×10^{-3} (6.60×10^{-4})	2.27×10^{-2} (7.33×10^{-3})	1.94×10^{-2} (6.91×10^{-3})	16.2
500 mM ribose	1.79×10^{-5} (1.93×10^{-5})	2.33×10^{-4} (2.75×10^{-5})	5.97×10^{-5} (8.25×10^{-6})	80.5

[a] Mean values of two replications with ± the range shown in parentheses.

Table 3. Onset Temperature (T_o) and Temperature at Peak Maximum (T_{max}) for Denaturation[a]

solution	T_{max} (°C)	T_o (°C)
no sugar	58.0 ± 0.4	49.8 ± 0.5
500 mM lactose	65.0 ± 0.3	53.9 ± 0.4
500 mM ribose	61.1 ± 0.3	51.6 ± 0.4

[a] Mean values ± standard errors.

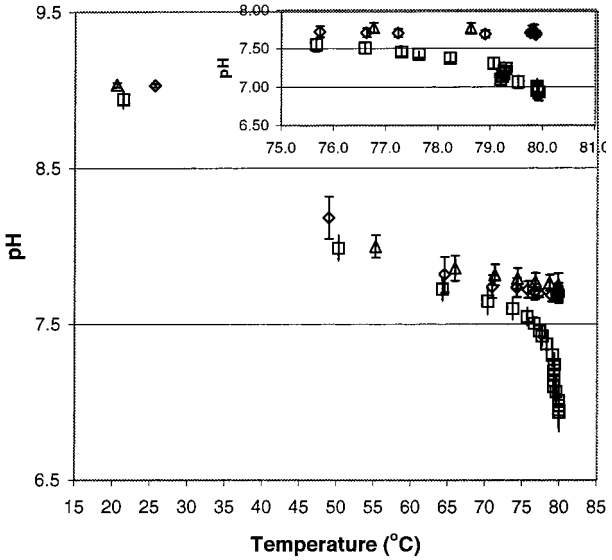

Figure 5. Influence of sugar type on pH as a function of temperature: (◇) lactose; (□) ribose; (△) no sugar added. Error bars indicate standard deviations.

The type of sugar tested (lactose or ribose) and the treatment used (unheated or heated) had a statistically significant effect ($P < 0.05$) on the percent available amine. The largest drop in available amine was seen with the heated sample containing 500 mM ribose (Table 1). This result coincides with the color development observed in the gels (Table 1). The gels containing ribose ranged from light orange to dark brown depending on the ribose concentration. Although the gels containing lactose did not have any color generation, the percent available amine did decrease before and after heating. Due to the color development seen in the gels and the decrease in available amine, it would seem that the Maillard reaction is progressing further in the gels containing ribose.

DSC. The type of sugar (lactose or ribose) had a statistically significant effect ($P < 0.05$) on the peak temperature (T_{max}) and the onset temperature of denaturation (T_o) for WPI solutions (Table 3). Protein solutions at pH 9.0 with no sugar added had an average T_{max} of 58 °C. The addition of ribose or lactose increased

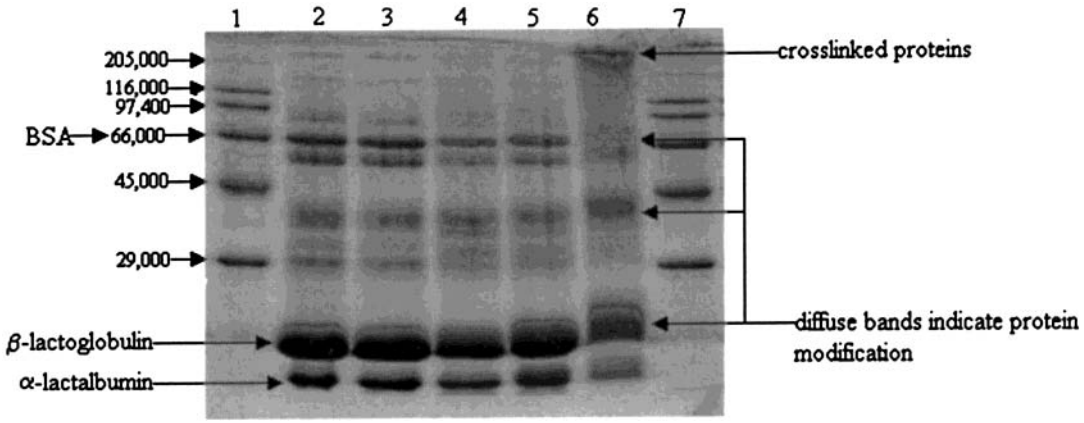

Figure 6. SDS−PAGE of whey protein. Samples: molecular weight standards (lane 1); WPI in deoionized water (lane 2); WPI in urea buffer (lane 3); solubilized WPI gel (lane 4); solubilized WPI/lactose gel (lane 5); solubilized WPI/ribose gel (lane 6); and high molecular weight standards (lane 7).

peak denaturation temperature. WPI solutions containing 500 mM ribose or 500 mM lactose had average T_{max} values of 61 and 65 °C, respectively. Jou and Harper (1996) observed the same trend. The addition of sucrose, trehalose, maltose, and lactose increased the T_o and T_{max} of whey proteins.

The ability of sugars to inhibit heat-induced aggregation of proteins has been known for many years (Ball et al., 1943). Arakawa and Timasheff (1982) believe the stabilization of proteins by sugars is due to a preferential interaction of proteins with solvent components. They found the proteins were preferentially hydrated in aqueous sugar solutions. The mechanism of preferential hydration is due to the positive effect sugars have on the surface tension of water. Other contributing factors include the exclusion volume of the sugars and the chemical nature of the protein surface. Although both sugars increased T_o and T_{max}, lactose enhanced protection of the protein due to its increased molecular weight compared to ribose (Arakawa and Timasheff, 1982). This explains the increased gel point seen in the gels containing lactose (Figure 2).

Heat and pH Study. Hill et al. (1992) found that the Maillard reaction decreased pH through the development of acidic products. They hypothesized that the pH decrease occurred after protein denaturation, and they demonstrated a strong correlation between pH drop and strength of the gels. Mat Easa et al. (1996) found that the pH drop caused by the Maillard reaction is dependent on the concentration and reactivity of the sugar. Armstrong et al. (1994) also studied the effect of the pH drop in the gels. In their study, the drop seemed to have more of an effect on syneresis than on gel strength.

All three solutions showed a pH drop of ∼1 unit in the first 30 °C increase of temperature and the same pH changes until ∼75 °C (Figure 5). The solutions containing ribose then began to show steady decreases in pH, whereas those with lactose or no added sugar stayed at a constant pH. The gels containing ribose had a pH of almost 1 unit less than the other two gels after 30 min of heating. DSC data showed that solutions containing 500 mM ribose had an average peak denaturation temperature of 61 °C, thus putting the drop in pH after protein denaturation. Small-strain data showed that gelation in solutions containing ribose occurred between 68 and 70 °C. This shows that the pH decrease seen in the gels containing ribose occurred after gelation had begun and that the pH would have little if any effect on the initial gel network formation. However, it could explain the increase in G' and decrease in G'' observed during the 30 min hold at 80 °C (Figure 3).

SDS—PAGE. SDS−PAGE showed that proteins from the gels containing ribose (in which the Maillard reaction more nearly reaches completion) have been modified. Tight, dense protein bands were present for the gels containing lactose and for the gels with no sugar added (Figure 6). Proteins from the gels containing ribose did not show tight bands. Some bands of proteins from the gels containing ribose, particularly BSA, were lost. This indicated that the proteins were covalently cross-linked. The gels containing ribose were less soluble in the 2% SDS, 8 M urea, 2% mercaptoethanol, and 20 mM Tris-HCl (pH 8.0) buffer than the gels containing lactose or the gels with no added sugar. After centrifugation, the protein solutions from the gels containing ribose contained 5−10% less protein than the protein solutions from the gels with added lactose and the gels with no sugar added. Armstrong et al. (1994) also reported that the Maillard gels were less soluble in SDS and β-mercaptoethanol than gels without added reducing sugar, which would also suggest that the gels containing ribose contained additional covalent cross-links.

Conclusion. Ribose and lactose had different effects on WPI gelation. Lactose stabilized the WPI to denaturation, which increased the time and temperature required for gelation, thus decreasing the fracture modulus of the gel. The decrease in primary amines without subsequent color development suggested that the Maillard reaction did not proceed very far in protein solutions/gels containing lactose. Ribose, however, covalently cross-linked some proteins, which increased the fracture modulus and gel elasticity. The decreased pH caused by the Maillard reaction in the gels containing ribose occurred after protein denaturation and the initiation of gelation.

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