Textural Characteristics and Dynamic Oscillatory Rheology of Maturation of Milk Gelatin Gels with Low Acidity

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ABSTRACT

The potential was assessed for using gelatin to form milk gels in the pH range outside that at which acid-induced coagulation of milk takes place, and performance of these gels was compared with aqueous gelatin gels. We studied gels containing two types of gelatin (A and B) at concentrations of 1.5, 3.0, 4.5, and 6.67% and within a pH range of 5.3 to 6.6. Bloom strength, breaking force, and hardness depended principally on the gelatin concentration and were independent of pH values. Type B gelatin produced weaker gels than did type A gelatin. As determined by instrumental texture profile analysis, values for retarded recoverable springiness and cohesiveness were very high for all samples. The values for storage modulus over maturation time were greater for milk gels than for water gels, suggesting a stabilization of the network from the milk components. The maturation mechanism and melting temperatures of milk gels proved to be similar to those of water gels. Gelatin has the potential for use in the preparation of a wide range of milk gels with low acidity and interesting textural properties.
(**Key words**: texture, gelatin, acidic milk gels, glucono-δ-lactone)

Abbreviation key: **G'** = storage modulus, **G"** = loss modulus, **GDL** = glucono-δ-lactone, **RM** = reconstituted NDM.

INTRODUCTION

At present, the commercial market is continuously increasing for gelled products based on acidified milk. New textures, new types of presentation, new flavors, and a choice of caloric level are being sought in the formulations that are being developed. Two processes are being used to obtain these kinds of products: the

fermentation of milk by lactic acid bacteria and the direct acidification of milk by the addition of an edible acid or by glucono-δ-lactone (**GDL**) (3), which is the preferred acidulant because of its slow release of gluconic acid. Both methods involve lowering the pH to near the isoelectric point of casein. Factors governing the formation of gels from reconstituted NDM (**RM**) by these two methods have been studied by many researchers (1, 2, 3, 14, 15). A recent paper (1) compared the two methods and concluded that chemical acidification with GDL could be used to prepare acidic milk gels with a weak texture similar to those prepared by traditional fermentation; however, the milk needed preheating (90°C) to produce good textural properties in the final gels.

Recently, milk-based products have appeared on the market that do not have the acidic taste or weak, creamy texture of traditional yogurt. These products have stimulated interest in studying methods for the production and stabilization of this kind of gelled product. Functional ingredients are needed that can achieve the required performance, and hydrocolloids have been increasingly useful in this area and have been used in the formation of milk gels (13, 32). Carrageenan, guar gum, and xanthan gum have produced higher viscosities in milk than in water (29), and carrageenan has also been used to form gels with milk (21). Pectins also have been studied for their capacity to develop good texture in a wide range of acidic milk products from desserts to cream cheese (1, 22).

The functional properties of gelatin permit wide application in the food industry, where its characteristics are used to gel, thicken, and stabilize. Gelatin has also been utilized as a stabilizer in some milk formulations (4, 5, 6, 11, 31), but its use in the manufacturing of milk gels has not been sufficiently studied. As a protein, gelatin is also a nutritive ingredient and is not subject to the legal restrictions of an additive, although maximum doses are legally regulated. The compatibility of gelatin with milk proteins makes it well suited for use as a functional ingredient (18, 25, 27, 28). Gelatin makes an outstanding contribution to product texture and mouthfeel because of

its unique property of melting at mouth temperature, giving a fat-like sensory perception as well as a neutral flavor.

The objective of the present study was to analyze the potential use of two types of commercial gelatin for the production of acidic and neutral milk gels by investigating several textural characteristics of the final gels and the kinetics of their maturation and melting point by using oscillatory dynamic techniques.

MATERIALS AND METHODS

Preparation of Samples

Milk. Nonfat dry milk (Nestlé, Boué, France) was reconstituted to a concentration of 10% (wt/wt) solids in distilled water. The reconstituted milk (**RM**) was cooled rapidly to 10°C, the temperature of acidification.

Acidification procedure. To dissolve gelatin dispersions completely, they must be heated at 40°C for 15 min. We found that, in these conditions, acid coagulation of RM does not take place at a pH >5.2. For the present study, we therefore selected a pH range of 5.3 to 6.6. The GDL (Merck, Darmstadt, Germany) was used as the acidifying agent. A preliminary study was carried out to ascertain the amount of GDL that was needed to obtain final pH values of 5.8 and 5.3. The amounts required were 0.4 and 0.8% (wt/vol) crystalline GDL, which was quickly stirred into the RM at 10°C and stored for 24 h. When the addition of gelatin changed the pH of the final system, the amounts of GDL were modified in order to obtain the required pH values. The final pH of the samples was measured using a micropH 2001-Crison pH meter (Crison Instruments, S.A., Alella, Spain).

Preparation of milk and water gels. Two commercial gelatins were used: processed acidic pigskin (type A) and processed limed hide (type B); both gelatins had a nominal Bloom strength of 230° (SBI-Systems Bio-Industries, Baupte, France). Both types of gelatin were added at 1.5, 3, 4.5, and 6.67% (wt/wt) to plain and acidified RM or to distilled water and left to swell for 1 h. The samples were then stirred and heated in a thermostatically controlled water bath to 40°C for 15 min to dissolve the gelatin totally. Froth produced during these steps was removed from the surface of the solutions.

Large Deformation
Rheological Measurements

For assessment of gel strength (the Bloom strength) and breaking force, hot (40°C) RM samples mixed with gelatin were put into Bloom glass jars that had an external diameter of 66 mm, an internal diameter of 59 mm, a height of 85 mm, and a capacity of 150 ml; these samples were matured for 17 h at 10°C. Bloom strength of the gels was measured at 10°C using a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK) with a flat-ended cylindrical plunger that was 12.7 mm in diameter at a crosshead speed of 1 mm/s. Bloom strength is defined as the mass in grams that is necessary to depress a standard plunger 4 mm into the gel (12). The breaking force was obtained by recording the first significant inflection point of the curve that was produced as the plunger penetrated into the gel to a depth of 20 mm.

To perform instrumental texture profile analysis, RM samples at 40°C that had been mixed with gelatin were put into glass molds and allowed to mature for 17 h at 10°C. After the gels were formed and stabilized, they were cut into 17-mm diameter cylindrical shapes using a stainless-steel core-borer. The gel cylinders were placed in a hollow steel device 17 mm in height and with parallel bases and then were cut with a thin razor blade (9). These cylindrical samples were compressed using a TA-XT2 Texturometer with a plunger 75 mm in diameter. The crosshead moved at a constant speed of 1 mm/s. Two cycles of 40% compression were run to obtain an instrumental TPA. Five seconds elapsed between cycles. The parameters recorded were hardness, the force needed to attain the given deformation; retarded recoverable springiness, the ratio of the distance recorded during the second compression of the sample to that recorded during the first compression (24); and cohesiveness, the ratio of the area under the second peak to the area under the first peak.

Small Deformation
Rheological Measurements

Dynamic oscillatory measurements were performed in the following manner. Aliquots of samples were poured while hot (40°C) onto a bottom plate of the rheometer (Physica Rheolab MC120; Paas Physica, Stuttgart, Germany) equipped with a 5-cm, 2° cone-plate measuring system. To prevent evaporation and to protect against dehydration during gel maturation, liquid paraffin was applied to the exposed surfaces of the sample. The temperature was lower to 10°C at a constant rate of 1°C/min, promoting gel formation. Preliminary tests for strain amplitude sweep were performed and showed that a strain amplitude of 0.10 was within the linear viscoelastic behavior for all samples for the experimental frequency selected. Oscillatory tests were made at 10°C for 17 h to assess

the maturation kinetics of the gelled samples [i.e., evolution of storage modulus (G') as a function of time]. Afterward, the melting characteristics of the gels were determined by oscillatory tests while a temperature ramp was applied; the samples were heated from 10 to 60°C at a speed of 1°C/min, and the temperature at which G' values crossed the loss modulus (G'') values was registered (8, 21). All of the oscillatory tests were run at a fixed frequency of 1 Hz and a strain amplitude of 0.10. Good reproducibility of results was obtained for all samples; the coefficient of variation of the replicates for each experimental point was <2%.

Statistical Analyses

Three different batches of each composition were prepared on different days, and all measurements were performed at least in duplicate. An ANOVA of results using the multiple-comparison test was performed for means of least significant difference, and significance was declared at $P < 0.05$ using the Stat-

graphics program (30). The same program was used to calculate the linear regressions.

RESULTS AND DISCUSSION

Using turbidometric techniques, Banon and Hardy (2, 3) reported that the pH value at which the acid-induced coagulation point of RM occurs increases as the temperature increases (i.e., coagulation of RM takes place at pH 5.35 at 30°C and at pH 5.50 at 42°C). Also, Kim and Kinsella (17) found that, if the amount of GDL added was sufficient to reach pH 5.1, the gelation of RM started after 2 h at 40°C.

In the present study, RM samples had to be heated at 40°C for 15 min to dissolve the added gelatin. Different amounts of GDL were added to RM samples to explore the minimum pH that could be reached by addition of GDL without coagulation of RM when heated at 40°C. This minimum pH value was determined to be 5.3 and could be obtained by addition of 0.8% of GDL to the RM samples at 10°C after 18 h of gradual hydrolysis of the lactone.

TABLE 1. Bloom strength, breaking force, and displacement at breaking of two types of gelatin gels made with reconstituted NDM or with neutral water.

Gelatin	Bloom strength		Breaking force		Displacement	
	Milk	Water	Milk	Water	Milk	Water
	——— (g) ———		——— (N) ———		——— (mm) ———	
Type A						
1.50%						
X̄	17[a]	19[a]	0.75[a]	0.96[a]	16.2[a]	17.5[a]
SD	1	1	0.03	0.14	0.7	1.2
3.00%						
X̄	67[b]	78[a]	3.88[a]	5.08[a]	16.1[a]	17.6[a]
SD	4	6	0.30	0.16	0.9	0.1
4.50%						
X̄	128[b]	152[a]	7.05[a]	9.07[a]	16.6[b]	17.7[a]
SD	1	7	0.13	0.61	0.5	0.5
6.67%						
X̄	300[a]	309[a]	12.89[b]	16.82[a]	16.0[b]	18.1[a]
SD	4	5	0.09	0.83	0.9	0.6
Type B						
1.50%						
X̄	14[b]	16[a]	0.56[a]	0.57[a]	15.3[a]	16.3[a]
SD	1	1	0.04	0.02	0.2	1.2
3.00%						
X̄	57[a]	61[a]	2.96[a]	3.23[a]	16.6[a]	17.1[a]
SD	4	1	0.16	0.09	1.4	0.2
4.50%						
X̄	123[b]	133[a]	5.42[b]	7.84[a]	15.8[b]	18.7[a]
SD	9	1	0.35	0.05	0.8	0.6
6.67%						
X̄	242[a]	257[a]	10.71[b]	16.82[a]	15.4[b]	20.0[a]
SD	7	4	0.72	0.86	0.3	0.8

[a,b]Means within rows and the same test procedure without a common superscript letter differ ($P < 0.05$) according to the least significant difference multiple range test.
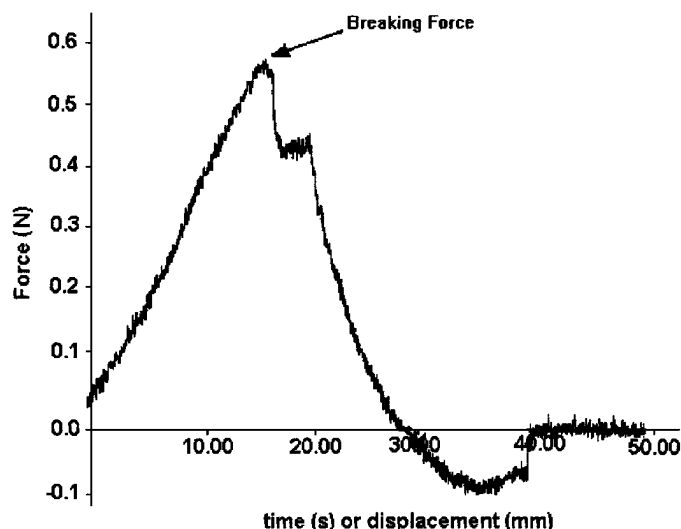
Figure 1. Typical profile for breaking force and penetration of a milk gel formed by gelatin (1.5% gelatin type A, pH 6.6).

Acid-induced gelation did not take place at pH 5.3, even after storage of RM samples for 48 h at 10°C. Therefore, gelatin was responsible for the final gel formation and the structure of samples. The three pH levels selected for this study were 5.3, 5.8, and 6.6 (pH 6.6 is the pH of RM without added acid). Gelatin concentrations of 1.5, 3.0, 4.5, and 6.67% (wt/wt) were selected to obtain a wide range of textures in the final gels. The 6.67% level is the gelatin concentration used by the official method for measuring Bloom strength (12). Two types of commercial gelatin were studied: type A, acid-treated pigskin, and type B, limed hide.

Gelatin types A and B lowered the pH of RM, especially at higher concentrations. These shifts in pH were taken into account when the pH values were adjusted, and different amounts of GDL were added to attain the desired final pH values of all samples.

Bloom Strength and Breaking Force

Comparison between water and neutral RM gels. Bloom strength values were slightly higher for water gels than for neutral RM gels for all concentrations of gelatin. The differences in absolute values of degrees Bloom were small yet significant ($P < 0.05$) in half of the cases (Table 1). The presence of RM components in the solvent medium may have caused this loss of rigidity of the gel. The effect of these components could relate to changes in the availability or mobility of the molecules of water in the medium (20). The mechanical parameters analyzed provide

macrostructural information about the system measured, and it is difficult to interpret changes in their values in terms of fine structure or physicochemical interactions between the components.

Breaking force showed a trend similar to that for Bloom strength when values for water and RM gels were compared (Table 1). At higher concentrations of gelatin, the plunger displacement values at which breaking took place were higher ($P < 0.05$) for water gels than for RM gels, indicating that the water gels had greater mechanical resistance (10). Values for both Bloom strength and breaking force corresponding to gelatin type B were lower than those of gelatin type A. Differences based on the type of gelatin were not easy to analyze because of the complexity of gelatin composition. The principal differences between gelatins of types A and B in isoelectric point and viscosity are well known (25), but it is difficult to analyze how such complex factors as molecular mass distribution and composition and sequence of amino acids can affect their behavior (19). Our results suggest that it might be worthwhile to consider possible differences in performance, depending on origin and pretreatment, when new products are being developed.

Influence of pH and of type and concentration of the gelatin on the texture of RM gels. Figure 1 shows a profile obtained by penetrometry of a gelatin RM gel. Curve shapes were similar for the complete range of gelatin concentrations analyzed. The curves showed a breaking peak, indicating the formation of firm set gels even using the lowest concentration (1.5%) of gelatin. Values corresponding to the latter were comparable with those found by Harwalkar et al. (14) for acid-induced RM gels in experimental conditions that, although not identical, were sufficiently similar for a comparison to be of interest. When gelatin content increased, firmer gels were produced; texture varied up to a very firm, self-standing product.

The values of Bloom strength and of force at the breaking point, as expected, increased as gelatin concentration increased (Table 2). Gels with type B gelatin had lower ($P < 0.05$) values for breaking force, but Bloom strength values were not always significantly lower within the same concentration of gelatin; differences were greater at higher gelatin concentrations. However, differences because of changes in pH were not significant ($P > 0.05$). According to Ledward (19), the junction zones of protein chains of gelatin gels are relatively nonpolar in nature; therefore, small changes in pH and in the protein charge would not have a very marked effect.

Syneresis was not observed in any of the gels. This advantage over the acid-induced RM gels, which exhibited a high degree of syneresis, depended on conditions of setting, such as processing heat treatment, final pH, and concentration of RM solids (14, 15, 16, 26).

Instrumental Texture Profile Analysis

Because the instrumental technique for texture profile analysis is empirical, it provides useful results when the test conditions are carefully standardized (7, 24). Preliminary trials were conducted in order to select the extent of deformation to be imposed on all samples, and, thus, the results obtained were comparable. The gels corresponding to 1.5% gelatin could not be tested because these weak gels could not be removed from the glass beaker without destroying the sample.

A deformation of 40% was chosen to be applied to all samples in order to avoid breaking gels during the test. At this level, valuable information could be obtained on important parameters such as hardness, springiness, and cohesiveness. Usually, at higher deformation, the gel sample is broken completely during the first bite, producing portions or small pieces of the initial bite, making parameters such as springiness and cohesiveness meaningless (24).

The hardness parameter showed a pattern that was similar to that of Bloom strength and force at the breaking point. Hardness was primarily dependent on gelatin concentration, but no significant ($P > 0.05$)

TABLE 2. Bloom strength, breaking force and displacement at breaking of two types of gelatin milk gels prepared at three pH values.

pH	Gelatin	Bloom strength		Breaking force		Displacement	
		Type A	Type B	Type A	Type B	Type A	Type B
		(g)		(N)		(mm)	
6.6	1.50%						
	X	17[a]	14[a]	0.75[a]	0.56[b]	16.2[a]	15.3[a]
	SD	1	1	0.03	0.04	0.7	0.2
	3.00%						
	X	67[a]	57[a]	3.88[a]	2.96[b]	16.1[a]	16.6[a]
	SD	4	4	0.30	0.16	0.9	1.4
	4.50%						
	X	128[a]	123[a]	7.05[a]	5.42[b]	16.6[a]	15.8[a]
	SD	1	9	0.13	0.35	0.5	0.8
	6.67%						
	X	300[a]	242[a]	12.89[a]	10.71[b]	16.0[a]	15.4[a]
	SD	4	7	0.09	0.72	0.9	0.3
5.8	1.50%						
	X	15[a]	15[a]	0.83[a]	0.71[b]	16.9[a]	17.0[a]
	SD	1	1	0.02	0.05	1.4	1.7
	3.00%						
	X	63[a]	53[a]	3.51[a]	2.71[b]	15.8[a]	16.1[a]
	SD	7	5	0.21	0.32	1.1	1.2
	4.50%						
	X	137[a]	118[a]	7.61[a]	5.18[b]	16.5[a]	14.9[b]
	SD	14	10	0.41	0.57	0.3	0.6
	6.67%						
	X	302[a]	259[b]	11.62[a]	10.05[b]	15.2[a]	15.6[a]
	SD	3	1	0.97	0.17	0.4	0.6
5.3	1.50%						
	X	18[a]	15[b]	0.97[a]	0.55[b]	15.6[a]	14.8[a]
	SD	1	1	0.01	0.04	1.0	1.2
	3.00%						
	X	74[a]	54[a]	3.64[a]	2.05[b]	14.9[a]	13.4[a]
	SD	5	5	0.16	0.17	0.6	1.2
	4.50%						
	X	147[a]	118[a]	7.09[a]	4.94[b]	14.9[a]	14.2[a]
	SD	11	3	0.50	0.38	1.3	0.8
	6.67%						
	X	298[a]	234[b]	13.3[a]	9.57[b]	14.4[a]	14.4[a]
	SD	6	6	0.93	0.73	1.2	0.8

[a,b]Means within rows and the same test procedure without a common superscript letter differ ($P < 0.05$) according to the least significant difference multiple range test.

TABLE 3. Hardness of two types of gelatin milk gels prepared at three pH values.

pH	Gelatin	Hardness			
		Type A		Type B	
		(N)			
		$\overline{X}$	SD	$\overline{X}$	SD
6.6	3.00%	0.94[a]	0.07	0.74[b]	0.06
	4.50%	1.76[a]	0.09	1.16[b]	0.09
	6.67%	2.75[a]	0.17	2.43[b]	0.09
5.8	3.00%	0.72[a]	0.03	0.68[b]	0.08
	4.50%	1.80[a]	0.04	1.06[b]	0.05
	6.67%	2.46[a]	0.16	2.46[a]	0.17
5.3	3.00%	1.04[a]	0.03	0.57[b]	0.02
	4.50%	1.64[a]	0.08	1.21[b]	0.02
	6.67%	2.98[a]	0.12	1.99[b]	0.07

[a,b]Means within rows without a common superscript letter differ ($P < 0.05$) according to the least significant difference multiple range test.

effect of pH values was found. Again, type B gelatin conferred significantly lower mechanical resistance than did type A gelatin, and these differences were greater at lower pH (pH 5.3) (Table 3).

Values for retarded recoverable springiness were very high, ranging from 0.87 to 0.98, indicating that samples recovered most of their initial height before the second stroke began. Cohesiveness values ranged from 0.83 to 0.90. No significant patterns of behavior ($P > 0.05$) were found with respect to the composition factor analyzed, indicating an important elastic component in the structure of the network, regardless of range of pH or the type and concentration of gelatin.

Small Deformation Rheological Measurements

Once the solution reached 10°C, the evolution of G′ over time of gel maturation, corresponded to typical curves for gelatin gels. The G′ values rose more rapidly at the beginning of the test; as maturation time increased, the slope of the curve decreased, reaching equilibrium value. This shape indicated that the gelatin chains in a first stage of maturation were probably still mobile inside a structure that was not completely set and that junction sites were constantly being created. Afterward, the chains became less mobile, and no new junction sites were created (23). The G′ values were higher for RM gels than for water gels, indicating a probable stabilization of the network as a structural effect attributable to changes in the hydrogen bonding environment, which is the basis of the formation of the gelatin network. This hypothesis is supported by the more pronounced differences in the weaker gels with lower gelatin concentrations; for those gels, electrostatic forces are of greater impor-
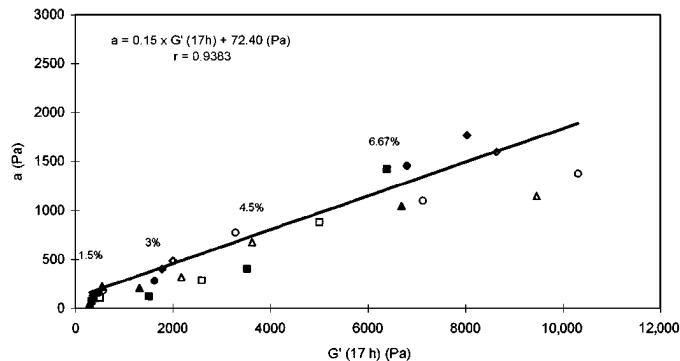

Figure 2. Linear correlation between $\Delta G'/\Delta\log$ time (a) where Δ = change, and G′ = storage modulus (17 h). Open symbols correspond to gelatin type A, and solid symbols correspond to gelatin type B. The pH values for milk gels are pH 6.6 (○,●), pH 5.8 (△, ▲), and pH 5.3 (□, ■); water gels (◊,♦) are also shown.

tance in connecting the more mobile chains.

To study the evolution of the maturation when gels were formed with RM, the rate of increase of G′, measured as the first derivative [parameter a, or $\Delta G'/\Delta\log$ time, proposed by Normand (23)], was calculated from these curves for all samples. A linear regression was made of all of the parameter values and their corresponding G′ values (17 h) for each sample (Figure 2). The fit was highly correlated (r = 0.938). These analyses suggest that the maturation pattern of gelatin gels is independent of gel type (water or RM), gelatin type or concentration, or pH.

Another characteristic of the maturation process of the gels is the relationship that exists between the values of G′ at 17 h and G′ at 1 h. Figure 3 illustrates this relationship for all samples; the correlation coefficient was high (r = 0.975). This correlation between values is important for industrial applications because it allows the prediction of G′ at 17 h
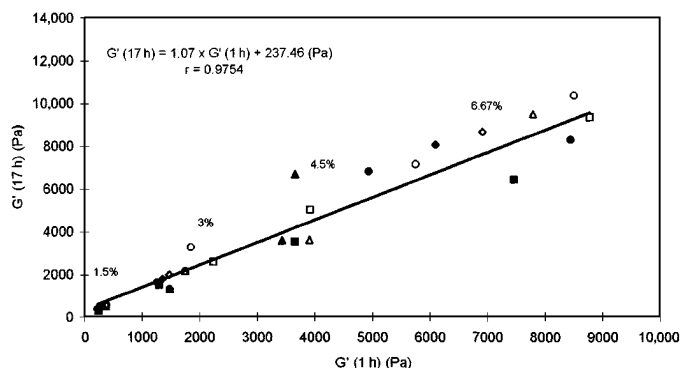

Figure 3. Linear correlation between the storage modulus (G′) values at 17 and 1 h. Open symbols correspond to gelatin type A, and solid symbols correspond to gelatin type B. The pH values for milk gels are pH 6.6 (○, ●), pH 5.8 (△, ▲), and pH 5.3 (□, ■); water gels (◊, ♦) are also shown.

from the value at 1 h of maturation. The values of the intercept and slope calculated from experimental results are shown in Figure 3. These calculations also were made by Normand (23) over a 20-h maturation for six different extracts of gelatin and various concentrations of gelatin.

Melting temperature was determined from the crosspoint of G′ and G″ values. The data were obtained from a temperature sweep test of samples from 10 to 60°C. Differences between water or RM gels for the same concentration of gelatin were not significant ($P > 0.05$). Neither pH nor type of gelatin significantly affected melting temperature, although melting temperatures were highest at higher concentrations of gelatin. Melting temperatures ranged from 27.0°C for the water gel with 1.5% gelatin to 33.5°C for RM gel with 6.67% gelatin at pH 5.3 (values not shown). In all cases, the gels should melt at body temperature, which confers good mouthfeel to final products by influencing the perception of a smooth texture.

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