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Thermally Induced Gelation of Succinylated Canola Protein Isolate

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Thermally induced gelation of unmodified and succinylated canola protein isolate (54% and 84% modification of free amino groups) was examined over a wide range of sodium chloride concentrations (0.0–0.7 M) and pH (3.5–11.0). Protein dispersions were heated at 72 °C for 30 min to simulate cooking conditions for a comminuted meat product. Succinylation improved gelation. For unmodified isolate, gels formed at only 4 of 18 combinations of pH and NaCl, while 12 gels formed from each level of succinylation under the same conditions. Gels from unmodified isolate formed only at high pH (≥9.5) whereas those from succinylated isolate formed from pH 5.0 to 11.0. Above pH 6.5, succinylated protein formed gels only in the presence of NaCl. In general, the firmest gels were obtained with moderate succinylation. All gels from unmodified isolate and those at pH 5.0 from succinylated isolate formed opaque gels due to the presence of insoluble particulates; all others were translucent. Translucent and opaque gels responded differently to rheological tests and were related in different ways to physicochemical and rheological properties of protein dispersions. Bonds involved in gel formation and stability were tentatively identified as hydrophobic interactions and hydrogen bonds.

Food gels consist of a continuous phase of interconnected particles and/or macromolecules intermingled with a continuous liquid phase such as water (Powrie and Tung, 1976). Gelling agents are generally present at levels of 10% or less and form a three-dimensional matrix such that the system behaves as a soft solid yet retains many properties characteristic of the fluid component and are thus termed "viscoelastic". As a rule, to obtain gels from globular proteins requires protein concentrations 1 order of magnitude higher than is required for gelation of polysaccharide or gelatin dispersions.

The mechanisms of gelation of globular proteins are not yet completely understood. The most generally accepted hypothesis was proposed by Ferry (1948) who suggested a two-step mechanism beginning with an initiation step involving unfolding or dissociation of the protein molecules, followed by aggregation and association and, under appropriate thermodynamic conditions, formation of a gel. Hermansson (1978, 1979a,b) described a globular protein gel as a state intermediate between a protein sol and precipitate, where a gel may form if a proper balance between protein–protein and protein–solvent interactions is achieved. Tombs (1970, 1974) suggested that gels are formed from globular proteins as a result of aggregation of protein molecules into strands followed by interaction of the strands to form a gel network. Bonds in protein gels vary quantitatively and qualitatively with type of protein and gelation environment and may include hydrophobic interactions, ionic attractions, hydrogen bonds, or disulfide linkages.

Although much work has been done on examination of gelation behavior of soy protein, little has been reported on gelation of canola protein. Sosulski et al. (1976) reported that rapeseed flours, concentrates, and isolate had poor gelation properties. Similarly, Thompson et al. (1982) also reported poor gelation of rapeseed protein concentrate. In contrast, Gill and Tung (1976, 1978) examined gelation behavior of the 12S glycoprotein fraction of rapeseed by both rheological and microscopical techniques and reported gelation at protein concentrations as low as 4.5%, with measurable thickening at 1% protein. Although gelation mechanism and the bonds involved in gel formation and stability were not fully elucidated, the authors concluded

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that some disulfide bonding was involved but that ionic and hydrogen bonds were not likely to be major factors in gel cross-links.

Chemical modifications of proteins with succinic anhydride has been demonstrated to improve protein solubility (Franzen and Kinsella, 1976) and to enhance thermal stability (Sato and Nakamura, 1977). There have been few reports, however, on functional properties of succinylated canola protein. Canola is the name given to varieties of rapeseed low in glucosinolates. Canola is the most important oilseed crop in Canada and northern Europe and is potentially an important protein source. As thermally induced gelation is an important functional property in many food systems, the present study examined gelation properties of unmodified and succinylated canola protein isolate over a wide range of sodium chloride concentrations and pH and related these properties to physicochemical and rheological properties of the isolate under the same environmental conditions.

MATERIALS AND METHODS

Succinylation. Canola (var. Tower) isolate was succinylated with 5.2% or 14.2% succinic anhydride (calculated on a protein basis), which resulted in 54% and 84% modification of free amino groups, respectively (Paulson and Tung, 1987). Unmodified and succinylated isolate (designated 0%, 5.2%, and 14.2% SA-treated) were examined for gelation behavior as well as protein solubility, surface hydrophobicity, ζ potential, and steady shear rheology. These properties were determined as a function of pH (3.5, 5.0, 6.5, 8.0, 9.5, 11.0) and NaCl concentration (0.0, 0.35, 0.7 M).

Dynamic Shear Properties of Thermally Induced Gels. As a potentially important use for plant proteins is to replace or extend meat protein in comminuted meat products, a temperature of 72 °C was used to induce gelation. This is within the temperature range generally used for comminuted meat products (70–75 °C) but lower than temperatures usually used to study plant protein gelation.

Seven to eight grams of 11.4% isolate dispersions prepared as previously described (Paulson and Tung, 1987) were heated in capped cylindrical plastic vials (i.d. = 22 mm, 20-mL capacity; Fisher Scientific) for 30 min in a 72 °C water bath. The samples were cooled under cold running tap water and then allowed to equilibrate to room temperature for approximately 2 h. Dynamic viscoelastic properties of the gels were obtained on a Weissenberg rheogoniometer equipped with 5-cm-diameter parallel plate fixtures at a gap thickness of 1 mm. The gels were carefully removed from the vials; undisturbed portions were sliced to a thickness of slightly greater than 1 mm and placed on the bottom platen. The top platen, supported by a No. 7 torsion bar (9.4 Pa cm^3 μm^{-1}), was then carefully lowered to a gap thickness of 1 mm to avoid air pockets between the platens. Evaporation of water from the sample was avoided by applying a thin layer of silicone oil to the exposed edge of the gel. A small sinusoidally varying oscillatory strain of maximum amplitude of 1.88% (which was determined to be within the linear viscoelastic range for these samples) was imposed over a frequency (ω) range of 0.19–19 s^{-1}. The amplitudes of the input (strain) and output (stress) voltage signals, and the phase difference between them, were monitored with a Tronotec Model 703A digital phasemeter. From these data, values of storage modulus (G', Pa; a measure of the energy stored elastically per cycle of sinusoidal deformation), loss modulus (G'', Pa; the energy dissipated as heat per cycle), loss tangent (the tangent of the phase angle between the stress and strain waves and numerically equal to G''/G', thus reflecting the relative proportions of viscous to elastic components of the samples), and dynamic viscosity (η', Pa s, where $\eta' = G''/\omega$) were calculated from the equations of Walters (1968) using a program written for a microcomputer. As plots of G' or η' versus ω were linear on logarithmic coordinates, the slope and intercept of each line were determined by least-squares linear regression. Values of G', G'', and loss tangent at a frequency of 10 s^{-1} were calculated from

$$G' = a\omega^b \quad (1)$$

where a (storage coefficient) is the intercept (at ω = 1 s^{-1}), b (storage index) is the slope of a log–log plot, and

$$\eta' = c\omega^{d-1} \quad (2)$$

where c is the dynamic shear consistency coefficient (Pa s^d) and d is the dynamic shear flow behavior index.

Protein Content of Gel Exudate (Gel Solubility). The gels were centrifuged at 27000g for 30 min; protein in the exudate was determined as for unheated dispersions (Paulson and Tung, 1987) and expressed as a percentage of total gel protein.

Steady Shear Rheology. Flow properties of 11.4% (w/w) canola isolate dispersions under steady shear were evaluated over a shear rate range of 4.3–1000 s^{-1} at 21 °C with cone/plate fixtures with a Model R.19 Weissenberg rheogoniometer (Paulson and Tung, 1988a). All dispersions followed power law or power law plastic behavior, and the apparent viscosity at a shear rate of 10 s^{-1} was calculated from the fitted flow models. This shear rate corresponds to the oscillatory frequency used for dynamic shear calculations.

Physicochemical Properties. Protein solubility, surface hydrophobicity (S_0), and ζ were determined as previously described (Paulson and Tung, 1987). Hydrophobicity was also determined after heating 0.1% (w/w) protein dispersions at 72 °C for 30 min. The hydrophobicity measured was that which was "exposed" by heating and was designated S_e.

Statistical Analyses. Backward stepwise multiple regression analyses were used to examine effects of protein solubility, hydrophobicity, ζ potential, and steady shear rheological properties on dynamic shear parameters of thermally induced gels using the MIDAS statistical computer program (Fox and Guire, 1976) on an Amdahl 470/V8 mainframe computer.

RESULTS AND DISCUSSION

Thermally Induced Gelation. Thermally induced gels or gellike materials formed at 28 of a possible 54 combinations of succinylation, NaCl concentration, and pH. Visually, they appeared as translucent gels, opaque gels, or gellike precipitates containing insoluble particulate materials. This was similar to the effects of neutral salts and pH on the appearance of ovalbumin gels (Hegg et al., 1979). For unsuccinylated canola isolate only 4 of a possible 18 gels formed, while 12 gels formed from each of the two levels of succinylation.

Storage modulus (G') of each gel increased slightly with oscillatory frequency, while dynamic viscosity (η') decreased in a linear manner when plotted on logarithmic coordinates. A representative rheogram is shown in Figure 1; all samples had similar straight-line dynamic shear behavior. The equation of each line was determined by least-squares linear regression (Tables I and II), from which storage modulus, loss modulus (G''), and loss tangent were calculated for a frequency of 10 s^{-1}.

For unsuccinylated isolate, gels were formed at pH 9.5 in the absence of NaCl and at pH 11 at all NaCl concen-

Table I. Dynamic Shear Storage Parameters of Canola Isolate Gels

succinic anhydride, %	pH	NaCl concentration, M: 0.0 a, Pa s^b	0.0 b	0.0 r^2	0.35 a, Pa s^b	0.35 b	0.35 r^2	0.70 a, Pa s^b	0.70 b	0.70 r^2
0	9.5	70.77	0.130	0.987	–	–	–	–	–	–
	11.0	108.2	0.109	0.952	670.4	0.103	0.988	517.7	0.105	0.968
5.2	5.0	10.44	0.144	0.891	328.9	0.095	0.990	150.7	0.096	0.972
	6.5	28.36	0.155	0.950	510.0	0.120	0.992	565.6	0.133	0.992
	8.0	–[a]	–	–	224.2	0.113	0.988	395.9	0.118	0.988
	9.5	–	–	–	230.9	0.100	0.992	435.8	0.108	0.982
	11.0	–	–	–	178.0	0.071	0.980	489.3	0.077	0.986
14.2	5.0	531.1	0.130	0.984	556.7	0.131	0.986	484.0	0.121	0.986
	6.5	10.30	0.127	0.822	212.9	0.096	0.996	362.4	0.104	0.986
	8.0	–	–	–	59.24	0.056	0.974	188.7	0.101	0.996
	9.5	–	–	–	65.60	0.109	0.984	205.0	0.101	0.988
	11.0	–	–	–	54.21	0.109	0.976	187.9	0.086	0.992

[a] Did not gel.

Table II. Dynamic Shear Flow Parameters of Canola Isolate Gels

succinic anhydride, %	pH	NaCl concentration, M: 0.0 c, Pa s^d	0.0 d	0.0 r^2	0.35 c, Pa s^d	0.35 d	0.35 r^2	0.70 c, Pa s^d	0.70 d	0.70 r^2
0	9.5	21.09	0.210	0.996	–	–	–	–	–	–
	11.0	13.65	0.163	0.986	107.2	0.142	0.999	98.25	0.142	0.999
5.2	5.0	2.45	0.270	0.990	80.19	0.123	0.992	37.90	0.157	0.990
	6.5	5.80	0.245	0.982	88.39	0.115	0.992	167.9	0.084	0.999
	8.0	–[a]	–	–	40.74	0.116	0.999	80.88	0.111	0.999
	9.5	–	–	–	36.83	0.126	0.999	84.42	0.119	0.999
	11.0	–	–	–	25.50	0.089	0.988	68.39	0.033	0.999
14.2	5.0	138.5	0.139	0.999	202.9	0.092	0.999	138.7	0.111	0.999
	6.5	3.56	0.230	0.974	33.48	0.098	0.999	66.97	0.096	0.999
	8.0	–	–	–	10.30	0.105	0.999	31.46	0.112	0.996
	9.5	–	–	–	12.27	0.118	0.996	30.90	0.112	0.996
	11.0	–	–	–	10.39	0.072	0.999	28.82	0.105	0.992

[a] Did not gel.

trations. All gels were opaque although the gel at pH 11.0 without NaCl had a great deal of translucent character. As seen in Figure 2A, as pH increased from 9.5 to 11.0, G' increased slightly for gels without NaCl. At pH 11.0, G' increased dramatically and then decreased as NaCl concentration increased to 0.35 M and then to 0.70 M. Treatment effects on the loss modulus of each gel paralleled the effects on storage modulus except for gels without NaCl where a slight decrease in G'' was seen as pH was raised from 9.5 to 11.0 (Figure 3A). The loss tangent of gels without NaCl decreased as pH increased from 9.5 to 11.0 (Figure 4A), whereas at pH 11.0 the loss tangent increased with NaCl concentration. Since the loss tangent reflects the proportion of viscous to elastic character in a viscoelastic material (Ferry, 1980), the gels became proportionately more elastic as pH increased to 11.0 and as NaCl concentration decreased at pH 11.0.

For canola protein isolate modified with 5.2% succinic anhydride (54% modification of amino groups), gels were formed at pH 5.0 and 6.5 both with and without NaCl, while from pH 8.5 to 11.0 gels formed only in the presence of NaCl. Gels at pH 5.0 were opaque and pasty whereas all others were translucent and springy. For pH 5.0 gels, increasing NaCl concentration to 0.35 M increased G' and G'' followed by a decrease in these parameters at 0.70 M NaCl (Figures 2B and 3B). For translucent gels (pH 6.5 and above), G' and G'' increased as NaCl concentration increased. Both G' and G'' were greatest at pH 6.5 for each level of NaCl. With the exception of the sample at pH 6.5 with 0.35 M NaCl, the loss tangent decreased, indicating that the elastic component of each gel increased as pH increased. Loss tangent was dependent on NaCl concentration as well (Figure 4B). Gels without NaCl had a higher loss tangent than gels with 0.35 M NaCl, while at each pH except 11.0 gels with 0.70 M NaCl had a higher loss tangent than gels with 0.35 M NaCl.

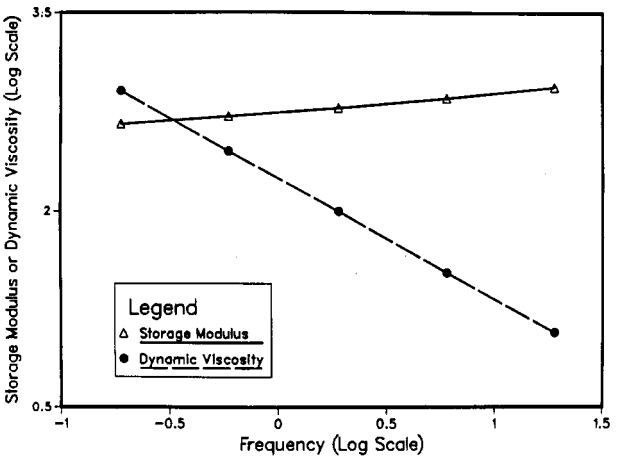

Figure 1. Storage moduli and dynamic viscosities of 5.2% SA-treated, pH 6.5, 0.7 M NaCl canola isolate gel as a function of oscillatory frequency.

Canola protein isolate succinylated with 14.2% succinic anhydride (84% modification of amino groups) had gelation behavior similar to that of the 5.2% SA-treated isolate. Opaque, pasty gels were formed at pH 5.0 at all NaCl concentrations whereas translucent gels formed at pH 6.5 and above. From pH 8.5 to 11.0, gels formed only in the presence of NaCl, whereas at pH 6.5 the sample without NaCl did not quite form a self-supporting gel but thickened considerably upon heating and appeared translucent and elastic. For translucent gels, both G' and G'' increased with NaCl concentration, whereas for opaque gels G' and G'' were highest at 0.35 M NaCl (Figures 2C and 3C).

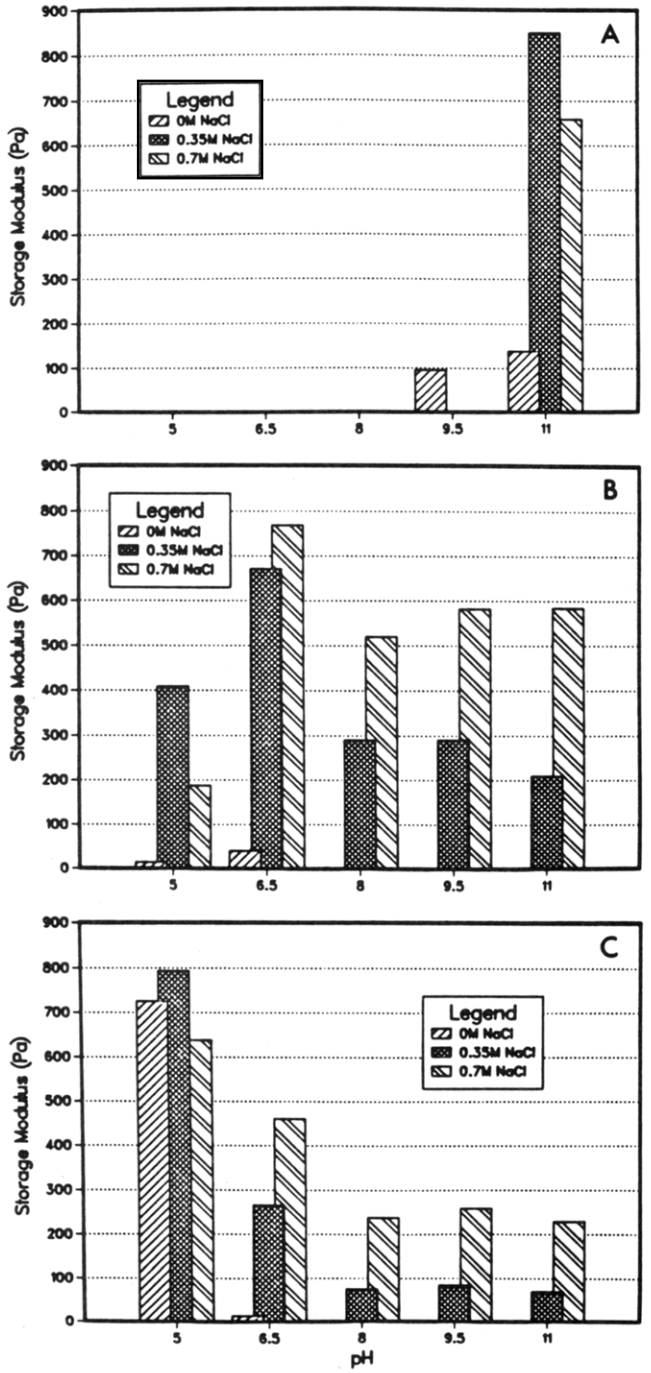

Figure 2. Storage modulus at 10 s^{-1} of 11.4% canola isolate gels: (A) unmodified; (B) 5.2% SA-treated; (C) 14.2% SA-treated.

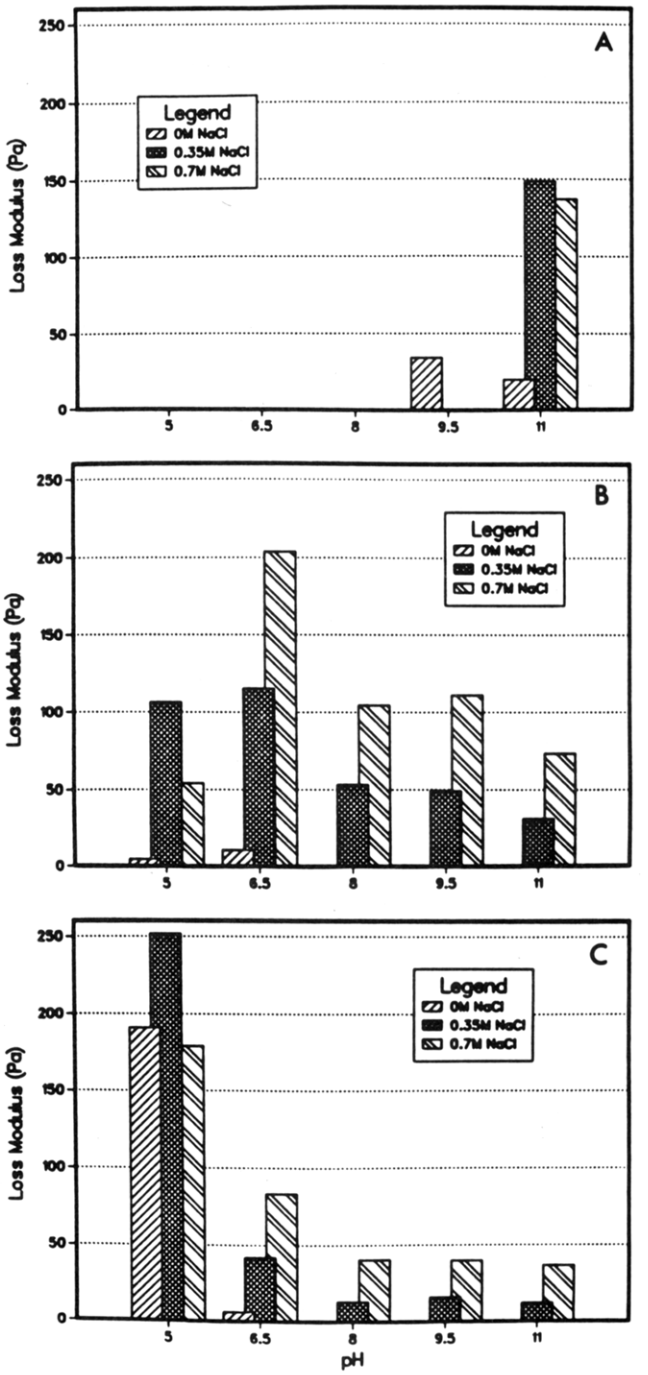

Figure 3. Loss modulus at 10 s^{-1} of 11.4% canola isolate gels: (A) unmodified; (B) 5.2% SA-treated; (C) 14.2% SA-treated.

Thus, for all opaque gels, G' and G'' first increased and then decreased as NaCl increased, while for all translucent gels these parameters increased as NaCl increased. For all gels there was a close association between G' and G'' where both the elastic and viscous components increased simultaneously.

Effects of pH and NaCl on loss tangent of 14.2% SA-treated gels are shown in Figure 4C. As expected, the loss tangent for the gel at pH 6.5 without NaCl, which appeared to be on the gelation threshold, was appreciably higher than for the self-supporting gels. All loss tangents were substantially less than 1.0, however, indicating that all gels were proportionately more elastic than viscous. For the self-supporting translucent gels, neither pH nor ionic strength greatly affected the loss tangent. Opaque gels had a higher loss tangent than self-supporting translucent gels; thus even though both G' and G'' were higher for opaque gels, there was proportionately less elastic than viscous character.

The high viscoelastic moduli of opaque gels appeared to contradict the results of a puncture probe test as well as visual observation. With a puncture test (Paulson and Tung, 1988b) the force required to rupture opaque gels was of the same order or less than for translucent gels. Opaque gels appeared pasty, lacked springiness, and syneresed readily. A similar effect was noted by Gill and Tung (1978) with thermally induced gels from the 12S fraction of rapeseed. They found that gels at pH 6.0 had lower apparent viscosity in steady shear but higher viscoelastic parameters in dynamic shear than gels at higher pH. Microscopic examination showed the presence of aggregates in the pH 6.0 gel, and the authors hypothesized that the aggregates were responsible for highly elastic recoveries under the

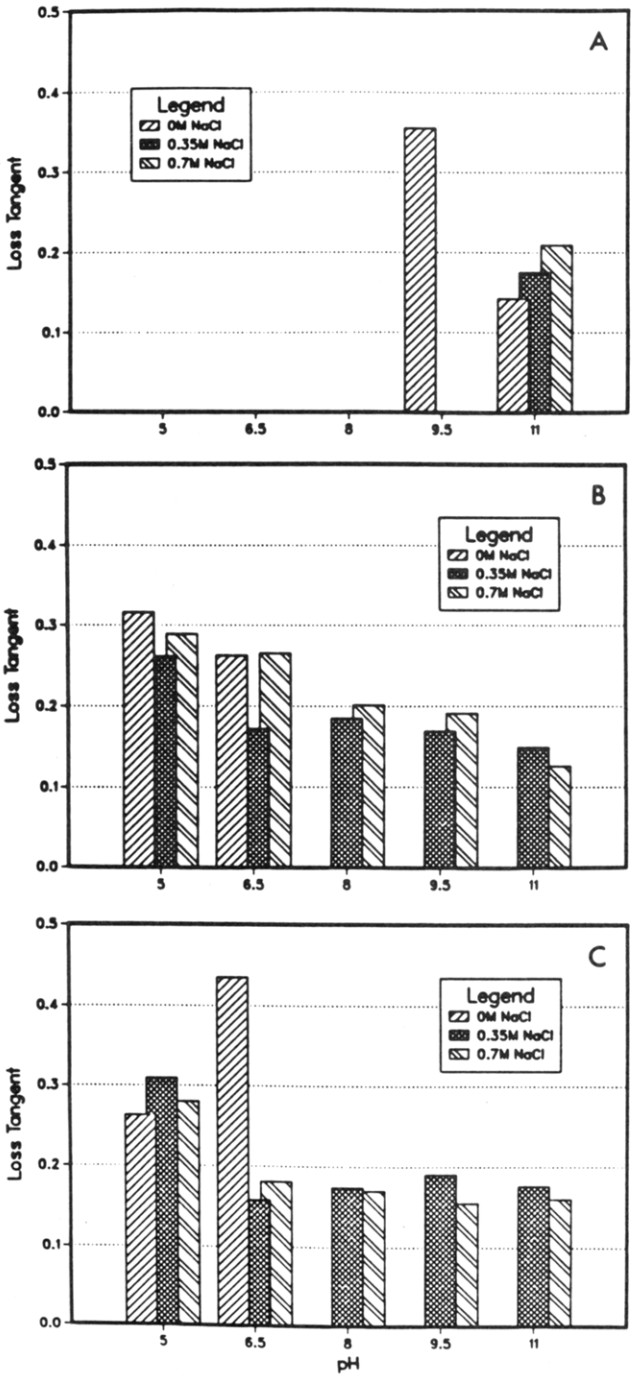

Figure 4. Loss tangent at 10 s^{-1} of 11.4% canola isolate gels: (A) unmodified; (B) 5.2% SA-treated; (C) 14.2% SA-treated.

nondestructive small deformations applied to dynamic testing. With steady shear conditions, however, the forces between aggregates would be broken and the aggregates would then be able to move readily with respect to one another. Dispersions that formed opaque gels in the present study all contained relatively large protein aggregates, and these gels would be expected to behave differently under nondestructive and destructive testing than translucent gels, which appeared to have a relatively homogeneous gel matrix.

To date, nearly all reports published on the thermal response of succinylated food proteins have indicated increased heat stability and a decrease or loss of gelation ability (Kinsella and Shetty, 1979), presumably as a result of increased charge repulsion between molecules (Ma and Holme, 1982; Sato and Nakamura, 1977). In contrast, Miller and Groninger (1976) found that gelation of fish protein concentrate was improved when 43–59% of the amino groups were succinylated. Choi et al. (1981) reported that limited succinylation of cottonseed protein increased gel strength of 20% protein dispersions, and Montejano et al. (1984) found that succinylated egg white required higher heating temperatures for gelation, but the gels had significantly greater strength and deformability at failure than those from native egg white. In the present study, the enhanced electronegativity of succinylated protein molecules appeared to reduce or prevent gelation in the absence of NaCl, but the addition of NaCl overcame charge repulsion, thus allowing close approach and aggregation of the protein molecules into a gel upon heating. This hypothesis is supported by the observed effects of succinylation level and ionic strength where G' and G'' decreased as succinylation increased from 54% to 84% of amino groups. Succinylation increased the electronegativity of the protein molecules, while an increase in NaCl concentration progressively overcame this effect. The effect of pH also supports this hypothesis, as unsuccinylated isolate formed gels only at high pH whereas succinylated isolates formed firmest gels in the lower end of the pH range in which gels formed. There appeared to be an optimum NaCl concentration for maximization of the viscoelastic moduli as indicated by the effect of NaCl on the opaque gels; the moduli first increased as NaCl concentration increased from 0.0 to 0.35 M and then decreased with 0.70 M NaCl. The optimum did not appear to be reached with the translucent gels, however.

It was apparent that extent of succinylation, NaCl concentration, and pH were all important variables in the gel-forming ability of canola isolate and the viscoelastic properties of the gels. As these variables also influenced protein solubility, hydrophobicity, ζ potential, and flow behavior (Paulson and Tung, 1987, 1988a), which in turn were thought to influence viscoelastic properties of the gels, relationships among these factors were examined by multiple regression analysis. Since the gels dissolved in 8 M urea or 6 M guanidine hydrochloride, covalent bonds such as disulfide were believed not to be involved in gel formation or stabilization.

For multiple regression analysis, viscoelastic parameters of the gels were used as dependent variables while potential independent variables included power law flow parameters from 11.4% isolate dispersions (Paulson and Tung, 1988a), ζ potential, surface hydrophobicity before (S_0) or after (S_e) heating, and protein solubility. Hydrophobicities of heated dispersions (S_e) were overall slightly higher than before heating (S_0) ($p < 0.01$) although this did not hold true for all environmental conditions (Table III). These small differences did not necessarily mean that heating produced little change in protein conformation, however, as relatively few peptide residues need to be exposed to the solvent in order to render the native conformation of the protein unstable (Franks and Eagland, 1975).

Schmidt (1981) emphasized that the importance of subjective or qualitative evaluation of protein gels should not be underestimated, as measurably strong protein gels may range in appearance from translucent and elastic to curdlike and opaque. As previously noted, the gels could be divided into two major classes based on visual observation: translucent and opaque. It has been hypothesized that, due to their microstructure, viscoelastic properties of these classes of gels may differ. Translucent gels varied in character from firm and springy to a thick material that was gellike and springy but was not self-supporting. Opaque gels ranged from gellike pasty precipitates with

Table III. Effects of Succinylation, pH, NaCl, and Heating on Surface Hydrophobicity of Canola Isolate

succinic anhydride, %	pH	NaCl concentration, M: 0.0 S_0[a]	0.0 S_e[b]	0.35 S_0	0.35 S_e	0.70 S_0	0.70 S_e
0	3.5	533.6	586.0	457.5	505.4	440.3	482.0
	5.0	263.3	249.5	222.9	230.1	236.4	214.8
	6.5	81.6	112.6	146.9	115.7	160.5	146.5
	8.0	68.0	78.3	133.3	139.8	126.2	145.6
	9.5	57.3	78.2	129.3	111.8	122.2	129.8
	11.0	33.4	32.6	62.8	69.5	91.7	85.4
5.2	3.5	578.5	605.7	429.0	461.3	382.2	477.3
	5.0	105.8	119.4	144.6	168.1	202.2	149.3
	6.5	52.8	49.5	109.3	103.9	125.4	136.1
	8.0	34.6	43.1	80.5	96.1	97.1	116.4
	9.5	37.9	37.3	82.2	80.1	78.3	106.9
	11.0	21.9	16.8	66.7	59.7	83.7	77.8
14.2	3.5	520.9	609.5	433.4	480.4	331.2	340.9
	5.0	77.5	117.3	143.4	145.5	144.8	138.3
	6.5	36.6	37.7	73.9	83.0	106.8	108.6
	8.0	23.2	20.2	65.7	78.0	103.4	103.6
	9.5	25.2	22.5	61.7	69.0	89.9	92.2
	11.0	21.1	15.4	71.3	58.5	73.0	63.9

[a] Hydrophobicity of unheated dispersions. [b] Hydrophobicity of heated dispersions.

Table IV. Multiple Regression Models for Prediction of Viscoelastic Parameters of Translucent Gels (n = 18)

dependent variable	independent variable	coefficient	F prob
storage modulus	solubility	−25.755	0.016
R^2 = 0.775	S_e	4.089	0.024
SE[a] = 116.74	constant	2.329×10^3	
F prob = 0.000			
storage modulus	S_e	7.662	0.000
R^2 = 0.770	η_{10}	0.750	0.019
SE = 117.89	constant	−491.380	
F prob = 0.000			
storage modulus	exudate protein	−44.062	0.000
R^2 = 0.963	exudate protein^2	0.234	0.000
SE = 47.32	constant	2.099×10^3	
F prob = 0.000			
loss modulus	S_e^2	0.222×10^{-1}	0.000
R^2 = 0.882	solubility × S_e	-0.266×10^{-1}	0.005
SE = 18.48	constant	89.538	
F prob = 0.000			
loss modulus	S_e	−2.360	0.042
R^2 = 0.888	S_e^2	0.239×10^{-1}	0.002
SE = 18.62	η_{10}	0.155	0.023
F prob = 0.000	constant	47.714	
loss tangent	S_e	-0.387×10^{-1}	0.000
R^2 = 0.900	S_e^2	0.382×10^{-3}	0.000
SE = 0.024	S_e^3	-0.117×10^{-5}	0.000
F prob = 0.000	constant	1.390	

[a] Standard error of estimate.

very little elasticity to one that was largely translucent with some opaque character. Translucent gels included all gels formed from each succinylated isolate at pH 6.5 or higher (18 total) whereas opaque gels included those formed at pH 5.0 from each succinylated isolate plus gels from unmodified isolate (10 total).

For translucent gels, G' was well described by solubility and S_e, where G' was inversely related to solubility and positively related to S_e (R^2 = 0.775; Table IV). When apparent viscosity at 10 s^{-1} (η_{10}) of unheated dispersions was allowed as an independent variable, η_{10} and S_e together accounted for 77.0% of the variation in G'. G' increased as η_{10} and S_e increased.

Although the water-holding capacity of gels has been examined extensively, little has been reported on the relationship between protein content of the gel exudate and textural properties. The storage modulus of translucent

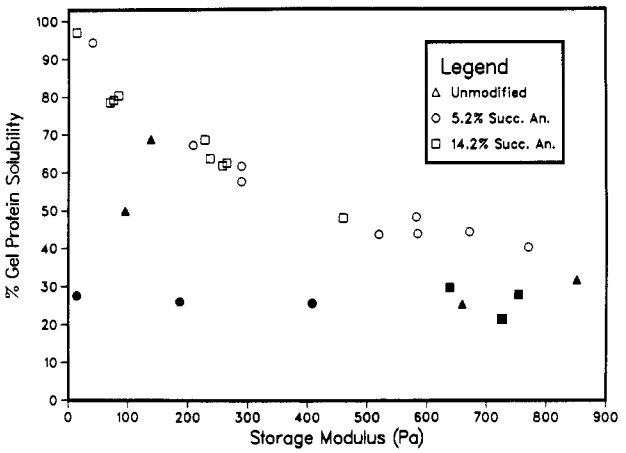

Figure 5. Gel exudate protein solubility vs. storage modulus of canola isolate gels (solid symbols indicate opaque gels).

gels followed a curvilinear relationship with exudate protein that accounted for more than 96% of the variation in G' (Table IV; Figure 5, open symbols). As exudate protein decreased, G' increased in a curvilinear manner. Therefore, for these gels it appeared as if G' was related to the number of protein molecules taking part in junction zones and cross-links and was probably the result of a greater number of similar bonds rather than a few covalent linkages. For opaque gels there were no significant relationships between gel exudate protein and the viscoelastic parameters (Figure 5, solid symbols).

The loss modulus (G'') of translucent gels was well described by S_e^2 plus the interaction of η_{10} and S_e (R^2 = 0.882, Table IV). G'' increased in an upward curvilinear manner with S_e, and G'' generally increased as solubility decreased. Alternatively, when η_{10} was allowed as an independent variable, η_{10}, S_e, and S_e^2 accounted for 88.8% of the variation in G''. As with G', G'' followed a curvilinear relationship with exudate protein (R^2 = 0.836), where G'' increased at a faster rate as protein in the gel exudate decreased. This may be the result of limited polymerization of protein molecules producing larger aggregates or longer strands but without complete cross-linking or integration into the three-dimensional gel matrix.

Loss tangent of translucent gels was described by a cubic fit of S_e (R^2 = 0.900; Table IV) where minimum values of loss tangent were found at intermediate S_e, while loss tangent increased at lower and higher values of S_e. This implied that even though both G' and G'' increased with S_e, optimum development of the elastic portion of a gel as compared to the viscous portion occurred when S_e was neither high nor low. With low hydrophobicity, there would be few areas on the surfaces of the protein molecules for three-dimensional network formation, but limited hydrophobic interactions would allow for aggregation of protein molecules to form strands as proposed by Tombs (1970, 1974), thus increasing the viscous component of the system. Alternatively, if protein hydrophobicity was very high and a relatively large proportion of the surface was able to take part in hydrophobic interactions, aggregation would be expected to be less ordered, giving larger more approximately spherical particles and a coarser, less well-oriented gel network structure. The larger the aggregate size, the smaller would be the contribution from each particle in the gel network (Tombs, 1974); hence, the proportion of elastic to viscous components of the system would be expected to decrease even though both G' and G'' would increase.

For opaque gels, G' was affected by S_e, ζ potential, and η_{10} (Table V), while significant variables influencing G''

Table V. Multiple Regression Models for Prediction of Viscoelastic Parameters of Opaque Gels (n = 10)

dependent variable	independent variable	coefficient	F prob
storage modulus	S_e	−12.650	0.004
R^2 = 0.919	ζ potential	44.716	0.002
SE[a] = 120.17	η_{10}	1.588	0.002
F prob = 0.006	$S_e \times \zeta$	−0.338	0.008
	constant	1.724×10^3	
loss modulus	S_e	−1.381	0.043
R^2 = 0.886	solubility^2	-0.494×10^{-1}	0.006
SE = 34.25	η_{10}	0.576	0.001
F prob = 0.003	constant	197.01	
loss tangent	solubility^2	-0.238×10^{-4}	0.034
R^2 = 0.449	constant	0.308	
SE = 0.054			
F prob = 0.034			

[a] Standard error of estimate.

were protein solubility, S_e, and η_{10} (R^2 = 0.886). For the loss tangent, only the square of solubility was a significant independent variable and accounted for less than 45% of the variation in this parameter. Viscoelastic properties of the opaque gels appeared to be influenced not only by the gel matrix but by aggregates that also lend opacity to the gels. Thus, the analysis of factors influencing viscoelasticity of opaque gels was complicated not only by the properties of the continuous phase but also by such factors as the size, shape, number, and internal structure of the aggregates. These factors may in turn depend on extent of succinylation, pH, and ionic strength. Since the factors influencing formation and physical properties of the aggregates would not necessarily be the same as those for the gel matrix, and may in fact have opposing influences, it is difficult to make a meaningful interpretation of the mode of action of the predictive factors on the system as a whole.

Gelation is a protein aggregation phenomenon where attractive and repulsive forces between protein molecules and solvent are so balanced that a well-ordered three-dimensional network or matrix is formed that is capable of trapping or immobilizing large amounts of solvent. Ionizable amino acids play an important role in determining electrostatic interactions between protein molecules, and therefore factors such as salts, pH, and temperature influence the balance between attractive and repulsive forces in the system (Wall, 1979). During denaturation, hydrophobic and hydrogen bonds buried in the interior of the molecule become exposed and re-form in a manner different from the native structure (Buttkus, 1974). Thus, regions of the molecule originally involved in stabilizing native structure become available for intermolecular interactions. A three-dimensional network can then form, provided that there are at least two attractive sites per molecule (Clark et al., 1981). Therefore, it appears as if optimum gelation conditions occur when attractive forces released by protein denaturation are just strong enough to counteract electrostatic repulsion, and an ordered limited aggregation can take place resulting in a gel network. Gel characteristics would be determined by the number of bonding sites available on each protein molecule, their spacial distribution, and relative bonding strengths under the prevailing conditions of pH and ionic strength. Hegg et al. (1979) noted that ovalbumin gels were formed at an intermediate state between high charge repulsion, which gave solubility, and low repulsion that resulted in precipitation. In a medium where ovalbumin underwent gelation, however, gels became increasingly transparent as net charge repulsion increased such as by increasing pH or decreasing the ionic strength.

Previous studies with succinylated protein have demonstrated increased thermal stability and decreased or retarded heat-induced gelation or coagulation (Ma and Holme, 1982). In the present study, the solubility of canola protein under slightly acidic and alkaline conditions was greatly improved by succinylation as a result of increased electronegativity of the protein molecules, but this also appeared to increase thermostability and reduce gelation at low ionic strength. With the addition of NaCl, however, intermolecular charge repulsion was reduced, which allowed for gelation when protein–protein and protein–solvent interactions were properly balanced. NaCl promotes aggregation due to reduction of the diffuse part of the electric double layer and may also induce conformational changes in protein molecules (Fennema, 1977) which may alter the number of bonding sites for gelation. Heating protein dispersions also induces conformational changes that tend to increase protein–protein interactions (Tombs, 1974). NaCl has also been found to improve the gel strength of many other thermally processed protein systems (Hermansson, 1982; Schmidt and Illingworth, 1978; Schmidt et al., 1978; Shimada and Matsushita, 1981).

Although not examined in depth, low levels of divalent cations were able to induce gelation in heated dispersions of succinylated canola isolate. This was suspected to be a result not only of enhanced depression of the electric double layer but also of the capacity of divalent cations to link two protein molecules together by their carboxyl groups. Preliminary experiments demonstrated that 0.035 M $CaCl_2$ in 11.4% dispersions of both 5.2% and 14.2% SA-treated canola isolates at pH 6.8 gave similar gel strengths, as determined by a puncture test, to those obtained with 0.70 M NaCl. Protein precipitation and therefore no gelation occurred with 0.35 M $CaCl_2$. A similar effect of ion type on gelation of whey protein concentrate was found by Schmidt et al. (1978). This finding may have practical importance as it demonstrates that the gelation ability of succinylated proteins may be utilized in products where high levels of salts such as NaCl may be detrimental to product quality or undesirable for health reasons.

The major bonds involved in gel formation and stability were tentatively identified as hydrophobic interactions and hydrogen bonds. It seems likely that hydrophobic groups largely contributed to gel formation since the gels formed during heating. In this temperature range, hydrophobic interactions increase with temperature (Ben-Naim, 1980). Schmidt (1981) suggested that hydrophobic interactions are important to dissociative–associative reactions that initiate the gelation process and contribute to layering or thickening of the gel matrix strands upon cooling. Shimada and Matsushita (1980) found a relationship between the proportion of hydrophobic groups in the amino acid profile of proteins and the type of gel formed upon heating. Hydrophobic and acidic residues dominate the amino acid profile of rapeseed proteins, whereas basic amino acids are in relatively low concentration (Sosulski and Sarwar, 1973). In the present study, the gels increased in firmness upon cooling, thus implicating the involvement of hydrogen bonds that are weakened by increasing temperature (Joesten and Schaad, 1974). Oakenfull and Scott (1984) reported that a combination of hydrogen bonds and hydrophobic interactions stabilized gels formed from high-methoxyl pectins. Schmidt (1981) suggested that hydrogen bonds stabilize gel structure and allow for a more open orientation necessary for water immobilization.

Although the effects of other heating conditions were not examined in detail, it was noted that temperatures up

to 160 °C did not destroy the gelation ability of the isolates. This is in contrast to soy protein, for which temperatures in excess of 125 °C have been reported to result in a metasol that did not form a gel upon cooling (Catsimpoolas and Meyer, 1970). This is of potential importance for the use of succinylated canola protein in a retorted product. Other authors have reported on the effects of heating conditions on gel formation, with varied results depending on type of protein, ionic environment, and heating conditions (Hermansson, 1982; Tombs, 1970; Schmidt and Illingworth, 1978; Schmidt et al., 1978). In view of these reports, the effects of heating conditions on the gelation properties of succinylated canola protein could form the basis of further studies.

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