Heat-Induced Gelation of Rapeseed Proteins: Effect of Protein Interaction and Acetylation

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ABSTRACT: The gel-forming abilities of a rapeseed protein isolate, composed of 70% globulin (cruciferin) and 30% albumin (napin), and their individual protein components, were investigated. The influence of acetylation upon the gelation properties was also studied. Highest gel strength (measured as shear modulus) of the isolate was obtained at pH values around 9, which is between the isoelectric points of both major proteins. Purified cruciferin gave the highest shear modulus values, with maxima at pH 6 and 8. Weak and poorly stable gels exhibiting strong hysteresis were obtained with isolated napin. Acetylation resulted in a pH shift of the shear modulus maximum of the protein isolate to about 6. The gelation temperature of the acetylated isolate had the highest pH and concentration dependence compared with the other proteins.

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KEY WORDS: Acetylation, gelation, protein gels, protein isolate, rapeseed proteins, rheological properties, shear modulus.

The ability to form gels is a key functional property in utilizing plant proteins. The gel-forming properties of proteins from soybeans and other legume and oilseed crops have been extensively studied (1–7).

Gill and Tung (8) first demonstrated the ability of a highly glycosylated 12S rapeseed protein to form gels on heating at pH > 4. The strongest gels were formed at high pH and ionic strength. The high level of carbohydrate (12.9%) led the authors to assume protein–carbohydrate interaction during gel formation. Thompson *et al.* (9) observed increased viscosity of a hexametaphosphate-extracted rapeseed protein isolate on heating to 80°C but did not obtain a gel. Paulson and Tung (10) studied thermally induced gelation of a rapeseed (canola) protein isolate on heating to 72°C. Gels formed only at high pH (>9.5).

Léger and Arntfield (11) studied gelation of 12S canola globulin. Gels prepared with 6% protein under alkaline conditions were superior to gels prepared from acidic solutions. The effects of pH, salts, and denaturing and reducing agents on gelation properties led the authors to conclude that hydrophobic forces and electrostatic interactions were responsible for establishing the gel network, while gel stabilization and strengthening were attributed to disulfide bonding, electrostatic interactions, and hydrogen bonding.

Succinylation has been used to improve the gel-forming properties of a canola protein isolate by Paulson and Tung (10). In this way, the pH range of gel formation was extended from the alkaline region (pH > 9.5) to 5.0.

Comparison of the gel-forming properties of the different rapeseed protein preparations is difficult owing to differences in protein composition and purity. Moreover, data on the protein composition of the isolates studied are scanty.

The present paper deals with the influence of the major protein components of rapeseed protein isolate [12S globulin "cruciferin" and 2S protein "napin" (12)] and of acetylation on the gel-forming properties of the isolate. The pH and concentration dependencies on the gelation temperature and the shear modulus of purified cruciferin and napin as well as nonmodified and acetylated isolates containing both proteins were studied.

MATERIALS AND METHODS

Protein preparation and characterization. Rapeseed flour (*Brassica napus* var. Lirajet) was prepared from dehulled meal by defatting with hexane, desolventizing by drying at room temperature, and screening (particle size <315 μm). Protein was extracted at room temperature by stirring the flour 30 min with water (wt/vol, 1:20) maintained at pH 8.5 by adding 1 N NaOH. After centrifugation (13500 × *g*, 20 min), the extract was exhaustively dialyzed against distilled water, to remove low-molecular-weight material, and freeze-dried. The nitrogen content was determined by means of Kjeldahl digestion, followed by indophenol blue reaction with commercially available test cuvettes (No. LCK 304, Dr. Lange, Düsseldorf, Germany). The nitrogen–protein conversion factor of 5.7 was used for estimating protein content (13,14).

The protein composition of the isolate was determined by means of sodium dodecyl sulfate–polyacrylamide gel electrophoresis. A combined Weber and Osborn (15) and Laemmli (16) system was used. The separation and sample gel were prepared according to Weber and Osborn; the electrode buffer and the sample buffer were according to Laemmli.

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The isolate was acetylated by adding acetic anhydride to a 5% (wt/vol) protein solution in phosphate buffer pH 8.0 (0.05 M phosphate, brought to an ionic strength of 0.5 with NaCl) at ambient temperature. The pH was maintained at 7.5–7.8. The degree of acetylation was determined by means of 2,4,6-trinitrobenzensulfonic acid as described elsewhere (17). The 12S globulin and the 2S protein fraction were prepared according to Raab and Schwenke (18).

Gel preparation and characterization. Proteins were dispersed in distilled water by stirring, and the desired pH was adjusted by adding 1 N NaOH or 1 N HCl and monitoring for 30 min. The dispersion was poured into the measuring system. The kinetics of elasticity development of the sample at the beginning of gelation was observed by dynamic shear modulus measurements (19) based on shear deformation in a tube (20). A sinusoidal pressure impulse was applied to the sample in 1-min time intervals while the sample temperature was steadily increased (1°C/min) to estimate the gelation temperature, or kept constant to estimate reaction time. Values of 0.5 to 200 Pa for the shear moduli were measurable with the equipment. The sample tube had an inner diameter of 10 mm. The sample column had a length of *ca.* 74 mm.

For measuring gel strength, the gels were prepared in the measuring systems at conditions for optimal gel strength, i.e., the pH-controlled protein dispersion was transferred to the preheated system, kept for 20 min at 10°C above the gelation temperature, then cooled down to room temperature, and maintained 15 h for ripening. The viscoelastic properties of gels were investigated by shear deformation between parallel plates with antislip lamellae (19). Stepwise constant loading (6 s) and relaxation (60 s) were used for deforming the gel fracture. The shear modulus was estimated from the initially linear-elastic region of the stress–strain curve.

The parallel-plate shear device was operated within a region of G = 10–20000 Pa. The horizontally arranged gel slices were 28 mm in diameter and 2.2 mm high. The measuring systems used prevented predeformation from sample handling.

The data are the mean values of quadruplicate (isolate and acetylated isolate) or duplicate (cruciferin and napin) analysis. The relative error was ±5% and ±1°C for the determination of the moduli and gelation temperature, respectively.

RESULTS AND DISCUSSION

Protein characterization. Figure 1 shows the electrophoretograms of the protein isolate, and 12S globulin (cruciferin) and 2S protein (napin) fractions. The cruciferin and napin contents amounted to 70 ± 2 and 30 ± 2%, respectively. Analysis of the acetylated protein isolate revealed 93% modification. The isoelectric points (IP) of the components were determined by isoelectric focusing to be around pH 6. IP values of the native protein components cruciferin and napin were previously shown (13,14) to be about 7 and >10, respectively.

Gelation temperature. A typical curve of kinetics of the gelation onset is given in Figure 2. The smallest measurable increase in shear modulus (1 Pa/°C/min) was taken as the starting point of gelation. The gelation temperature determined for the given example (15% protein, pH 9.0) was 69°C. Gelation could be induced, however, at lower temperature by subjecting the system to longer heating. When it was heated, for example, for 1 h and the temperature was maintained 4°C below the above-determined gelation temperature, gelation occurred. This temperature was considerably lower than the denaturation temperature of 78.5°C for an isoelectric canola isolate and of 88.0°C for a "micelle" isolate determined by Murray *et al.* (21). The denaturation temperature of a 12S globulin preparation was found by Léger and Arntfield (11) to range between 80 and 81.3°C at pH 6–10.

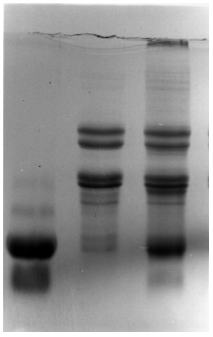

FIG. 1. Electrophoretograms (sodium dodecylsulfate-polyacrylamide gel electrophoresis) of rapeseed proteins: 1 napin; 2 cruciferin; 3 protein isolate.

Figure 3 shows the dependence of the gelation temperature on the protein concentration at about pH 9 and 6 for the unmodified proteins and the acetylated isolate, respectively. Generally, increasing protein concentration led to earlier onset of gelation during heating. Among the unmodified proteins, this dependence was most pronounced for the protein isolate; the gelation temperature drops from 79.5°C in a 7.5% dispersion to 66°C at 20% concentration. The smallest temperature change, from 72 to 70°C, was observed for cruciferin, while for the napin fraction, the temperature dropped from 86 to 82°C. The concentration dependence of the isolate demonstrated the influence of both protein components as well as the dominating effect of the globulin. A problem in the measurement of the napin gels was their weakness at lower pH values and their tendency for syneresis. The latter has not been observed in the gels of the isolate, although they had napin contents of about 30%.

The highest concentration dependence of gelation temperature was found for the acetylated protein isolate when measured at pH close to the isoelectric region. The gelation temperature decreased from 74°C in 8% protein dispersions to 43°C in 15% dispersions (Fig. 3).

Figure 4 gives the pH dependencies of gelation temperature for 12.5% dispersions of the different proteins. The maximal gelation temperature was measured for both cruciferin (72°C) and the isolate (77°C) around a pH of 7, which corresponded to the isoelectric region of cruciferin. The gelation temperature of the strongly basic napin fraction increased from 80°C at pH 10 to 95°C at pH 7. This behavior corresponded to the increasing electrostatic repulsion with distance

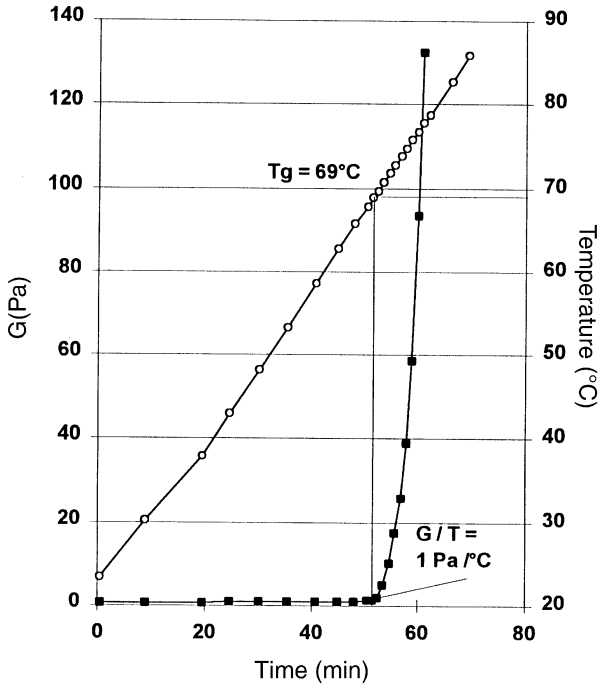

FIG. 2. Kinetics of the gelation onset of rapeseed protein isolate. $C_{protein}$ = 15%, pH = 9.0; ■, shear modulus; ○, sample temperature. G, shear modulus; Tg, gelation temperature; T, temperature.

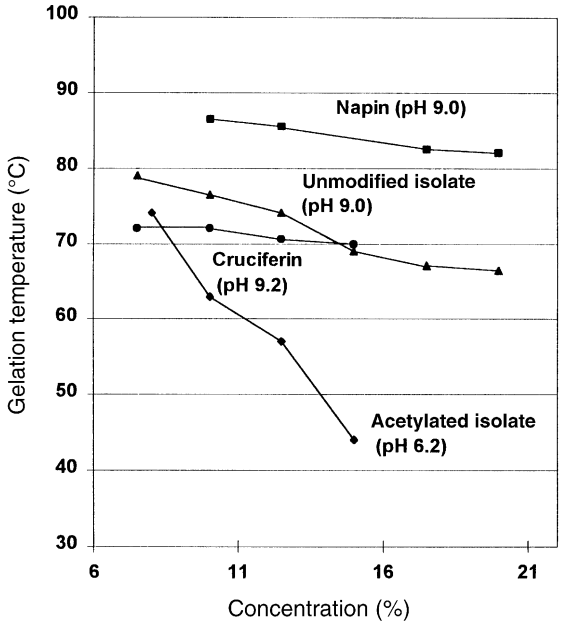

FIG. 3. Concentration dependence of the gelation temperature of rapeseed proteins.

from the isoelectric point, which renders the aggregation of polypeptide chains more difficult.

The acetylated isolate exhibited the strongest pH dependence for gelation temperature, increasing from 53°C at pH 6 to 95°C at pH 9.5. This is due to an increasing repulsive effect of the negatively charged carboxyl groups, the neutralization of which by the positively charged amino groups was drastically diminished after acetylation of the latter.

When the dispersion of the acetylated isolate was brought to pH 5.5 and slowly heated, weak setting occurred at 32°C. Further heating to 40°C resulted in isoelectric flocculation to produce a coarse and pasty material. Translucent gels were obtained at pH > 6 in contrast to the gels from the unmodified isolate, which were opaque. This different behavior reflects structural differences of these proteins.

Although there is a lack of detailed information on conformational changes of the rapeseed globulin cruciferin after acetylation, the existing knowledge on structural changes in succinylated cruciferin (22) and acetylated napin (23), as well as those in acetylated plant globulin homologous to cruciferin (24), may be used to interpret the functionality of acetylated rapeseed protein isolates. These data point to considerable changes in the globulin spatial structure, whereas that of napin changed only slightly owing to stabilization by disulfide bridges. A marked increase of the surface hydrophobicity at high degrees of acetylation, which has been observed for napin (23) and faba bean 11S globulin (24), can also be assumed for the rapeseed globulin component. In the latter, the hydrophobicity increase should be due both to the attachment of hydrophobic acetyl residues and to intrinsic factors that became evident by exposing buried hydrophobic residues after unfolding of the protein. Loosening of the spatial structure facilitates the formation of gels. Thus, the pH range of possible gel formation of canola protein isolate could be enlarged after succinylation from pH > 9.5 to pH 5.0 (10). Succinylated faba bean protein isolates were able to form gels without prior heating (25). Similar effects have been observed with the acetylated rapeseed protein isolate in this study. Therefore, one has to take into consideration different mechanisms of gel formation for the unmodified and the modified isolate.

The opaque appearance of the gels from unmodified isolate suggests a coagulate structure, corresponding to random aggregates of protein molecules in the gel network (26,27). Random aggregation of only partially unfolded protein molecules can explain the gelation onset below the denaturation temperature. Contrary to that, acetylated proteins are substantially unfolded, which may be described as the "molten globule" state (28). The structures of the translucent or even transparent gels, formed by the modified protein isolates at pH above 6, may correspond to a network built up of "string of beads" polymers (27).

This type of network is formed by a rather ordered aggregation of unfolded polypeptide chains, which is favored by a certain extent of electrostatic repulsions (26,27). The latter are effective in the acetylated isolate at pH values above the IP. A similar mechanism is also proposed for the gelation of napin, although it formed transparent weak gels at pH 7–9.

Gel strength (shear modulus). Figure 5 shows the dependence of the shear modulus on the pH value of the protein dis-

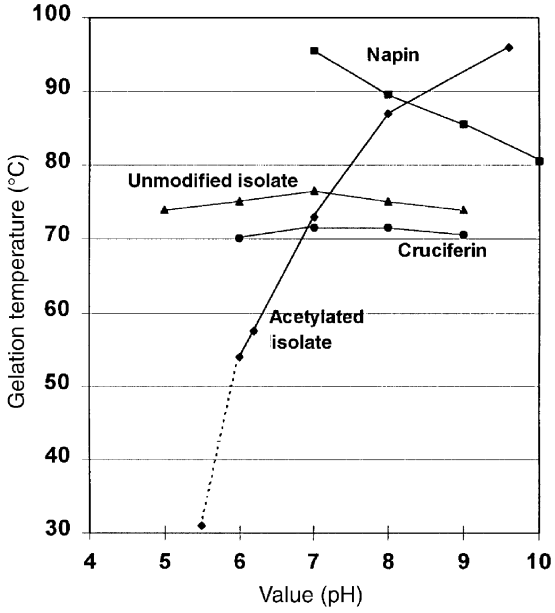

FIG. 4. The pH dependence of the gelation temperature of rapeseed proteins. $C_{protein}$ = 12.5%.

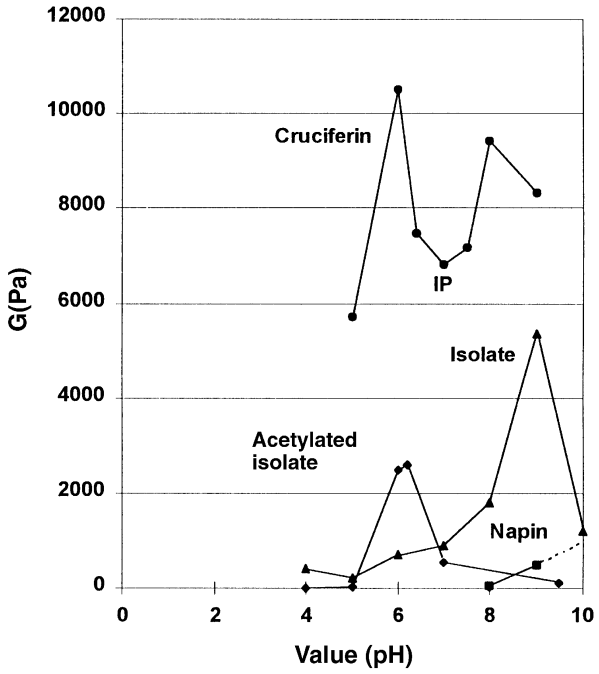

FIG. 5. The pH dependence of the shear modulus of rapeseed protein gels. $C_{protein}$ = 12.5%; IP, isoelectric points.

persion. Different curves resulted from gels of the various proteins. Napin formed the weakest gels, the shear modulus of which increased when approaching the IP (>10). Intensive syneresis was observed for these gels at pH 10. The developing gel was homogeneous near the gelation temperature, but the gel volume reduced to 10% during cooling. A realistic measurement of the modulus of napin gels at room temperature was therefore not possible.

Gels of the globulin (cruciferin) gave increasing modulus values when the pH was approaching the IP (about 7), both from the acidic (pH 5) and alkaline (pH 9) regions. However, in the vicinity of the IP (pH 6.5–7.5), the modulus decreased and reached a minimum value at the IP. Increasing syneresis of these gels around the IP allowed us to deduce that the gel network collapsed into aggregates during cooling and aging.

Although cruciferin is the main protein component in the isolate, gels of the latter showed different pH dependence for shear modulus. Maximum values of the modulus were measured at pH ~9. This suggested a synergistic effect of both protein fractions on gel formation. Because this pH was in the region where both protein components bear opposite net charges, an electrostatic interaction between cruciferin and napin, which favors the association of both proteins, could account for the observed effect.

Gels of the acetylated isolate gave maximum modulus values in 12.5% protein dispersions at pH values close to the isoelectric region, i.e., at pH 6–6.3. At pH 5.5, gel formed that shrank just after formation, exhibited syneresis, and partly decomposed into coagulates. At pH 4, gels with measurable elastic properties (measuring limit 6–10 Pa) could not be obtained. Compared with the unmodified protein isolate, the acetylated isolate produced much stronger gels at pH 6 with more than twofold higher shear moduli. In contrast, the gels of the acetylated protein were so weak at pH 9.0–9.5, where those of the unmodified isolate have maximum modulus values, that the moduli of the modified protein gels could not be determined. This behavior reflects electrostatic effects on the gel structure. In both, strongest gels were obtained when electrostatic repulsions were minimized. This occurred in the unmodified isolate by mutual charge neutralization between both protein components. The resulting gels were opaque and should correspond to a randomly aggregated network structure. For the acetylated protein isolate, maximum gel strength was attained close to the IP. However, the gels obtained were translucent or even transparent as already discussed. Because optimal gelation of the unmodified protein isolate was found at alkaline conditions (pH 9), chemical reactions that lead to covalent crosslinks, such as the formation of lysinoalanine after alkaline splitting of cysteine disulfide bonds (29), should be taken into consideration.

The gelation temperature and gel strength of the protein isolate were essentially determined by the high-molecular-weight component, cruciferin. The poor gelling properties of napin were overcome by interaction with the globulin component.

Napin is rich in helical structure and is stabilized by disulfide bonds (30). Without splitting of disulfide bonds, napin tends to renaturation after being denatured. Partial renaturation of the heat-denatured napin during recooling to form a less flexible product rich in helical structure may be the rea-

son for instability and hysteresis of napin gels.

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