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Texture Characteristics, Protein Solubility, and Sulfhydryl Group/Disulfide Bond Contents of Heat-Induced Gels of Whey Protein Isolate

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The total sulfhydryl (SH) group and half-cystine contents and various properties (water-holding capacity, transmittance, firmness, elasticity, protein solubility) of gels of whey protein isolate (WPI) prepared at different protein concentrations, pHs, and temperatures have been investigated. Assays were also carried out in the presence of *N*-ethylmaleimide or of sodium chloride. Gel texture (firmness, elasticity) is not strictly related to the total number of disulfide (S–S) bonds. At neutral and alkaline pHs, gel firmness and total SH groups decreased with increasing pH (6.5–9.5), while gel elasticity, transmittance, and protein solubility (in buffers without S–S bond-reducing agent) increased. Hydrophobic interactions and intermolecular S–S bonds caused by SH/S–S interchange reactions predominate in the WPI gel network and are probably responsible for (1) the firmer gel structure at increasing protein concentration and (2) the heat resistance at high temperature (ca. 135 °C). The high elasticity of gels at neutral and alkaline pHs may result from intermolecular S–S bonds. The gels formed at acid pHs are nonelastic and markedly solubilized in denaturant-containing buffers. Hydrogen bonds may represent the main molecular bonds in the network of such acid gels.

Whey protein concentrates (WPCs) and whey protein isolates (WPIs) containing from 25 to 95% protein in the dry state are produced from cheese whey by several techniques including gel filtration, ion exchange, ultrafiltration, adsorption, polyphosphate precipitation (Cheftel and Lorient, 1982; Modler, 1985). The functional properties of whey proteins are affected not only by the processing history of WPCs and WPIs but also by the nature and the amount of both the protein and the nonprotein components (de Wit et al., 1983, 1986). One of these functional properties is the ability to form heat-induced gels under appropriate conditions. The gelation of WPC and of its constitutive proteins has been investigated under various experimental conditions (de Wit and de Boer, 1975; Hermansson, 1975; Hermansson and Åkesson, 1975; Hegg, 1982; Harwalkar and Kalab, 1985 a,b; Paulsson et al., 1986; Dumay, 1987). The dependence of gelation on disulfide (S–S) bonds has been demonstrated by the addition of reducing agents (Dunkerley and Zadow, 1984; To et al., 1985). Addition of cysteine at low concentration enhanced gel firmness, while higher concentrations reduced it (Schmidt et al., 1978, 1979). Gel transparency was related to the total sulfhydryl (SH) group content in WPC (Hillier et al., 1980). Voutsinas et al. (1983) observed that the thermal functional properties of WPC (thickening, coagulation, gelation) were significantly correlated with a composite of the content of SH group before heating and of protein hydrophobicity. In contrast, Kohnhorst and Mangino (1985) found that the SH group content in WPC powder was not a good indicator for gel strength.

Little is known about SH group and S–S bond contents in gels obtained from WPC or from the individual whey proteins (Li-Chan, 1983; Beveridge et al., 1984). In the

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present study, an attempt has been made to determine the changes in SH group and S–S bond contents in whey protein isolate as a result of heat-induced gelation. These changes have been studied as a function of protein concentration, pH, and temperature. Other gelation experiments were carried out in the presence of *N*-ethylmaleimide or sodium chloride in order to obtain additional information on the molecular forces involved in gel texture. The WPI selected for this study is an industrial preparation of high solubility, high gelation ability, and high β-lactoglobulin content.

MATERIALS AND METHODS

Materials. Whey protein isolate (WPI), prepared by anion-exchange chromatography using Sphérosil QMA resin, was a gift from Union Laitière Normande (Condé sur Vire, France). It contained 5% moisture, 88% protein ((total nitrogen – nonprotein nitrogen) × 6.38) (dry basis), 3.7% total minerals (dry basis), and 1.6% lactose (dry basis). Fast liquid chromatography on a Mono Q anion-exchange column (Pharmacia Co., Ltd.) of proteins soluble in a 0.02 M piperazine buffer, pH 6.0, indicated that WPI contained 72 g of β-lactoglobulin (β-LG) and 5 g of α-lactalbumin (α-LA)/100 g of total protein. The nitrogen solubility indices (grams soluble nitrogen/100 g of total nitrogen) were 83, 91, and 95.5 at pHs 4.6, 6.0, and 7.0, respectively.

Sephadex G-25 was obtained from Pharmacia. The other chemicals used were of reagent grade.

Heat Treatment of WPI Dispersions and Preparation of Gels. The aqueous dispersions of WPI (1–9.9% protein) were adjusted to the required pH values with 6 N NaOH or 6 N HCl, treated for partial deaeration (to avoid air bubbles in the subsequent gels) with a water pump for 2 min, and placed in glass tubes (2.2-cm i.d. × 4.5-cm height) with tightly closed stoppers. The tubes were heated for 45 min in a water bath (75–95 °C) or in an autoclave (105–135 °C) and then cooled rapidly to room temperature in tap water. In some cases, *N*-ethylmaleimide (NEM) was added to the aqueous dispersions at a concentration of 36 mM and the pH was adjusted to 7.0 with 6 N NaOH. After incubation at room temperature for 1 h, the pH was adjusted to the required value and the NEM-modified WPI dispersion was heated. The cooled gel samples were kept at 4 °C for 15–20 h before all analyses.

Transmittance of Gel (T_{660}). Heat treatment was carried out on 5 mL of WPI dispersion in a glass tube (10 × 120 mm) with a stopper as described in the previous paragraph. After the mixture was cooled to room temperature, the gel transmittance was measured at 660 nm against distilled water with a Bausch and Lomb Spectronic 88 colorimeter.

Determination of Gel Texture. Texture analysis was carried out on gel sections (2.2-cm diameter × 1.0-cm height) at 25 °C with a Stevens-LFRA Texture Analyzer or with an Instron Universal Testing Instrument, Model 1140, when gel firmness was more than 10 N. Compression was exerted by a cylindric plunger with a flat section (5.1-cm diameter) at a displacement speed of 1.0 mm/s. Gel firmness was defined as the force F_0 (in Newtons) exerted by the gel immediately after a 4-mm compression. The plunger was then kept at this 4-mm position, and the force F_5 was measured again after 5 min. The gel elasticity was taken as $1 - (F_0 - F_5)/F_0$. Six determinations were carried out with each gel, and the averages ± standard deviation were calculated.

Determination of the Water-Holding Capacity (WHC) of Gels. Gel (1–1.5 g) was placed on a filter cloth fixed in the middle position of a 50-mL centrifuge tube. Centrifugation was carried out in a Sorvall superspeed centrifuge RC 2-B with an angle rotor (SS-34) for 5 min at 120 *g*. WHC was expressed as percent of the initial gel water remaining in the gel after centrifugation. Four determinations were carried out with each gel, and the averages ± standard deviation were calculated.

Determination of Protein Solubility, Total SH Groups, and Half-Cystine (SH +(2 × S–S)). WPI and gels were solubilized either in 0.086 M Tris–0.09 M glycine–4 mM ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), pH 8.0 buffer (standard buffer) (analysis of protein solubility), in the same standard buffer containing also 8 M urea and 0.5% (17.3 mM) SDS (analyses of protein solubility and of total free SH groups), or in the same standard buffer containing 8 M urea, 0.5% SDS, and 10 mM dithiothreitol (DTT) (analyses of protein solubility and of total SH + (2 × S–S)). The protein solutions were adjusted to 0.1% (0.05 g of protein/50 mL of buffer), homogenized with an Ultra-Turrax below 25 °C for 3 min, and then centrifuged at 20000 *g* for 15 min. The supernatant fractions were analyzed for protein solubility and for the contents of SH groups and of half-cystine. The supernatants were transparent or slightly opalescent (transmittance at 600 nm was always above 95%). Protein solubility was calculated as 100 × protein content of the supernatant/total protein content.

Total free SH groups were determined according to Ellman (1959) as previously indicated (Shimada and Cheftel, 1988) except that the concentration of denaturants was 8 M urea and 0.5% SDS and that the reaction of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) with SH groups was carried out at room temperature for 30 min, instead of 15 min with soy proteins (because the SH groups of WPI reacted slowly; Shimada and Cheftel, unpublished data). The SH content was expressed in micromoles per gram of protein solubilized in the buffer containing 8 M urea and 0.5% SDS. It was checked that keeping WPI gels at 4 °C for 15–20 h did not modify significantly the SH group content. In contrast, the SH group content decreased when a 1% protein solution was kept at 4 °C after heat processing.

The half-cystine content was determined with DTNB after removal of DTT by a Sephadex G-25 column, as previously described (Shimada and Cheftel, 1988), except that elution was carried out with the standard buffer containing 8 M urea and 0.5% SDS. The half-cystine content was expressed in micromoles per gram of protein solubilized in the buffer containing denaturants and DTT (protein thus solubilized is very close to total protein).

Each gel was independently extracted three times with each of the three buffer solutions. Average values ± standard deviation were calculated for protein solubility and for the contents of total SH groups and of half-cystine.

The S–S bond content was calculated as the difference between half-cystine and SH group contents. It should be considered as an apparent content because WPI gels were not completely dissolved in the buffer containing denaturants. Since the SH group content of dissolved protein was probably higher than that of total protein, the calculated S–S bond content somewhat underestimates the S–S bond content of the total protein in the gels.

Protein Determination. Protein concentration was determined spectrophotometrically with $E^{1\%}_{1cm} = 10.2$ at 280 nm. The $E^{1\%}_{1cm}$ value of WPI was checked in the standard buffer containing denaturants with or without DTT. For protein determination, these buffers were used as blanks. In order to determine the protein concentration

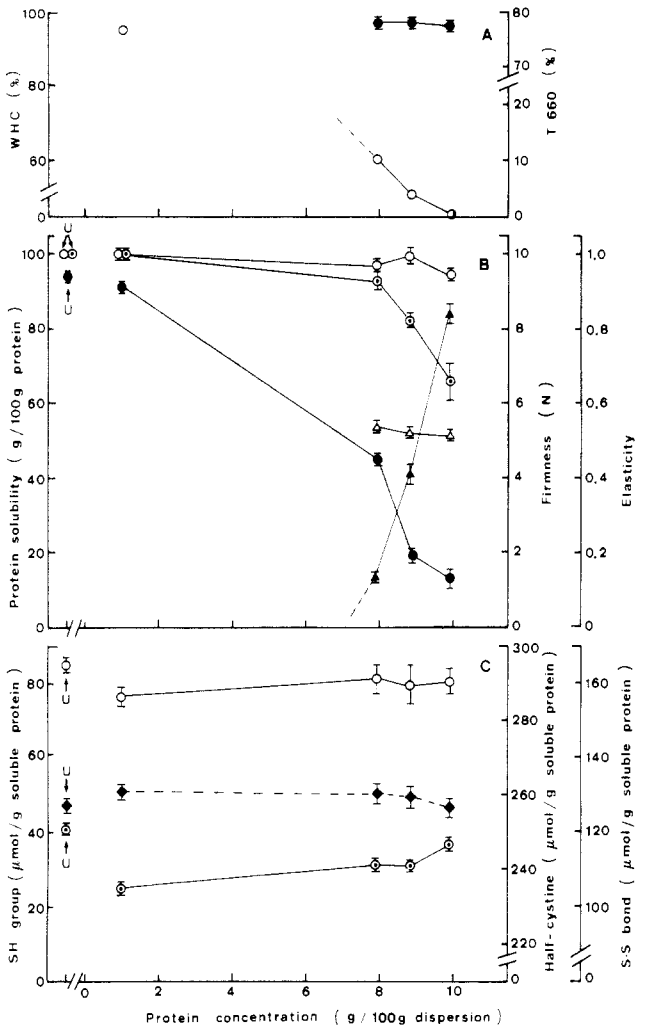

Figure 1. Water-holding capacity (WHC), transmittance (T_{660}), protein solubility, texture characteristics, and contents of SH groups, half-cystine, and S–S bonds of WPI dispersions or gels as a function of protein concentration during heating. Heat processing at 85 °C for 45 min (pH 7.5). U = unheated WPI. Concentration for all solubilization assays: 0.1 g of protein/100 mL. A: (●) WHC, (○) T_{660}. B: (▲) firmness, (△) elasticity. Buffers used for protein solubilization: (●) standard buffer, (⊙) standard buffer containing 8 M urea and 0.5% SDS, (○) standard buffer containing 8 M urea, 0.5% SDS, and 10 mM DTT. C: (⊙) total SH, (○) half-cystine, (♦) S–S bonds (by difference). The bars show standard deviation.

of opalescent solutions, 30 μL of 2-ME was added to 3 mL of protein solution and the mixture was incubated at room temperature for 1 h in order to obtain complete transparency. The concentration of protein solutions containing NEM was determined both by spectrophotometry and according to Lowry et al. as modified by Bensadoun and Weinstein (1976). Both methods gave identical results.

RESULTS AND DISCUSSION

Influence of Protein Concentration on the Characteristics of WPI Gels. The texture characteristics, the protein solubility, and the SH group and half-cystine contents of dispersions or of gels obtained by heating (85 °C, 45 min, pH 7.5) diluted or concentrated dispersions of WPI are shown in Figure 1. When unheated WPI (U) was solubilized at a concentration of 0.1 g of protein/100 mL, the proportions of protein soluble in the standard buffer alone and in the buffer containing 8 M urea plus 0.5% SDS without or with DTT were 94, 100, or 100%, respectively (Figure 1B). When a solution of WPI containing 1% protein was heated, protein solubility in the standard buffer decreased slightly to 90.5% but remained equal to 100% in the presence of denaturants (without or with DTT). When WPI dispersions containing more than 7% protein were heated, gels were formed. These gels had high water-holding capacity (WHC), and their transmittance decreased with increasing protein concentration (Figure 1A). Gel firmness increased with protein concentration, but all gels displayed a similar elasticity (Figure 1B). Protein solubility of gels in the standard buffer was low compared to that of the heated 1% protein solution and fell markedly with increasing protein concentration. This appears to indicate that intermolecular hydrophobic interactions dominate in the gel matrix as the protein concentration increases. Protein solubility of gels in the standard buffer containing denaturants decreased with increasing protein concentration but to a lesser extent: Because the protein solubility of gels was close to 100% in the presence of DTT, this lowered solubility (at high protein concentation) may be caused by intermolecular S–S bonds.

The total SH group and half-cystine contents of unheated and heated WPI are shown in Figure 1C. In unheated WPI, these contents were 40.6 and 295 μmol/g of protein, respectively. Pure β-Lg contains 54.6 μmol of SH groups and 273 μmol of half-cystine/g of protein (Brunner, 1977). The difference between both groups of values probably reflects the presence of α-La in WPI and possibly also oxidative changes (of SH into S–S) taking place during WPI preparation. When WPI dispersions (pH 7.5) were heated, the total content of SH groups decreased (as compared to that of unheated WPI), but less so at higher protein concentrations. However, SH group contents analyzed immediately after heat processing followed by cooling (ca. 1 h instead of 15 h in the standard analytical procedure) were 35 and 34 μmol/g of soluble protein at 1 and 9% protein concentrations, respectively (Shimada and Cheftel, unpublished data). The SH group content of the heated 1% protein solution decreased markedly during further storage at 4 °C, while the corresponding decrease in the stored gel was very slight. Thus, air oxidation of SH groups appears to take place progressively during storage at 4 °C in the case of protein solutions but not of gels. Since SH group contents are expressed per gram of protein soluble in the buffer containing denaturants, changes in SH group contents of WPI gels at high protein concentration may also reflect the preferential insolubilization of aggregates with a high S–S/SH ratio.

The contents of half-cystine did not change significantly as a result of heating (Figure 1C). Watanabe and Klostermeyer (1976) observed that when a 1% solution of β-Lg A (pH 6.9) was heated under air at 80 or 90 °C for 20 min, the decrease in half-cystine content was close to 1.5 or 3.3% and the decrease in SH group content was 35 or 51%, respectively. These were much smaller under anaerobic conditions. In the present study, the partial deaeration carried out before heating may account for the small decrease in SH group content.

The apparent content of S–S bonds, calculated from SH groups and half-cystine contents, remained almost constant (Figure 1C). This confirms that there was no significant oxidation of SH groups into S–S bonds. It therefore appears that the strengthened gel network observed at high protein concentrations does not depend on an increase in the total number of S–S bonds.

Influence of pH. The texture characteristics, the protein solubility, and the SH group and half-cystine contents of gels obtained by heating (85 °C, 45 min) WPI dispersions (9% protein, w/w) of various pHs are shown

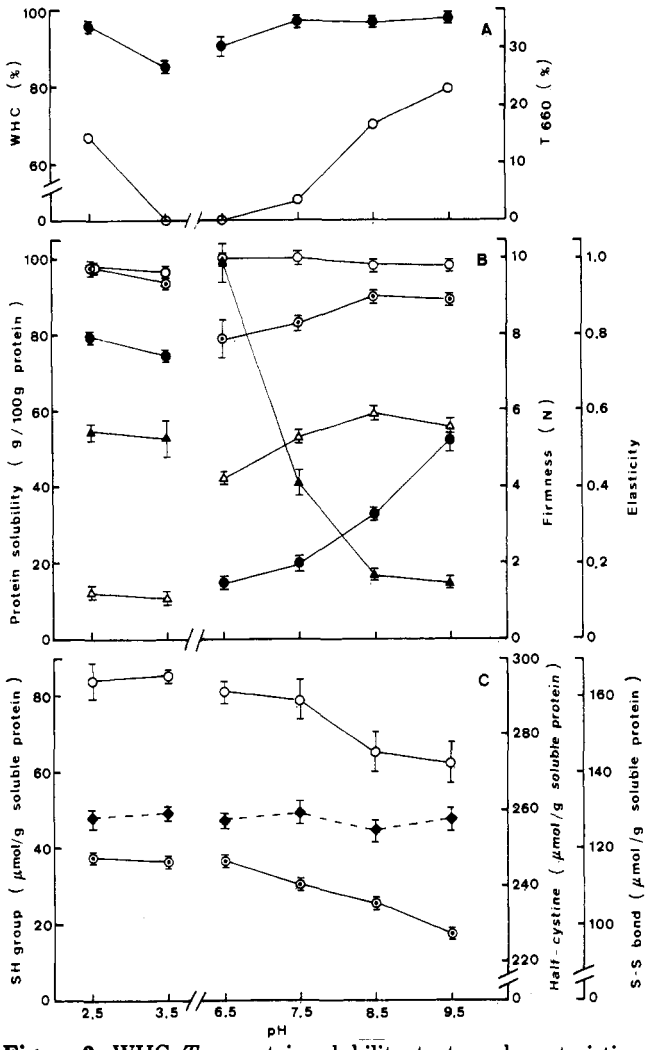

Figure 2. WHC, T_{660}, protein solubility, texture characteristics, and contents of SH groups, half-cystine, and S–S bonds of WPI gels as a function of pH during heating. WPI dispersions (9% protein concentration, w/w) were heated at 85 °C for 45 min. Symbols are the same as in Figure 1. The bars show standard deviation.

in Figure 2. The WHC values of gels prepared at pH 3.5 and 6.5 were somewhat decreased (Figure 2A). These same gels were opaque, and their transmittance was severely decreased. The increase in gel transmittance observed above pH 7 may be due to the N → R conformational change of β-Lg, which is known to modify the specific optical rotation (Tanford et al., 1959), to increase the reactivity of the SH group (Dunnill and Green, 1965), and to release a proton from a buried carboxyl group (Basch and Timasheff, 1967). This conformational change takes place near pH 6.8 in the native state. Gel transmittance may be influenced by the polarity of amino acids, exposed to the aqueous surface environment. When many nonpolar amino acid side chains are present at the surface of protein molecules and/or the net charge is low, protein aggregation is enhanced and gel transmittance decreases.

On the alkaline side of the isoelectric pH, gel firmness decreased markedly with increasing pH, while gel elasticity increased (Figure 2B). The protein solubility of gels in the buffers without or with denaturants increased from pH 6.5 to 9.5 (Figure 2B). All gels were dissolved almost completely in the buffer containing denaturants plus DTT.

Hydrophobic and electrostatic interactions are usually predominant in the network of gels obtained in the isoelectric pH range. The transmittance and protein solubility data suggest that hydrophobic interactions strongly contribute to the network of the pH 6.5 gel, while electrostatic repulsions weaken the firmness of gels obtained at higher pH.

The total SH group and half-cystine contents decreased in gels of increasing pH (Figure 2C). The losses of half-cystine observed in the present study may result mainly from the degradation of S–S bonds, because losses remained similar when gels were prepared at various pHs with NEM-modified WPI (Shimada and Cheftel, unpublished data). The effect of alkali on the degradation of cystine has been studied extensively in model systems as well as in proteins. Nashef et al. (1977) observed that S–S bonds in lysozyme were degraded by alkali treatment through a β-elimination mechanism that induced the formation of dehydroalanine, elemental sulfur, and H_2S. β-Elimination was directly dependent on hydroxide ion concentration. It is believed that the degradation of S–S bonds involves a SH group intermediate that is decomposed into H_2S and dehydroalanine. At alkaline pHs, SH groups (in the reactive PS^- form) may also readily oxidize into S–S bonds (or participate to SH/S–S interchange reactions). These opposite reactions may explain why, in the present study, the calculated contents of S–S bonds remained constant in gels of different pHs and identical with the content in unheated WPI (Figure 2C). These results also indicate that the formation of WPI gels requires no increase in the total number of S–S bonds.

On the acidic side of the isoelectric pH, nonelastic gels were formed and their protein constituents were more soluble in the buffers without and with denaturants than those of neutral or alkaline gels (Figure 2B). It is known that the reactivity of SH groups, which enhances both the oxidation of SH groups into S–S bonds and SH/S–S interchange reactions, decreases significantly under acidic conditions (McKenzie and Ralston, 1971; McKenzie et al., 1972). The SH group and half-cystine contents in acid gels remained identical with those of unheated WPI, and no additional S–S bonds were formed (Figure 2C).

These data may be explained by the following hypotheses: Low SH reactivity at acid pHs prevents SH/S–S interchange reactions, resulting in gels with low elasticity and high protein solubility. In contrast, at neutral pHs, intermolecular SH/S–S interchange reactions are enhanced, leading to elastic gels with lower protein solubility. At pH ≥8.5, reduced firmness and enhanced protein solubility (pH 8 buffer) of gels appear to be due to lowered aggregation reactions through electrostatic repulsions. At these alkaline pHs, heat processing causes a significant decrease in SH group and half-cystine contents. The next experiments were carried out in order to check some of these hypotheses.

Gel Formation in the Presence of *N*-Ethylmaleimide (NEM). NEM was added to WPI dispersions (9% protein) at a concentration of 36 mM. This corresponds to about 10 times the total SH group concentration. Incubation with NEM was carried out at pH 7.0 for 1 h at room temperature. The modified WPI dispersion was then adjusted to the required pH and heated at 85 °C for 45 min, as indicated in Materials and Methods. It was checked that the content of residual total SH groups was below 1 μmol/g of protein (except in the case of the pH 9.5 gel where the SH content was found to be equal to 4 μmol).

Gels obtained above pH 7.5 were more transparent in the presence than in the absence of NEM, and gels obtained at pH 6.5 and 3.5 were opaque in both cases, while the pH 2.5 gel became opaque in the presence of NEM

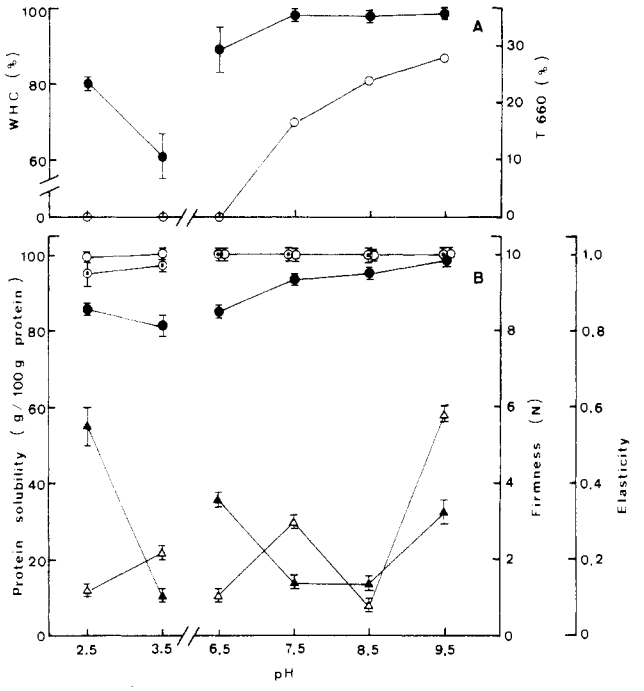

Figure 3. Effect of NEM on WHC, T_{660}, protein solubility, and texture characteristics of WPI gels as a function of pH during heating. WPI dispersions (9% protein, pH 7.0) were incubated for 1 h in the presence of 36 mM NEM at room temperature, adjusted to the required pH, and then heated at 85 °C for 45 min. Symbols are the same as in Figure 1. The bars show standard deviation.

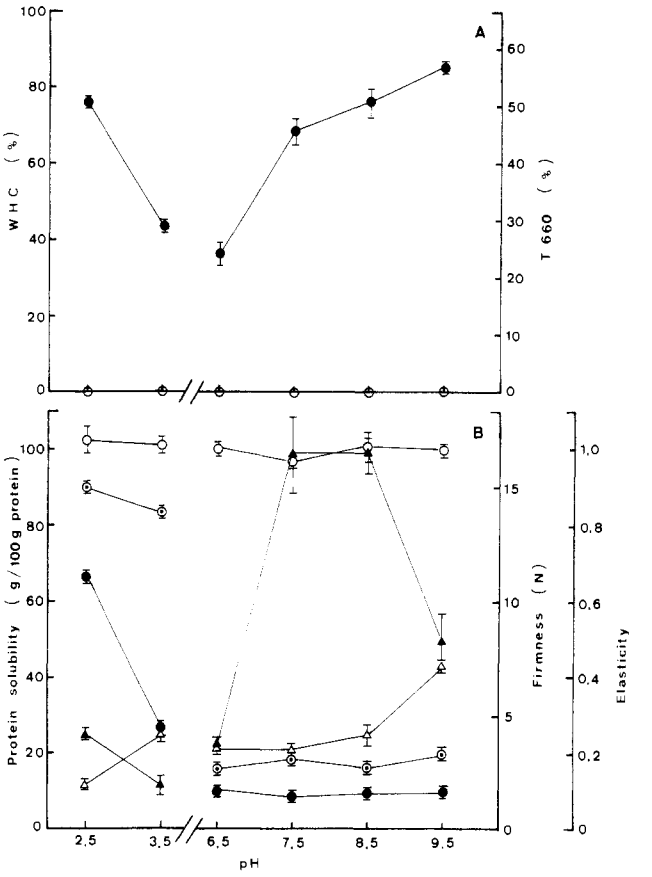

Figure 4. Effect of NaCl on WHC, T_{660}, protein solubility, and texture characteristics of WPI gels as a function of pH during heating. WPI dispersions (9% protein) were heated in the presence of 0.1 M NaCl at 85 °C for 45 min. Symbols are the same as in Figure 1. The bars show standard deviation.

(Figure 3A). The WHC of NEM-modified gels decreased on the acidic side of the isoelectric pH (Figure 3A). It therefore appears that the masking of SH groups by NEM facilitates the surface exposure of hydrophilic amino acids at pH ≥7.5 (lesser aggregation) and that of hydrophobic amino acids at pH 2.5.

The protein solubility of NEM-modified gels in the buffer without or with denaturants increased markedly at neutral and alkaline pHs, as compared to that of nonmodified gels (Figure 3B). All gels were completely dissolved in the buffer containing DTT. The comparison of protein solubility data from Figures 2B and 3B strongly suggests that SH/S–S interchange reactions are responsible for the low protein solubility of gels formed at neutral pH in the absence of NEM. Indeed NEM prevents both SH/S–S interchange reactions and protein insolubilization. On the whole, gel firmness and elasticity were reduced in the presence of NEM, excepted at pH 9.5 (firmness and elasticity) and at pH 2.5 (firmness). This weakening of the gel network may be partly attributed to the prevention of SH/S–S interchange reactions. Brewer and Riehm (1967) observed that residues of lysine and histidine were modified under high concentrations of NEM (0.1 M) and of alkaline agents (pH 8.0). The abnormal texture of NEM-modified gel at pH 9.5 may result from such side reactions with excess NEM at alkaline pH and at high temperature.

The increased transparency of NEM-modified gels at neutral and alkaline pHs indicates that if SH/S–S interchange reactions are prevented during heating, hydrophobic amino acid side chains are not exposed from the interior of protein molecules to the aqueous environment. Hydrophobic interactions stabilized by S–S bonds may contribute to the formation of very firm gels and result in low protein solubility in the buffers without DTT (e.g., pH 6.5 gel without NEM). On the opposite side, gels dependent on hydrophobic interactions without stabilization by S–S bonds are weak and easily dissolved even in the buffer without DTT (e.g., NEM-modified gels at pH 6.5 and 3.5).

The protein solubilities of acid gels were similar with or without NEM (Figures 2B and 3B). The low WHC and low firmness at pH 3.5 may result from enhanced protein aggregation. Gel firmness and elasticity at pH 2.5 were not modified by the presence of NEM. Thus, gel texture at pH 2.5 does not depend on the formation of intermolecular S–S bonds and probably depends mainly on the formation of hydrogen bonds (including those between protonated carboxyl groups). Hydrogen bonds appear to contribute to the firmness of the pH 2.5 gel but do not lead to increased elasticity. Evidence for the role of hydrogen bonds in acid gels has been given for insulin (Burke and Rougvie, 1972) and bovine serum albumin (Yasuda et al., 1986). In both cases, the gel network depended on the formation of β-sheets.

Gel Formation in the Presence of NaCl. In the range pH 2.5–9.5, the presence of 0.1 M NaCl caused a decrease in the WHC of gels and all gels were opaque (Figure 4A). Gel firmness markedly increased at neutral and alkaline pHs in the presence of NaCl, while elasticity decreased (Figure 4B). Such increases in the firmness of WPC gels in the presence of NaCl were also found by Hermansson and Åkesson (1975) and by Schmidt et al. (1978). Protein solubility in the buffers without and with denaturants decreased markedly in the case of gels prepared in the presence of NaCl (Figure 4B). However, all gels were dissolved completely in the buffer containing denaturants and DTT. At low concentrations, NaCl is known to affect electrostatic interactions between protein molecules by

neutralizing electrical charges, while at higher concentrations NaCl also stabilizes hydrophobic interactions (von Hippel and Schleich, 1969; Damodaran and Kinsella, 1981; Babajimopoulos et al., 1983). It is apparent from the results of Figure 4A,B (decreased WHC, transparency, protein solubility) that the addition of 0.1 M NaCl stabilized hydrophobic interactions in the gels. In the presence of NaCl at pH 6.5, a coarse protein aggregate was formed as a result of strong hydrophobic interactions and of low net charge. The increase in firmness (and the decrease in elasticity) at neutral and alkaline pH probably results from the stabilization of intermolecular hydrophobic interactions by NaCl.

Altogether, the results from Figures 2–4 (influence of pH with or without NEM or NaCl) at neutral and alkaline pHs indicate that hydrophobic interactions and S–S bonds between polypeptide chains predominate in the network structure of WPI gels, while electrostatic repulsions become active in reducing gel firmness at alkaline pHs.

At acid pHs, firmness was not enhanced in the presence of NaCl (pH 3.5 gel was a coarse protein aggregate) although NaCl enhanced hydrophobic interactions, as judged from the decrease in solubility (Figure 4B). As already discussed, hydrophobic interactions not stabilized by S–S bonds do not appear to contribute greatly to the firmness of gels. The results from Figures 2–4 support the hypothesis that WPI gels at acid pHs (without NEM or NaCl) are held mainly through hydrogen bonds. Such bonds do not lead to high elasticity values.

Influence of Temperature. The texture characteristics, the protein solubility, and the SH group and half-cystine contents of gels obtained by heating WPI dispersions (9% protein, pH 7.5) for 45 min at various temperatures (75–135 °C) are shown in Figure 5. WHC was high in all cases, although it decreased slightly with increasing temperature (Figure 5A). Gel transmittance was low in all cases but decreased from 75 to 115–125 °C and increased again at 135 °C (Figure 5A). Gel firmness increased from 75 to 125 °C and somewhat decreased at 135 °C, while gel elasticity remained constant over the whole temperature range (Figure 5B). Protein solubility in the standard buffer decreased from 75 to 105 °C and then increased gradually from 105 to 135 °C (Figure 5B). Protein solubility in the buffer containing denaturants decreased from 75 to 125 °C and increased at 135 °C. All gels dissolved completely in the buffer containing denaturants plus DTT. These data appear to indicate that the firmness of gels was strengthened through intermolecular hydrophobic interactions stabilized by S–S bonds as the gelling temperature increased up to 125 °C.

The total SH group content and above all the half-cystine content decreased markedly with increasing temperature (Figure 5C). The half-cystine content thus decreased from 290 to 220 μmol/g of total protein when the gelling temperature was changed from 75 to 135 °C. This decrease is almost double the initial SH content (40.6 μmol/g of protein). Such half-cystine losses are well documented in severely heated milk proteins (Payne-Botha and Bigwood, 1959; Dill et al., 1962; Kisza et al., 1970; Swaisgood and Cho, 1973; Patrick and Swaisgood, 1976; Watanabe and Klostermeyer, 1976; Aboshama and Hansen, 1977). The formation of H_2S and of other volatile sulfur-containing compounds has also been demonstrated and contribute to the flavor of cooked milk or dairy foods. The loss of half-cystine probably occurs through a mechanism similar to the one taking place during alkaline processing, i.e. desulfuration of disulfide bonds (Cheftel, 1977).

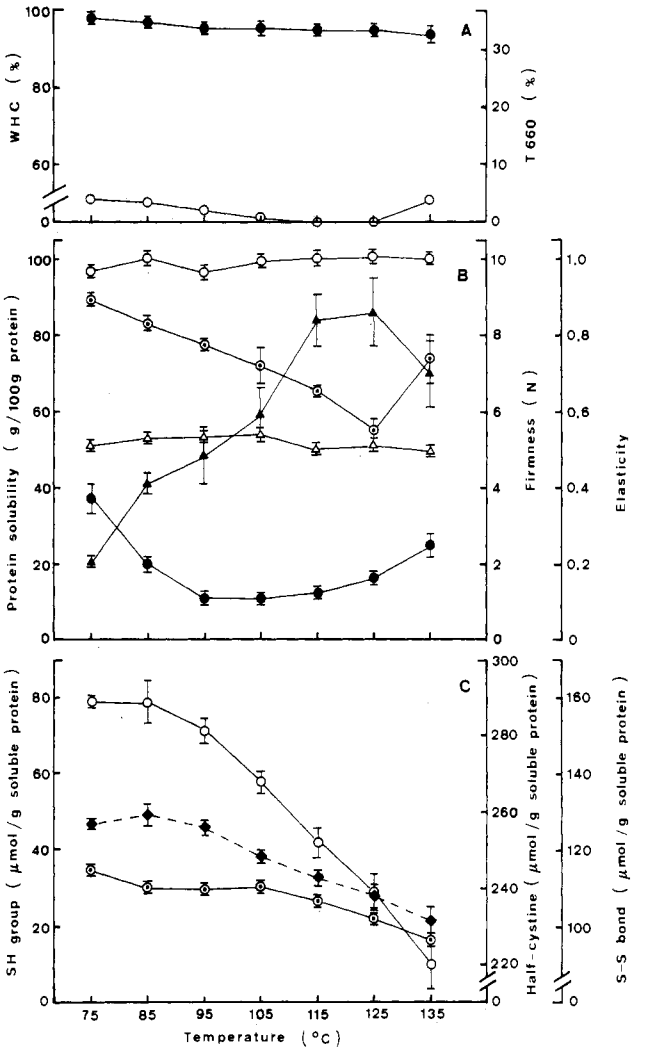

Figure 5. WHC, T_{660}, protein solubility, texture characteristics, and contents of SH groups, half-cystine, and S–S bonds of WPI gels as a function of processing temperature. WPI dispersions (9% protein, pH 7.5) were heated in a water bath (75–95 °C) or in an autoclave (105–135 °C) for 45 min. Symbols are the same as in Figure 1. The bars show standard deviation.

In spite of the drastic decrease in the S–S bond content at higher temperatures, gel firmness and elasticity did not decrease and even the gel obtained at 135 °C remained firm and elastic. In contrast, the firmness of gels prepared with soy protein isolate (SPI) was found to decrease drastically after heating at 130 °C for 30 min, and it was suggested that gel weakening resulted mainly from losses in half-cystine and in amide groups (Shimada and Cheftel, 1988). Unheated WPI contains much more half-cystine (ca. 295 μmol/g of protein) than unheated SPI (ca. 100 μmol/g of protein), and the half-cystine content remained about twice as high in WPI than in SPI even after heating at 135 °C for 45 min. This may explain the higher heat resistance of WPI gels. In parallel, the amide group content of β-Lg (ca. 11 mg of amide N/g of protein; Brunner, 1977) is lower than that of soy proteins (ca. 18 mg of amide N/g of protein; Catsimpoolas et al., 1967). WPI gels may therefore be less sensitive than SPI gels to deamidation reactions at high temperature. Such reactions normally lead to fewer hydrogen bonds and increased electrostatic repulsions. This may also contribute to the higher heat resistance of WPI gels.

De Wit and Klarenbeek (1981) reported that β-Lg displays two endothermal peaks near 70 and 130 °C (at pH 7.5) when studied by differential scanning calorimetry.

They speculated that the second peak (near 130 °C) corresponds to the unfolding of residual protein structure after protein denaturation at 70 °C and that this residual structure was stabilized by S–S bonds. The decrease of gel firmness at 135 °C observed in the present study may reflect the breakdown of intermolecular interactions, including S–S bonds and hydrophobic interactions.

It can be concluded from the data presented here that hydrophobic interactions and intermolecular S–S bonds are the main determinants of the network of WPI gels at neutral and alkaline pHs, while hydrogen bonds are responsible for the network of gels formed at acid pHs. In addition, intermolecular S–S bonds may directly influence gel elasticity.

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Registry No. NEM, 128-53-0; H_2O, 7732-18-5; NaCl, 7647-14-5.

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Investigation of Some Factors Influencing Isomalathion Formation in Malathion Products[1]

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Isomalathion contents of malathion powders prepared with attapulgite, bentonite, Celite, Fuller's earth, kaolinite, and silicate were influenced by the carrier exchangeable cation (Na, K, Ca), surfactant, relative humidity (RH) of the formulation and storage environment, and the container surface. The isomalathion content and the period for attaining its maximum on storage at 55 ± 1 °C depended on the carrier as well as the other factors stated above. The isomalathion formed from malathion applied on plant leaves was below detection level (0.2 ppm) over a period of 14 days.

In 1976 an epidemic of human poisoning from the handling of malathion water-dispersible powder (WDP) was reported from Pakistan (Baker et al., 1978). Investigations by these authors revealed that the mammalian toxicity correlated best with the isomalathion [*S*-methyl isomer of malathion; *O,S*-dimethyl *S*-1,2-dicarbethoxy ethyl phosphorodithioate] content of the powders. Subsequent studies confirmed this and also indicated that the auxiliaries employed in solid malathion formulations induced isomalathion formation in formulations during tropical storage and/or storage at elevated temperatures (Aldridge et al., 1979; Miles et al., 1979).

Systematic information on the effect of solid formulation carriers on the isomalathion content of malathion powders was reported by Halder and Parmar (1984). The isomalathion content varied with each carrier but did not correlate with the physicochemical characters of the carriers such as pH, pK_a, bulk and particle density, cation-exchange capacity, sorptivity, and specific surface area. In order to further understand the factors influencing the content of this isomer, studies on the role of exchangeable cations on the carrier, some surfactants used in water-dispersible powders, relative humidity of the environment (affecting hydration status of carrier and WDP), and the container and leaf surfaces were undertaken.

MATERIALS AND METHODS

Carrier. Industrial-grade attapulgite, bentonite, kaolinite, Fuller's earth (MCA Industries, New Delhi, India), Celite (Loba Chemie, Wien Fishamend, Austranal), silica gel H (Glaxo Laboratories Ltd., Bombay, India), and hydrated calcium silicate (Hindustan Insecticides Ltd., New Delhi, India) were investigated. Specific surface areas (Carter et al., 1965) (m^2/g): attapulgite, 116.8 (lit. 140; Escard, 1952); bentonite, 575 (lit. 580–750; Raman and Ghosh, 1974); kaolinite, 34.1 (lit. 37–45; Escard, 1952); Fuller's earth, 510 (lit. 500; Halder and Parmar, 1984); Celite, 169.2; silica gel H, 540.6; hydrated calcium silicate, 726.1. The differential thermal analysis of the calcium clay (DTA apparatus, Stanton-Redcraft, alumina reference, heating at 10 °C/min from 40 to 1100 °C in air) showed the following: attapulgite, 100, 570 (lit. 180, 280, 400, 800 (exo); Kulbicki and Grim, 1959); bentonite, 160, 700, 850–950 (S-shaped) (lit. 150, 700, 850–950 (S-shaped); Mackenzie, 1970); kaolinite 570, 1000 (exo) (lit. 550–580, 1000 (exo); Holdridge and Vaughan, 1957); Fuller's earth, 150, 550 (lit. 150, 550–600; Mackenzie, 1970); Celite, 120, 150, 750; hydrated calcium silicate, 80, 730, 875 (exo). The surface area and DTA values indicated that bentonite, kaolinite, and Fuller's earth were of good quality and attapulgite was of doubtful identity. Celite, silica gel H, and hydrated calcium silicate were pure-grade authentic materials, but their surface area and DTA values could not be verified from the literature.

Malathion. Technical-grade malathion (Hindustan Insecticides Ltd., Maharashtra, India), a colorless liquid (96.96%, moisture content 0.03%, acidity 0.15%), was used.

Surfactants. Acipon ADS 1, a mixture of alkyl sulfate, alkyl aryl sulfonates, and some synthetic dispersing agents (Ahura Chemical Products Pvt. Ltd.); Dispersol F Conc. PDR, a powder based on the sodium salt of methylenebis[naphthalenesulfonic acid] (Indian Explosives Ltd.); Idet 20, a mixture of alkyl aryl sulfonates (Hindustan Insecticides) and sodium lauryl sulfate (Hopkins and Williams), were used.

Preparation of Monoionic Clays. An appropriate quantity of attapulgite, bentonite, Celite, Fuller's earth,

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