Solubility Profile, Intrinsic Viscosity, and Optical Rotation Studies of Acid Precipitated Soy Protein and of Commercial Soy Isolate

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Solubility profiles have been compiled for freeze-dried, acid precipitated soy protein and for a commercial soy isolate as a function of ionic strength, pH, dialysis, pH 12 treatment, and Na_2SO_3 treatment. Intrinsic viscosity and optical rotation, $[\alpha]_D$, were used to follow the denaturation of these isolates by such denaturants and processes as guanidine hydrochloride, acid precipitation, and pH 12 treatment. The overall data of this work support the following conclusions: (1) A single solubility measurement at a given pH and ionic strength, such as the nitrogen solubility index, is insufficient to characterize the solubility of an isolate. (2) Some soy proteins are insolubilized by acid precipitation. Additional proteins are insolubilized by commercial processing. Dialysis prior to acid precipitation, however, prevents these insolubilization reactions. (3) Loss of solubility cannot be used as the sole criterion of soy protein denaturation, since some reactions at pH 12 treatment that denature the proteins actually increase the solubility.

Isolation and commercial processing of soy protein isolates will cause physical and chemical changes in the proteins. To study these changes, methods for characterizing the physical and chemical states of soy proteins are necessary. There are many methods available for characterizing soluble and crystalline proteins. Methods for characterizing protein slurries are, however, limited. Of the available methods, protein solubility is a widely used method.

A great quantity of soy protein solubility data is available in the literature. (See, for example, Paulsen et al., 1960; Nash and Wolf, 1967; Mattil, 1974.) Often, however, the measurements are made at only one pH. A better insight into the physical and chemical states of the proteins can be obtained by studying the solubility over a wide pH range. In this paper, we have compiled pH–solubility profiles for soy protein isolates. We have studied the effects of various processing conditions and sample treatments on the pH–solubility profiles of several soy protein isolates.

Optical rotation and intrinsic viscosity are established methods for studying the denaturation of native globular proteins. (See, for example, Tanford, 1968; Fukushima, 1968.) Using these methods upon the solutions from the solubility experiments, we have estimated the degree of loss of native secondary and tertiary structures suffered by these treated proteins. Loss of solubility has often been used as a criterion of soy protein denaturation. (See, for example, Smith et al., 1951; Fukushima, 1959; Wolf et al., 1964; Wu and Inglett, 1974.) We have compared this criterion with those of optical rotation and intrinsic viscosity in order to test its generality.

MATERIALS AND METHODS

Protein Isolates. The following three isolates were studied.

(A) *Laboratory Prepared Curd (Curd).* One part of commercial defatted soy flakes was extracted with ten parts of dilute NaOH (pH 9–10). After removal of spent flakes, the total extract was adjusted to pH 4.5 with dilute HCl to precipitate the curd. The curd was washed three times with H_2O, resuspended at pH 7.0, and freeze-dried.

(B) *Curd from Dialyzed Extract.* A portion of the above extract was dialyzed 4 days against a pH 9–10 NaOH solution containing 0.01 M Na_2SO_3 and 6 days against H_2O at 4 °C before acid precipitation. (Since mercaptoethanol interfered with the Biuret assay, Na_2SO_3 was used as a disulfide bond reducing agent.) The precipitated curd was washed three times with H_2O, resuspended at pH 7.0, and freeze-dried.

(C) *SPI.* A general purpose commercial soy protein isolate (SPI) manufactured by Ralston Purina Company under the trade name Edi-Pro N was used.

All the isolates were in the proteinate form. The pH of 0.9% w/w slurries in distilled water ranged from 6.8 to 7.0. All the samples were stored at room temperature in closed containers. No noticeable changes in solubility during the span of this study were observed.

Solubility Method. The solubility method developed in our previous paper (Shen, 1975) was used. Fifteen milliliters of H_2O containing enough NaCl and HCl or NaOH to adjust the final slurry to the desired pH and ionic strength was added to 0.1362 g of a soy isolate to make a 0.9% w/w slurry. The necessary amounts of HCl or NaOH to attain a given pH were determined by preliminary titrations. The slurry was equilibrated at 25 °C in a shaker bath for 2 h and then centrifuged at 42 000*g* for 20 min to remove the insoluble proteins from the "soluble" proteins. *Those proteins remaining in solution under the above condition will, henceforth, be labeled as soluble proteins.* Total and soluble proteins were determined by Biuret analyses before and after the centrifugation, respectively. The solubility is expressed as the percent of the total protein that remains in solution under the above conditions. The pH of the slurry was taken to be the final pH of the soluble protein solution. For ionic strength calculations, the definition of Tanford and Buzzell (1956) for dilute protein solutions was used. Statistical analysis on solubility data taken under similar conditions but on different samples of a given isolate and conducted on different days gave coefficients of variations for the method that ranged from 1.2 to 2%.

Intrinsic Viscosity Methods. Relative viscosities were measured in Cannon Fenske no. 50 capillary viscometers using the twin viscometer technique of McKie and Brants (1972). Flow times were measured to ±0.05 s by electric timers. Since flow times were greater than 150 s, kinetic energy corrections were neglected. Temperature was maintained at 35.00 ± 0.01 °C in an oil bath. Solutions of soluble proteins, 3 to 20 mg/ml concentration, were prepared according to the standard solubility method. The intrinsic viscosity was obtained by least-squares fits of the

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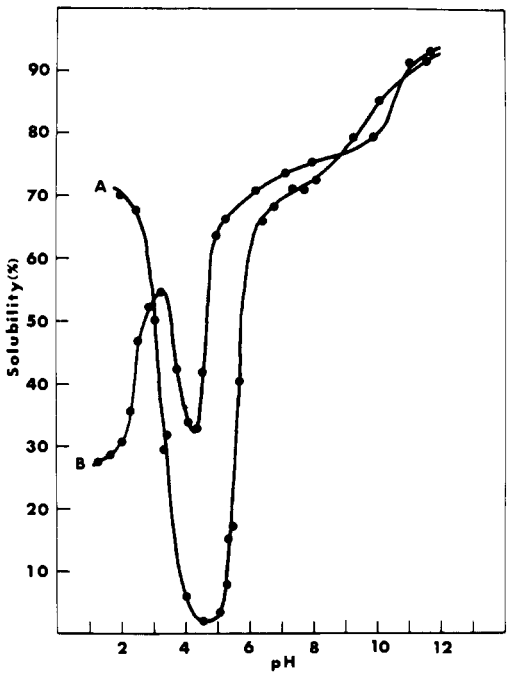

Figure 1. pH-solubility profile of curd in H_2O (A) and in 0.5 M ionic strength solution (B).

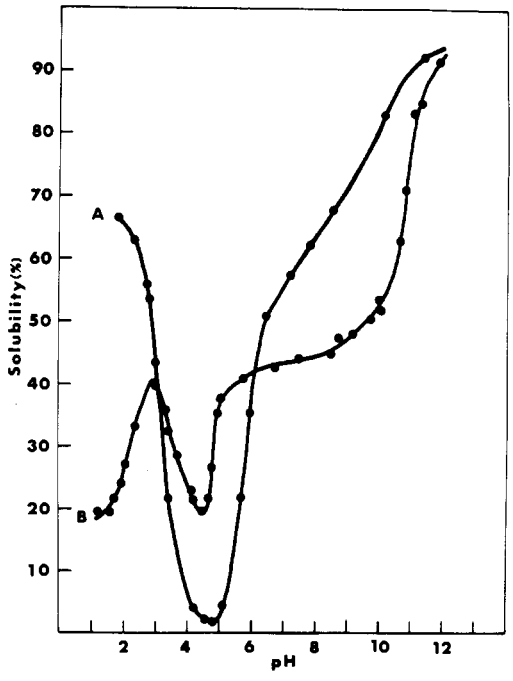

Figure 2. pH-solubility profile of SPI in H_2O (A) and in 0.5 M ionic strength solution (B).

data to both the Huggins (1942) and the Kraemer (1938) equations. No corrections on $[\eta]$ were made for the small differences between solution and the solvent densities.

Optical Rotation Methods. The optical rotations at the sodium D line, $[\alpha]_D$, of soluble protein solutions prepared by the standard solubility method were obtained using a Zeiss circle 0.01° polarimeter. A 2-dm path length cell was used. Protein concentrations were kept as high as 589-nm light transmission will allow. Typically, these concentrations ranged from 2.5% for very clear solutions to 0.5% for slightly scattering solutions.

RESULTS AND DISCUSSION

Effect of Ionic Strength. In Figure 1, the solubility profile of curd in H_2O is compared with the profile in 0.5 M ionic strength solvent. In Figure 2, the above comparison is made for SPI. For both samples, the profiles in H_2O are dramatically different from those in the 0.5 M ionic strength solvent. The broad minima between pH 4 and 5.2 in both of the H_2O profiles are changed to sharp dips in the 0.5 M ionic strength profiles. The minimum solubility for curd at pH 4.5 was raised from 2% in H_2O to 32% in 0.5 M ionic strength solvent. The corresponding increase in solubility for SPI is from 2 to 20%.

Below pH 4, a peak with local maximum at pH 3.3 and with 55% protein solubilized appears in the 0.5 M ionic strength profile, while the solubility of curd in H_2O rises almost vertically between pH 3 and 4. A similar difference is observed in SPI profiles. There, the local maximum in the 0.5 M ionic strength profile is at pH 2.9 with 40% of the protein solubilized. Above pH 6, both curd profiles have similar behavior, but the SPI profile in 0.5 M ionic strength solvent has a plateau from pH 6 to 10 that is not seen for the profile in H_2O.

These differences are more dramatic than those observed by Hermansson (1973) for a soy isolate prepared "under very mild conditions on a pilot plant scale". There, the H_2O profile was compared with one in a 0.2 M ionic strength solution.

The reason for these dramatic differences can be deduced from Figure 3. In Figure 3, the solubility of SPI at pH values of 2, 4.7, and 6.8 has been plotted as a function of ionic strength. The effect of increasing ionic strength at pH 2 is a sharp salting out effect that decreases the solubility from 67 to 20% before bottoming at an ionic strength of 0.65 M. At pH 6.8, there is a much smaller salting out effect that levels off at 0.2 M ionic strength. The effect at pH 4.7, however, is a large salting in effect that increases the solubility from 2 to 38% before leveling off at 0.65 M ionic strength. Thus, the lower solubilities observed at pH <3 and pH >6 in the 0.5 M ionic strength profiles as compared to the H_2O profiles can be explained by the salting out effect at these pH values, whereas the higher solubilities observed between pH 3 and 6 are explained by the salting in effect. Similar effects but of slightly different magnitude were seen by Hermansson (1973) for her isolate. The leveling off of the salting in effect at 0.65 M ionic strength agrees with the extraction data of Anderson et al. (1973).

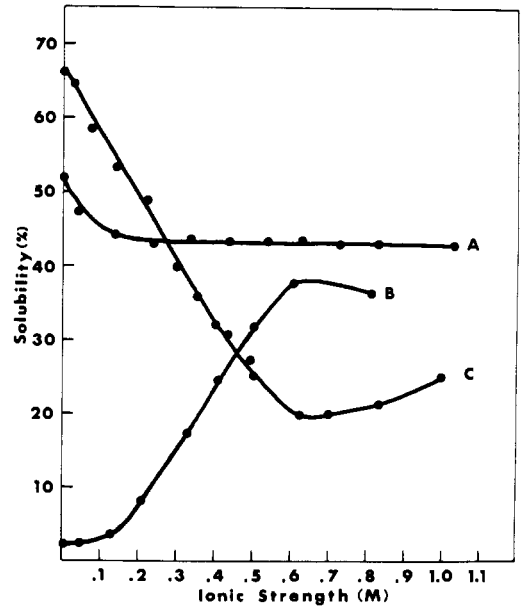

Figure 3. Ionic strength-solubility profile of SPI at pH 6.8 (A), 4.7 (B), and 2.0 (C).

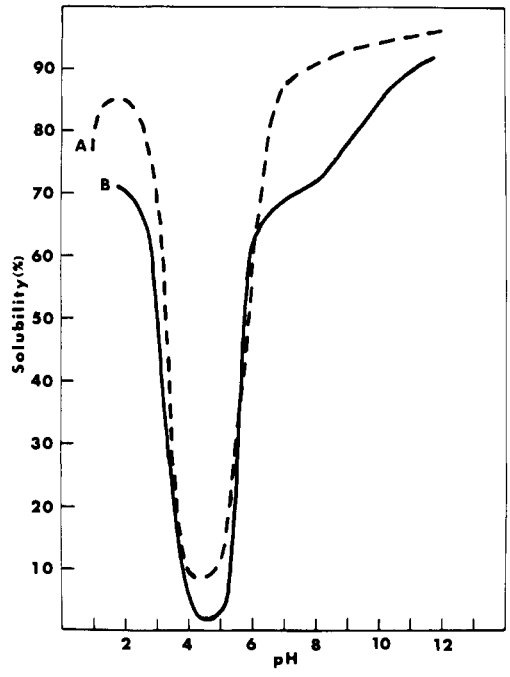

Figure 4. Comparison of the pH-solubility profile of curd in H_2O (B) with the soy meal extraction profile of Smith and Circle (1938) (A).

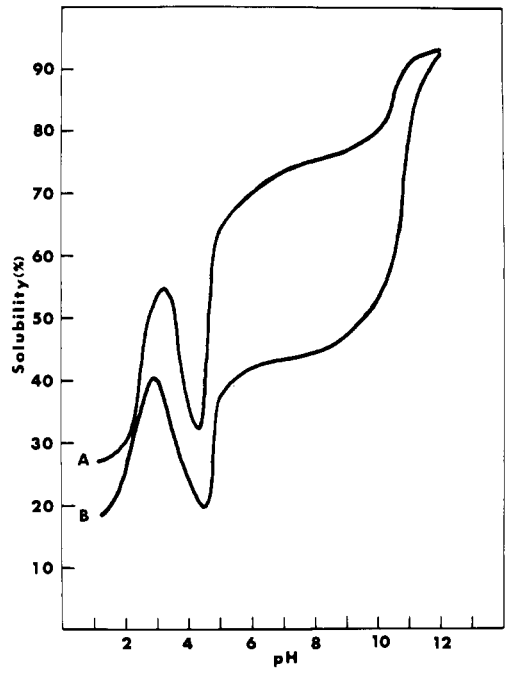

Figure 5. Comparison of the pH-solubility profiles of curd (A) and SPI (B) in 0.5 M ionic strength buffer.

Effect of Acid Precipitation. The effect of acid precipitation on the solubility profile can be seen in Figure 4, which compares the curd profile in H_2O with the extraction profile for meal protein from Smith and Circle (1938). The extracted protein, unlike the curd, has not been acid precipitated. These profiles are not directly comparable quantitatively because of the presence of whey proteins in the extraction profile and because of the different experimental methods used. However, the qualitative differences in the shape of the profiles are instructive in that they confirm the insolubilization effects of acid precipitation (Nash et al., 1971; Anderson, 1974).

Effect of Commercial Processing. In Figure 5, the pH–solubility profiles of curd and SPI are compared. Both samples were in 0.5 M ionic strength solution. SPI is less soluble than curd at all pH values below 12. The effect is especially pronounced in the pH 6–10 range where the solubility of SPI is 30% lower than that of curd. Thus, the effect of commercial processing is to insolubilize additional proteins not previously insolubilized by acid precipitation. (SPI should not be taken to represent all commercial soy isolates. The variability in solubility between different commercial soy isolates is well known; see Nash and Wolf (1967).)

The Effect of Dialysis Prior to Acid Precipitation. Dialysis might remove small molecular weight impurities such as phytate which might aid in insolubilizing proteins during acid precipitation. The profiles of the curds from undialyzed and dialyzed extracts are compared in Figure 6. Acid precipitation did not insolubilize any proteins in the curd from the dialyzed extract, since the profile for the curd from the dialyzed extract is quite similar to the meal extraction profile (Figure 4). Thus, the dialysis prior to acid precipitation apparently blocked the insolubilization reactions. The cause of this effect must, however, be attributed to three factors: the removal of phytate and other small molecular impurities that might help to insolubilize proteins during acid precipitation, prolonged dialysis against an alkaline solution (pH 9–10), and prolonged treatment with a disulfide reducing agent during dialysis. Unfortunately, a phytate analysis was not made

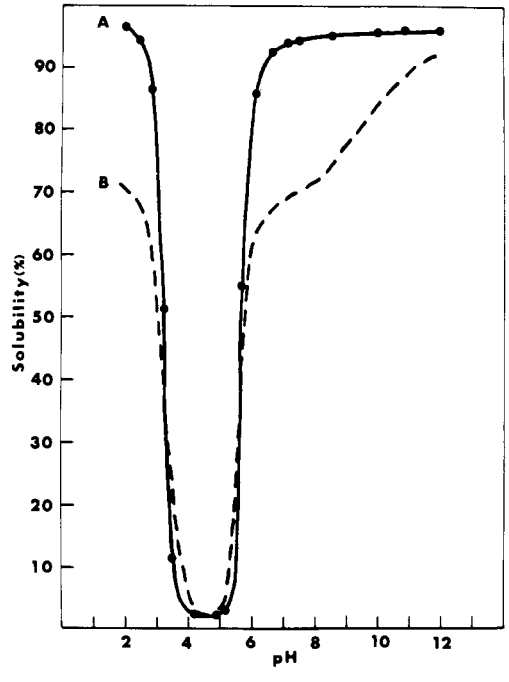

Figure 6. Comparison of the pH-solubility profiles of the curds from the dialyzed extract (A) and the nondialyzed extract (B) in H_2O.

on the dialyzed curd. Smith and Rackis (1957) noted that removal of phytate raised the "isoelectric point" by 0.8 pH unit. The fact that there is no appreciable shift in the isoelectric point observed here might indicate incomplete phytate removal. The solubilizing effects of the high pH treatment and of a disulfide reducing agent (Na_2SO_3) are now discussed separately.

The Effect of pH 12 Treatment. The dramatic increases in the solubilities of curd (Figure 1) and of SPI (Figure 2) as the pH is raised above 10 indicated that alkaline solutions are effective in resolubilizing soy proteins. The effect of extreme alkaline treatment on resolubilizing proteins was studied by treating SPI for 2 h at pH 12 (the most extreme pH used in this work) before

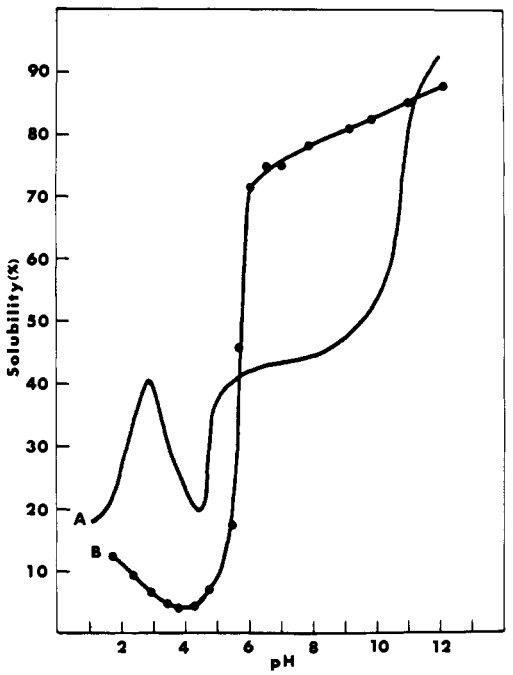

Figure 7. Comparison of the pH-solubility profiles of SPI (A) and pH 12 treated SPI (B) in 0.5 M ionic strength buffer.

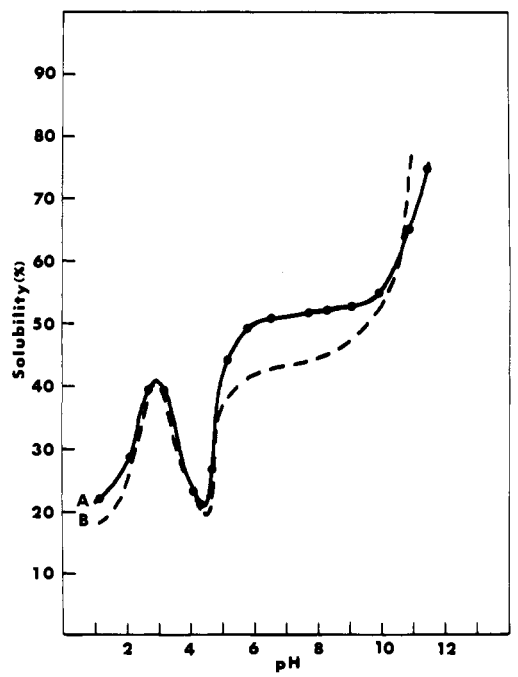

Figure 8. Comparison of the pH-solubility profiles of Na_2SO_3 treated SPI (A) and SPI (B) in 0.5 M ionic strength buffer.

lowering the pH and adjusting the ionic strength to 0.5 M. The profiles of the treated and the untreated samples are compared in Figure 7. The alkaline treatment was effective in raising the solubility of SPI for pH values greater than 6, but it was less effective than dialysis. Below pH 6, this treatment appreciably lowers the solubility. This behavior is quite different from that of the curd from the dialyzed extract.

The Effect of Disulfide Bond Reducing Agents. It has been shown that disulfide bond reducing agents resolubilize at pH 7.6 some proteins insolubilized by acid precipitation and commercial processing (Nash and Wolf, 1967). This effect was studied over a wide pH range by

Table I. Intrinsic Viscosity

Sample	$[\eta]$, cm^3/g
Curd	4.8 ± 0.1
SPI	5.6 ± 0.3
SPI in Gdn·HCl	22.0 ± 0.7
pH 12 treated SPI	14.1 ± 0.3
Curd from dialyzed extract	16.4 ± 0.4

Table II Optical Rotation

Sample	$[\alpha]_D$, deg/dm
Curd	−43.4
SPI	−46.8
SPI in Gdn·HCl	−89.8
pH 12 treated SPI	−69.2
Curd from dialyzed extract	−74.5

treating SPI at neutral pH with 0.025 M Na_2SO_3 for 2 h before adjusting the pH and ionic strength to the desired values. The profiles of the treated and the untreated samples are compared in Figure 8. All the samples were at 0.5 M ionic strength. Below pH 5, treatment with SO_3^{2-} had little effect on the solubility of SPI. Since the 2-h treatment at pH 7 should give ample time for complete disulfide bond reduction, it is unlikely that this result is due to ineffective disulfide bond reduction. It seems that disulfide bond reduction alone is not enough to resolubilize these proteins for pH below 5. From pH 5 to 10, the solubility of SPI is increased 10% by the Na_2SO_3 treatment. Thus, Na_2SO_3 resolubilizes only a small portion of the protein insolubilized by acid precipitation and commercial processing. Above pH 10, the effect of Na_2SO_3 is again negligible. Thus, the solubilizing effect of high pH treatment is not enhanced by the addition of Na_2SO_3.

Intrinsic Viscosity and Optical Rotation. The results of our intrinsic viscosity and optical rotation studies on the soluble proteins are given in Tables I and II, respectively. All samples contained 0.05 M Na_2SO_3. For experimental convenience, intrinsic viscosity data were taken at 35 °C, and optical rotation data were taken at 25 °C. All the data were obtained at pH 7.

The soluble portions of curd and SPI at pH 7 have intrinsic viscosities of 4.8 and 5.5 cm^3/g, respectively. Comparison of these values with the intrinsic viscosities of a series of globular proteins tabulated by Tanford (1968) indicates that the soluble portions of curd and SPI are essentially in the native globular state. Our value compares reasonably with the value of 5.2 cm^3/g obtained under similar solvent conditions at 25 °C by Wolf et al. (1963).

The specific rotations at the sodium D line of curd and SPI at pH 7 are −43.4 and −46.8 deg/dm. These values compare reasonably with the value −46.4 deg/dm obtained by Wolf et al. (1963) for native soy proteins. Thus, it seems that the soluble proteins of curd and SPI have their secondary (α-helix and β-pleated) structures essentially intact. This complements the intrinsic viscosity data which show the soluble proteins of curd and SPI to be essentially in their native globular states.

There is much evidence (Tanford, 1968a,b) which shows that globular proteins are completely denatured to random coils in 6 M guanidine hydrochloride (Gdn·HCl) solutions containing a disulfide bond reducing agent. Thus, the values of 22.0 cm^3/g and −89.8 deg/dm for SPI in 6 M Gdn·HCl and 0.05 M Na_2SO_3 at pH 7 would be, respectively, the intrinsic viscosity and the specific rotation of completely random coiled soy proteins. Comparing the above values with those for "native" soy proteins, we would expect partially denatured soy proteins to have intrinsic viscosity values between 4.8 and 22.0 cm^3/g and specific rotations between −40 and −90 deg/dm. The percent of

denaturation might be estimated as in Fukushima (1968) by the fractional change of the observed property:

$$\% \text{ of denaturation} = (A_{\text{sample}} - A_{\text{native}})(100)/(A_{\text{Gdn·HCl}} - A_{\text{native}})$$

where A is a measured physical property (intrinsic viscosity or specific rotation). As indicated by the data in Tables I and II, both techniques show pH 12 treated SPI and curd from the dialyzed extract to be considerably denatured. The estimated degrees of denaturation for SPI are 53% by intrinsic viscosity and 54% by optical rotation, whereas the degrees of denaturation for the curd from the dialyzed extract are 67 and 66%, respectively. It seems fortuitous that these two different physical measurements should estimate the same degree of denaturation for both samples.

SUMMARY AND CONCLUSIONS

The overall data of this work support the following general conclusions. One, a single solubility measurement at a given pH and ionic strength, such as the nitrogen solubility index, is insufficient to characterize the solubility of a soy protein isolate. (Mattil (1974) expressed a similar sentiment.) This is evident from the figures presented in this report. Many revealing details especially in the pH 2–6 region would be lost. Furthermore, it is important to gather the profiles at several ionic strengths. A corollary of the above conclusion is that pH and ionic strength should be specified with all solubility data.

Two, soy proteins are insolubilized by acid precipitation and commercial processing. Even mild extraction, precipitation, and freeze-drying conditions insolubilize some soy proteins. If commercial processing is used instead of freeze-drying, additional proteins are insolubilized. Dialysis at alkaline pH in the presence of Na_2SO_3 prior to acid precipitation apparently prevents this insolubilization by some unknown mechanism.

Three, loss of solubility cannot be used as the sole criterion of soy protein denaturation. Intrinsic viscosity and optical rotation measurements show that the soluble proteins from curd and SPI are essentially native. In those cases, loss of solubility is a measure of denaturation. However, for the curd from the dialyzed extract and the pH 12 treated SPI, even the soluble proteins are substantially denatured by the intrinsic viscosity and optical rotation criteria. These denatured samples at most pH values have higher solubilities than their undenatured counterparts. Thus, loss of solubility is not a general criterion of protein denaturation. Fukushima (1959) reached a similar conclusion using an enzyme assay to determine denatured proteins.

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Functional Properties of Succinylated and Acetylated Soy Protein

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The color and aqueous solubility of soy protein were markedly improved by succinylation of the ϵ-amino groups. Succinylation of over 90% of the available amino groups shifted the isoelectric point from 4.5 to 4.0. Both emulsifying activity and emulsion stability were improved by 30 and 21%, respectively, and emulsifying capacity was improved threefold. Foaming capacity and foam stability of succinylated soy protein were improved by 20 and 50%, respectively. Sodium chloride enhanced foaming capacity. The effects of pH on emulsifying and foaming properties of succinylated protein paralleled its effects on protein solubility. Acetylation of soy isolate caused negligible changes in the functional properties studied.

Because of the increasing cost of food grade proteins and the trend toward complete food formulation from refined ingredients, there is a growing need for less expensive proteins. These should possess requisite functional properties for their successful utilization in various food products (Kinsella, 1976). Traditional animal sources of protein, although nutritionally and functionally superior, cannot continue to adequately meet these needs because of their cost and limited supply (Lockmiller, 1972).

Hammonds and Call (1970) estimated the maximum

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