revealed as the second limiting amino acid, had the value 98.0 whereas all the other amino acids exceeded the respective levels in the reference pattern. For rainbow trout roe only tryptophan, which was the first limiting amino acid with the score 90.0, did not exceed the recommended level.

In most foods and diets lysine, total sulfur-containing amino acids, or tryptophan is found to be the first limiting component (FAO/WHO, 1973). In roe lysine seems to exist in relatively high proportions as in other fish protein. On the contrary, valine shows a low score in Baltic herring roe and in this aspect the results are comparable to those of mullet (*Mugil cephalus*) roe reported by Lu et al. (1979). The overall quality of the roe protein studied is comparable to the FAO/WHO amino acid pattern and to the egg protein which is often used as the reference.

ACKNOWLEDGMENT

The authors thank Kalatukkuliike A. Sandin, Hanko, and Saarioinen Oy, Sahalahti, for the fish samples.

LITERATURE CITED

Cantoni, C., Bianchi, M. A., Renon, P., Beretta, G., *Arch. Vet. Ital.* **26**, 181 (1975).
Davies, M. D., Thomas, A. J., *J. Sci. Food Agric.* **24**, 1525 (1973).
FAO/WHO, *WHO Tech. Rep. Ser.* **No. 522**, 118 (1973).
Gjessing, E. T., *Fiskeridir. Skr., Ser. Teknol. Unders.* **4**, No. 7, 8 (1963).
Graham, C. E., Smith, E. P., Hier, S. W., Klein, D., *J. Biol. Chem.* **168**, 711 (1947).
Lu, J. Y., Ma, Y. M., Williams, C., Chung, R. A., *J. Food Sci.* **44**, 676 (1979).
Medford, B. A., Mackay, W. C., *J. Fish. Res. Board Can.* **35**, 213 (1978).
Moore, S., Stein, W. H., *Methods Enzymol.* **6**, 819 (1963).
Satia, B. P., Donaldson, L. R., Smith, L. S., Nightingale, J. N., *J. Fish. Res. Board Can.* **31**, 1796 (1974).
Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57**, 33 (1954).
Seagran, H. L., Morey, D. E., Dassow, J. A., *J. Nutr.* **53**, 139 (1954).
Sikka, K. C., Johari, R. P., *J. Agric. Food Chem.* **27**, 962 (1979).
Vuorela, R., Kaitaranta, J., Linko, R. R., *Can. Inst. Food Sci. Technol. J.* **12**, 186 (1979).
Zaitsev, V., Kizevetter, I., Lagunov, L., Makarova, T., Minder, L., Podsevalov, V., in "Fish Curing and Prosessing", MIR Publishers, Moscow, 1969, p 74.

Received for review March 3, 1980. Accepted April 15, 1980. This investigation was supported by research grants from Suomen Luonnonvarain Tutkimussäätiö and Turun Yliopistosäätiö.

Protein Solubility Characteristics of an Ultrafiltered Full-Fat Soybean Product

Carol L. Lah and Munir Cheryan*

Protein dispersibility (PDI) as a function of pH and concentration of various salts was studied for a full-fat soy protein product produced by ultrafiltration (UF). For the acidic and neutral pH regions, PDI was higher than that of the raw material (ground whole soybeans) and a commercial soy isolate. A significant difference in salting out at pH 6.7 was observed depending on the order of mixing of ingredients; protein dispersed after NaCl was dispersed showed much larger salting out effects than if the protein was dispersed in water prior to NaCl solution. At pH 6.7, PDI of UF soy was 6–20% between 0.01 and 0.2 M $CaCl_2$. When tricalcium phosphate was used at 0.01–0.15 mol of calcium/L, PDI was 81–89%. Phytic acid had a significant effect on protein solubility in the acidic pH region, and its presence may also mask the true effects of low levels of Ca^{2+} on solubility characteristics.

Much attention has been focused on nontraditional protein sources such as alfalfa, cottonseed, algae, blood, and, of course, soybeans in order to augment the limited supply of protein in the world. A substantial amount of processing is usually necessary to convert these materials into more readily utilizable forms, and as a result the product may have less than desirable functional properties. Recently, ultrafiltration (UF) has been shown in our laboratory to be a viable means of producing purified protein–fat products from whole soybeans (Omosaiye et al., 1978; Omosaiye and Cheryan, 1979a,b). By selecting the appropriate membrane pore size and operating conditions, it is possible to simultaneously fractionate and concentrate water extracts of soybeans under mild operating conditions, using much less energy than that required by conventional processes that require heating and cooling. The functional properties of such a UF soy product warrant attention not only because of the novelty of the process and relatively mild processing conditions but also because the final product is greatly reduced in undesirable components such as oligosaccharides, phytic acid, and trypsin inhibitor compared to the original soybeans and has no lipoxygenase-induced "painty" off-flavors. A product with such a desirable combination of physical properties produced by a relatively simple process is uncommon, especially in the full-fat form.

The objective of this study was to evaluate protein solubility characteristics of a full-fat soy protein product produced by ultrafiltration. Solubility is a critical functional property, since a protein generally has to be in solution in order to exert its other desirable functional characteristics (Kinsella, 1976). Nitrogen Solubility Index and Protein Dispersibility Index (PDI) are the two most common methods of evaluating solubility characteristics. They differ chiefly in that the former is a low-shear, long-time method, while the latter is done at high shear for a short time. Because most food products are generally prepared commercially under high-shear, short-time conditions for production efficiency, PDI is considered a better indication of solubility behavior in such systems (Pour-El, 1976) and hence this test was used in our studies. The effect of pH, sodium chloride, calcium chloride, and calcium phosphate tribasic on PDI was studied. In addition, phytic acid, a common constituent of many vegetable protein products, has been shown to complex with proteins, resulting in lowered solubility and possible shifts in the pH–solubility profile (Smith and Rackis, 1957; Shen, 1976; Cheryan, 1979). Since many commercial soy products

Department of Food Science, University of Illinois, Urbana, Illinois 61801.

0021-8561/80/1428-0911$01.00/0

retain a substantial amount of the phytate originally present in the soybean, and ultrafiltration has been reported to be an effective means of removing phytic acid from soybean systems (Okubo et al., 1975; Omosaiye and Cheryan, 1979a), it was of interest to study its effect on protein solubility of the UF soy product.

MATERIALS AND METHODS

Production of UF Soy Product. The ultrafiltered soy product was prepared by a method developed at the University of Illinois by Omosaiye and Cheryan (1979b). Briefly, water extracts of soybeans were made by a series of unit operations involving soaking of soybeans, blanching, grinding, and filtration in a plate-and-frame filter press. Ultrafiltration of the water extracts was done at pH 6.6–6.8 and 50 °C in a Romicon pilot-scale hollow fiber unit equipped with the XM50 membrane (50 000 molecular weight cutoff). The retentate was recycled through the ultrafiltration unit to a fivefold volume reduction. It was then rediluted with water to its original volume and reultrafiltered to a 3.3 volume reduction. The retentate was freeze-dried and stored in covered plastic containers at 4 °C until evaluation.

Analytical Methods. Moisture content, ash, and nitrogen (Kjeldahl) were determined by standard methods (AOAC, 1970). Protein was calculated as N × 6.25. Lipid was determined as described by Omosaiye and Cheryan (1979b). Phytic acid was measured by using the methods of Wheeler and Ferrel (1971) and Earley and de Turk (1944); results with either method were essentially the same. Carbohydrate is expressed as the difference of protein, fat, ash, moisture, and phytic acid.

Protein Dispersibility Index (PDI). The PDI method No. Ba 10-65 (AOCS, 1970) was used to evaluate the solubility characteristics of the UF soy product, ground whole soybeans, and Promine-D (Central Soya, Ft. Wayne, IN). UF soy and Promine-D were evaluated within 6 months of manufacture, so effects of aging would not confound solubility comparisons. Twenty grams of soy product, weighed to 0.1-g accuracy, was added to 50 mL of deionized water in a Waring blender jar and stirred with a spatula so that the product was wetted. More deionized water was then added to total 250 mL of water. The slurry was then dispersed in a Waring blender connected to a variac which was set so that the blender shaft rotated at 8500 rpm according to phototachometer measurements. After 5 min of blending, 0.5 N NaOH or 0.5 N HCl was used to adjust to the desired pH and the total water volume was then brought up to 300 mL, taking into account the addition of acid or alkali acccordingly. The soy slurry was blended for an additional 5 min (for a total of 10 min), and the pH was rechecked at the end of the blending. The PDI test was done at room temperature, and no provision was made to control temperature during blending.

The soy slurry was allowed to settle for 10 min before decanting into two 50-mL centrifuge tubes. The tubes were centrifuged in a Servall SS-4 enclosed superspeed centrifuge (Ivan Sorvall, Inc., Norwall, CT) at 1400g for 10 min, and the nitrogen in the supernatant was determined. Since 15 mL of supernatant is equivalent to 1 g of dry sample, the PDI was calculated by using

$$\text{PDI (\%)} = \frac{\text{protein in 15 mL of supernatant}}{\text{protein in 1 g of dry sample}} \times 100$$

Whole soybeans were ground fresh in a CRC Micromill with circulating cold water for 1 min for each set of solubility determinations.

Table I. Proximate Analysis of Soy Protein Products (% Dry Basis)

component	whole soybeans	UF soy product	Promine-D
protein (N × 6.25)	43.3	59.6	93.0
fat	24.0	33.0	
ash	4.70	3.30	3.80
phytic acid			
solids basis	1.26	1.32	2.14
protein basis	2.91	2.21	2.30
carbohydrate[a]	26.7	2.80	1.10

[a] By difference.

Effect of Salts. Two methods of adding NaCl to the soy slurry were evaluated. In the first, NaCl was completely dissolved in the deionized water before addition of the UF soy, and PDI was measured as before. In the second method, the NaCl was added after 7 min of blending (i.e., the protein was dispersed in water prior to addition of salt). In either case, the pH was readjusted after 10 min of blending. The blender contents were left undisturbed for 10 min and then stirred with a spatula before decanting into two 50-mL centrifuge tubes. The stirring is important so as to avoid the problem of liquid-phase separation which occurs in soy proteins at certain critical salt concentrations and pH values (Van Megen, 1974). The molarities of the Na^+ ion ranged from 0.1 to 1.0 and were evaluated at pH 2, 4.7, and 6.7.

Calcium chloride, at concentrations of 0.01, 0.03, 0.05, 0.1, and 0.2 mol/L, was added to the UF soy slurry after 7 min of blending, as described above for NaCl. The pH was adjusted to 6.7 with NaOH at the end of blending. Blender contents were left undisturbed for 10 min before decanting into the centrifuge tubes.

When sodium chloride and calcium chloride were used in combination, 0.2 M sodium chloride was added after a 10-min blend of the soy slurry, followed by an additional 3-min blend. Calcium chloride was then added and blended for 3 min more. The pH was adjusted at the end of blending to 6.7.

Calcium phosphate (tribasic) at concentrations of 0.03, 0.09, and 0.15 mol of calcium per L of protein dispersion was first dispersed in deionized water before the UF soy was added. This is to ensure that the maximum possible amount of calcium phosphate tribasic was in solution prior to introducing the protein.

The effect of added phytic acid was studied by dissolving the equivalent of 2.7 g of phytic acid/100 g of protein in 300 mL of deionized water prior to adding UF soy. Sodium phytate was purchased from Sigma Chemicals, St. Louis, MO (Lot No. 67C-0183).

RESULTS AND DISCUSSION

The compositions of whole soybeans, Promine-D, and the UF soy product are shown in Table I. Compared to the soybean, the UF soy product has higher protein and fat contents and much lower carbohydrate content. The phytate content of the UF soy product, expressed on a protein basis, is lower than that of the original soybean and very similar to that of Promine-D. The phytate content of the UF soy product reported here is higher than that observed by Omosaiye and Cheryan (1979a), which could be because less ultrafiltration was done here in the second stage (a 3.3-fold reduction instead of a 5-fold reduction). Also, phytate removal depends on pH, type and concentration of ions, nature of the phytate–protein complex, possibly the age of the soybeans, and other unknown factors. Since the yield of UF soy product was typically 50% of the weight of the original soybeans, it means that,

Table II. Analysis of Variance for pH-Solubility Data

source	df	sum of squares	mean square	F value
		UF Soy		
treatment	13	98 726.9605	7594.3816	3820.51[a]
error	52	103.3651	1.9878	
total	65	98 830.3257		
		Ground Soybean		
treatment	6	28 119.7314	4686.6219	387.04[a]
error	21	254.2884	12.1089	
total	27	28 374.0198		
		UF Soy with Phytate		
treatment	4	21 346.9279	5336.7320	350.68[a]
error	15	228.2755	15.2184	
total	19	21 575.2035		
		Promine-D		
treatment	10	62 752.1139	6275.2114	568.02[a]
error	51	563.4220	11.0474	
total	61	63 315.5359		

[a] Significant at the 0.5% level.

on a mass balance basis, about 48–54% (range for three separate UF runs) of the phytate present in the original soybeans had been removed by ultrafiltration. This process is reported to yield product with a quite low residual trypsin inhibitor activity (Lowe et al., 1977; Omosaiye and Cheryan, 1979b). The minerals remaining in the UF soy product are probably bound to the protein (Omosaiye and Cheryan, 1979b).

Protein Dispersibility in Deionized Water. Comparing the PDI of UF soy with that of ground whole soybeans, the UF soy had higher solubility at the extremes of pH and lower solubility at the isoelectric point (Figure 1 and Table II). Prefiltration of ground soybean slurry through a filter press removed much of the insoluble proteins, which would explain the higher solubilities at the acidic and alkaline pH values. On the other hand, low molecular weight nitrogenous compounds such as amino acids and small peptides passed through the UF membrane into the permeate. These compounds are soluble at the isoelectric point. Since they have been selectively removed, it results in a lower PDI in the isoelectric region.

As this is a full-fat soy product, lipid–protein interactions could have an effect on the apparent solubility and stability of the proteins. Nelson et al. (1976) have hypothesized that the formation of hydrophilic protein–lipid complexes was responsible for the soy beverage–protein stability they observed, as evidenced by lack of settling of the protein. The phospholipids in soybeans may absorb or form other types of complexes with proteins (Ohtsuru et al., 1978; Markley, 1950). It has been postulated that lecithin in its natural state favors water dispersion of soy proteins as a lecithin–protein complex (Markley, 1950). Unfortunately, the detailed structure of any lipid–protein complex, even a model one, is not yet known (Rand, 1976).

Effect of Phytic Acid. Figure 1 also appears to indicate a shift of the pH–PDI profile to the right in the acidic range upon ultrafiltration of ground soybean extracts. This could possibly be due to the partial removal of phytic acid, as postulated by Smith and Rackis (1957). Of the 12 replaceable protons in the phytic acid molecule, 6 are strongly dissociated with a pK of 1.8 (Omosaiye and Cheryan, 1979a). Hence, phytic acid is a highly charged electronegative molecule which can strongly chelate or bind minerals and proteins. In the acidic range, proteins have a net positive charge and consequently a strong phytate–protein interaction occurs, leading probably to the formation of an unionized salt. The net charge on the acidic protein is diminished as a result of anion binding. In other

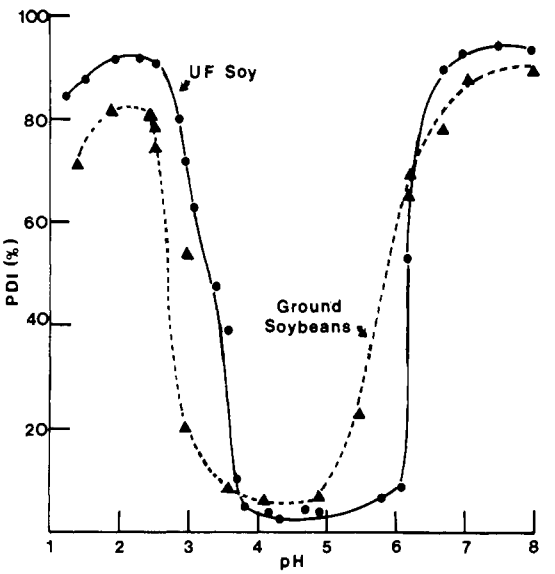

Figure 1. Solubility profile of UF soy product and ground whole soybeans in deionized water. Least significant difference (LSD) between products ($P < 0.001$) = 1.85%.

words, protein in a high phytate concentration environment (such as ground soybeans) would precipitate out at a lower pH than in a low phytate concentration system, such as dialyzed (Smith and Rackis, 1957) or ultrafiltered proteins.

Smith and Rackis (1957) determined that removal of 40% of the phytic acid from a soy isolate by dialysis resulted in a shift in the pH–solubility profile in the acidic range to the right by about 0.4 pH unit, and removal of 78% of the phytic acid caused a 1.0 pH unit shift. Addition of phytic acid back to the dialyzed protein practically restored the original solubility profile. Similarly, Iacobucci et al. (1973) observed a shift of almost 2 pH units when phytic acid was added to 11S soy protein at a level of 5 g of phytate phosphorus per 100 g of protein. The data in this study are consistent with these reports, even though the removal of phytic acid was much less, about 24% calculated on a protein basis.

For confirmation of these effects of phytic acid on the solubility profile, phytic acid was added back to the UF soy product so that the final level of phytate was 1.7 times that of the original soybean. Smith and Rackis (1957) used similar levels in their experiments. As shown in Figure 2, there is a noticeable shift of the pH–PDI curve of UF soy to the left by about 0.5 pH unit upon the addition of phytic acid. There was a slightly greater shift in the acidic side of the isoelectric point than in the alkaline side, which confirms the results of Fontaine et al. (1946a,b), who showed that phytate reduces the solubility of peanut and cottonseed proteins at pH values below the isoelectric point but does not appear to influence the solubility of these proteins at alkaline pH values. In a similar manner, it is interesting to note that, in the acidic range, the PDI curve of the ground soybeans (Figure 1) is almost superimposable on the PDI curve of the UF soy with the added phytate (Figure 2), but not in the alkaline region or above the isoelectric point.

Comparison with Promine-D. The objective measurement of PDI is still subject to many uncertainties associated with apparatus, techniques, analytical methodology, and other factors (Kinsella, 1976), which makes it difficult to compare solubility data of our products with literature values, unless a standard protein is simultaneously evaluated by each laboratory. Promine-D has been extensively studied, and much solubility data have been

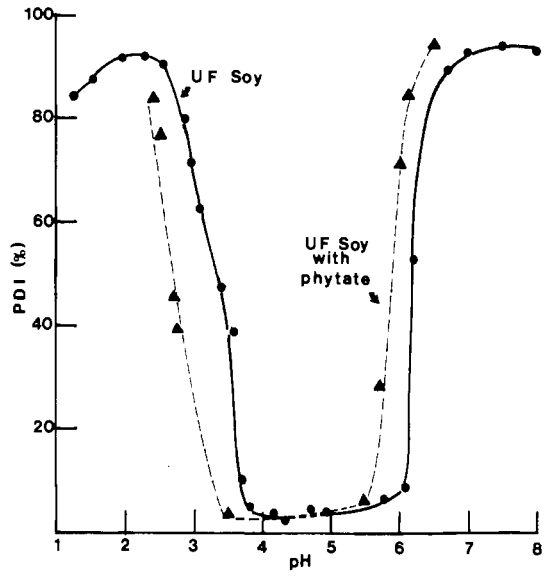

Figure 2. Effect of added phytic acid on the solubility characteristics of UF soy. Sodium phytate to equal 2.7 g of phytic acid/100 g of protein was added. LSD between treatments ($P < 0.001$) = 2.2%.

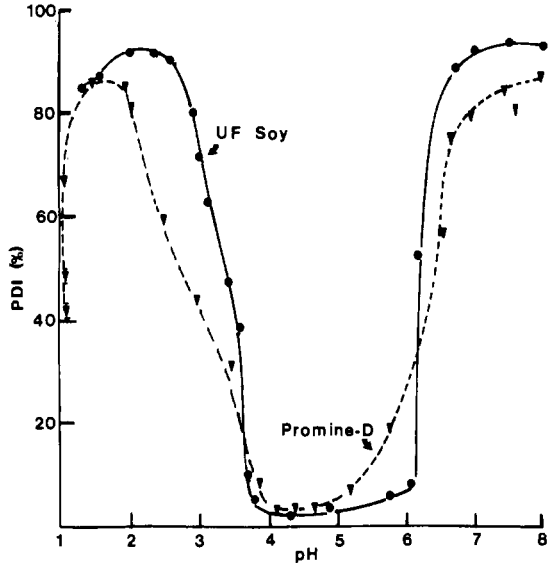

Figure 3. Comparison of pH–PDI profiles of UF soy and Promine-D in deionized water. LSD between products ($P < 0.001$) = 1.5%.

published in the literature; hence, it was used in this project as a "standard" so that better comparisons could be made with the UF soy product.

The UF soy had a higher solubility at the acidic and neutral pH ranges and a wider isoelectric range than Promine-D (Figure 3). There may be several reasons for the UF soy's higher solubility, but since the full process of making Promine-D is not known these explanations are only speculative. Unlike many commercial soy isolates, UF soy was produced with no pH adjustment and hence there were no irreversible protein aggregations that normally occur during isoelectric precipitation (Wolf, 1970), resulting in higher PDI for the UF soy product. In addition, the ultrafiltration process retains almost all the "whey" proteins, while the isoelectric precipitation process removed them. Soy whey proteins may aid in the dispersing of proteins, resulting in higher solubilities (Markley, 1950). Although Nash et al. (1971) found no clear-cut evidence for a protein-solubilizing factor in whey,

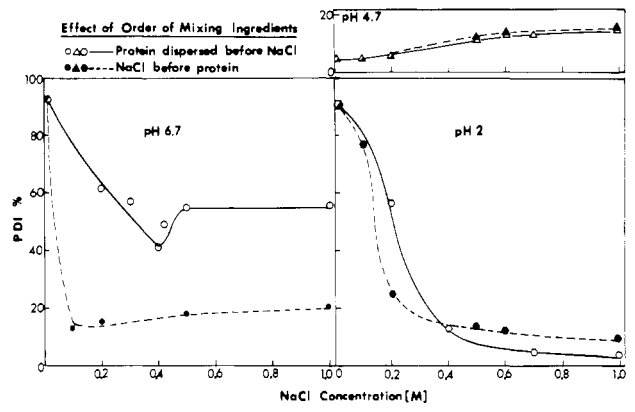

Figure 4. Effect of sodium chloride and the order of mixing of ingredients on solubility characteristics of UF soy. LSD between treatements ($P < 0.001$) = 0.53%.

the 2S and 11S fractions appeared more stable when whey proteins were present.

Shen (1976) observed less insolubilization of soy proteins if dehydration was done by freeze-drying (the method used in this project) as compared to commercial dehydration methods as would be used for Promine-D. This is confirmed to a certain extent by comparing PDI at pH 6.7 of UF soy dried by spray drying (84%; Lowe et al., 1977) to our value of 90% obtained by freeze-drying.

The wider isoelectric range of the UF soy may be due to the whey proteins (Fontaine et al., 1946b), which account for 6–8% of the total protein content of the soybean (Omosaiye and Cheryan, 1979b). Saio et al. (1973) also noted that the isoelectric range was narrower for the acid-precipitated protein than that of the water extract.

Effect of Salts. In general, the effect of adding NaCl to UF soy was to lower PDI at neutral and acidic pH (commonly referred to as the "salting out" phenomenon) and slightly increase it at the isoelectric point ("salting in"), as shown in Figure 4. There was a dramatic difference in salting out behavior depending on the order or time of addition of salt to the protein dispersion. At pH 6.7, when NaCl was dissolved in the water *before* dispersing the soy product, the PDI was as low as 14% at 0.1 M NaCl and it remained very low up to 1.0 M NaCl (broken line in Figure 4). However, when the salt was added to the slurry after the protein had been dispersed, the PDI was about 78% at 0.1 M NaCl and leveled off at 55% PDI at 1.0 M NaCl. This effect of order of addition of ingredients on PDI has rarely been mentioned in the literature, but it is of obvious significant if one is trying to incorporate soy proteins into real food systems which tend to have high salt contents, such as luncheon meats, or augment intact muscle by using techniques based on cured meat preparation. If solubility is important in the final application, then it is essential to completely hydrate and dissolve the protein prior to addition of salt. Decker and Kolar (1978) recently discussed some commercial implications of this problem. The minimum exhibited in PDI at pH 6.7 in Figure 4 has also been observed by Hermansson (1978).

A pronounced salting out effect was also observed at pH 2 (Figure 4). The time of addition did not appear to have a large effect on PDI, although the difference in PDI was "statistically significant". There was only a slight salting in at pH 4.7 (Figure 4), and again there was no large effect of the method of adding salt on the degree of salting in at the isoelectric point.

The effect of order or time of salt addition on solubility may be only an apparent difference. With more equilibration time, as occurs in many physical chemistry in-

vestigations, this difference could be less noticeable. However, this difference is significant when extrapolated to problems of food processing, as in these cases speed of preparation is of major importance.

The general explanation of the salting out phenomenon is that the Na^+ and Cl^- ions tend to screen the fixed charges of the amino acid side chains, preventing water–peptide interactions (Bello et al., 1966). However, it is generally assumed, when discussing macromolecule–ion interactions, that a hydrated ion is reacting with a hydrated macromolecule (Franks and Eagland, 1975). This may not be true in the case of dispersion of protein in NaCl solutions (i.e., adding NaCl to the water before the protein) because the macromolecule may not be fully "hydrated". When NaCl is dissolved in the water first, the resulting sodium ion has a slightly more positively charged hydrogen than the hydrogen of ordinary water and is therefore better able to compete for a receptor site on the protein (Bello et al., 1966), leading to more drastic salting out. However, in the reverse case, once water has been bound to the protein first, as would be the case for the protein dispersed in deionized water before salt addition, the Na^+ ions are less able to compete for sites on the protein or for water molecules directly bound to the amide dipoles (Franks and Eagland, 1975), thus resulting in less salting out. Hermansson (1972) found that soy proteins took a longer time to reach maximum hydration in salt solutions than in water, even though actual swelling ability was decreased in the presence of salt. The differences in salting out behavior due to the time of addition observed in this study between pH 2.0 and 6.7 are probably due to complex interactions, part of which are due to the roles Na^+ and Cl^- play according to the difference in charge of the amino acid side groups at the given pH.

In contrast to the salting out phenomenon, salting in is a nonspecific electrostatic interaction between a charged protein molecule and the ionic environment (Von Hippel and Schleich, 1969). These interactions cause a net decrease in the acitivity coefficient of the protein which is reflected as an increase in the net stability and solubility. Salting in is not dependent on ion type but on ionic strength. Considering this definition, it is not surprising to find that the time of addition of salt had little or no effect on the degree of salting in (Figure 4).

Effect of Calcium Salts. Similar effects on protein dispersibility were observed with calcium ions except that in this case the mechanism may be more complicated. Figure 5 shows the effect of $CaCl_2$ on PDI on pH 6.7. At 0.01 M Ca^{2+}, PDI dropped to 6.7%. At higher Ca^{2+} concentrations, there was an apparent salting in, resulting in a PDI of 19% at 0.2 M Ca^{2+}. These results are in general agreement with other researchers (Appurao and Narasinga Rao, 1975; Sakakibara and Noguchi, 1977; Hermansson, 1978; Van Megen, 1974) who observed protein precipitation at 0.01–0.03 M Ca^{2+} and salting in beginning around 0.05 M Ca^{2+}.

The most common explanation for these effects is that they are due to a change in the conformation of the protein as a result of direct binding of calcium by the protein, setting off a denaturing mechanism different from other ions (Bello et al., 1966; Franks and Eagland, 1975; Appurao and Narasinga Rao, 1975; Saio et al., 1967). Hermansson (1978) has suggested that charge neutralization of proteins by Ca^{2+} may also be important. Solubilities were higher for the UF soy in which 0.2 M NaCl had been added to the water dispersion before the addition of $CaCl_2$ (Figure 5), although the increase is minor. This is due possibly to the Na^+ ions partially shielding the interacting sites of

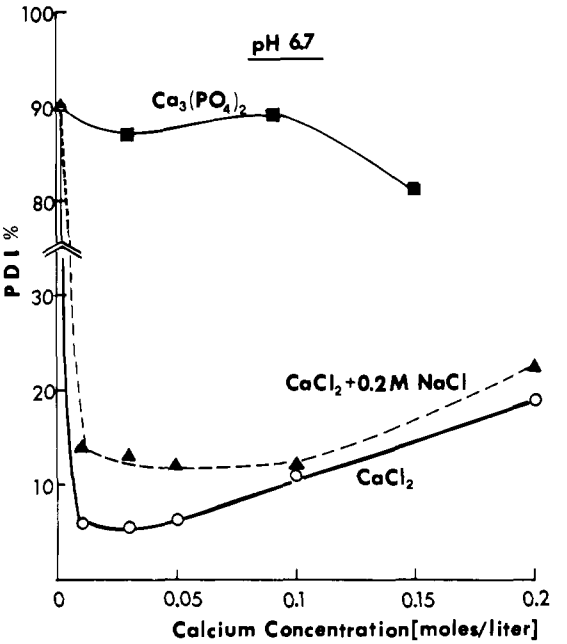

Figure 5. Effect of calcium salts on solubility characteristics of UF soy at pH 6.7. LSD between treatments ($P < 0.001$) = 0.8%.

the protein molecules from the calcium ions (Saio et al., 1973; Appurao and Narasinga Rao, 1975).

At higher $CaCl_2$ concentration (0.1 M Ca^{2+} and above), the literature shows greater salting in effects than were observed for the UF soy (Van Megen, 1974; Hermansson, 1978). No doubt part of this difference reflects the difference in protein fractions and processing methods used by the other experimenters. However, as previously discussed, the UF soy did not show an appreciable salting in effect in the isoelectric region in the presence of NaCl; therefore, the same mechanism may be at work in this case.

The effect of calcium salts on protein solubility is of commercial importance for two reasons: the manufacture of tofu, where low solubility is required, and for the fortification of soy-based infant formulas (to ensure sufficient intake of calcium), where good solubility in the presence of calcium is desirable. The latter is of special concern if it is the sole or major source of calcium in the infant's diet. The data in Figure 5 indicate that $CaCl_2$ is adequate for the former purpose but should not be used for the latter. However, when calcium was added to the UF soy in the form of calcium phosphate tribasic, the UF soy retained a PDI of 81–89% (Figure 5). Calcium phosphate tribasic is quite insoluble, and so the calcium in this form does not readily react with the protein. For this reason, soy beverages should be fortified with this form of calcium rather than with $CaCl_2$. Some settling of the calcium phosphate would occur, and this would necessitate adequate mixing or shaking of the container prior to consumption of the fortified beverage.

When sodium phytate was added to the UF soy dispersion, there was a distinct inhibition of protein precipitation by calcium. The added-phytate UF soy was 82.5% soluble at 0.01 M $CaCl_2$ as compared to 6.7% in the low phytate concentration system. At higher calcium levels, there was no significant difference between the high and low phytate concentration systems, agreeing with the findings of Appurao and Narasinga Rao (1975). Phytic acid forms extremely stable complexes with multivalent cations, and hence calcium binds preferentially to the phytic acid instead of the protein; the Ca^{2+} is unavailable for interaction with protein, thus preventing its precipitation. At higher Ca^{2+} levels, however, all the charged

groups of phytic acid are saturated with calcium. The excess free Ca^{2+} interacts with proteins and causes them to precipitate out. It appears that reduction of phytate makes soy proteins more sensitive to Ca^{2+}.

CONCLUSIONS

Protein dispersibility characteristics of ultrafiltered soybean products are interesting and somewhat unusual. The product is very sensitive to low levels of Na^+, displaying less of a salting in at the isoelectric point and more of a salting out at acidic and neutral pH than other forms of soy proteins. The method of studying salt–protein interactions is important. Proteins dispersed before NaCl showed higher solubilities than if NaCl was dispersed first. UF soy is also very sensitive to $CaCl_2$ but not to calcium phosphate tribasic, up to a concentration of 0.15 mol of calcium per L. Our studies reiterate the importance of considering effects of phytic acid whenever solubility characteristics of vegetable proteins are studied. Phytic acid was found to have a significant effect on solubility characteristics; the data suggest that its association with soy proteins results in a shift of the pH–solubility profile to the left by about 0.5 pH unit and may mask the true effects of Ca^{2+} on protein solubility, especially at low Ca^{2+} levels. Preliminary studies indicate that this product may be particularly useful as emulsification and whipping aids. These functional properties have also been studied and will be reported in forthcoming publications.

ACKNOWLEDGMENT

The authors acknowledge the analytical help of K. E. Weingartner with phytate assays and O. Omosaiye with phytate and lipid assays. The authors thank the Illinois Soybean Program Operating Board for a research grant and the New York Section of the Institute of Food Technologists and the FMC Educational Fund for fellowships to C.L.L. for graduate study in food science.

LITERATURE CITED

AOAC, "Official Methods to Analysis", 11th Ed., Association of Official Analytical Chemists, Washington, DC, 1970.
AOCS, "Official and Tentative Methods of the American Oil Chemists Society", 3rd Ed., American Oil Chemists Society, Chicago, IL, 1970.
Appurao, A. G., Narasinga Rao, M. S., *Cereal Chem.* **52**, 21 (1975).
Bello, J., Haas, D., Bello, H. R., *Biochemistry* **5**, 2539 (1966).
Cheryan, M., 177th National Meeting of the American Chemical Society, Honolulu, HI, April 1–6, 1979, Paper No, 51, Division of Agricultural and Food Chemistry.
Decker, C. D., Kolar, C. W., 5th International Congress of Food Science and Technology, Kyoto, Japan, Sept 17–23, 1978, Paper No, I-2-3.
Earley, E. B., de Turk, E. E., *J. Am. Soc. Agron.* **36**, 803 (1944).
Fontaine, T. D., Pons, W. A., Irving, E. W., *J. Biol. Chem.* **164**, 487 (1946a).
Fontaine, T. D., Irving, G. W., Markley, K. S., *Ind. Eng. Chem.* **38**, 658 (1946b).
Franks, F., Eagland, D. *CRC Crit. Rev. Biochem.* **3**, 165 (1975).
Hermansson, A. M., *Lebensm.-Wiss. Technol.* **5**, 24 (1972).
Hermansson, A. M., *J. Texture Stud.* **9**, 33 (1978).
Iacobucci, G. A., Myers, D. V., Okubo, K., U.S. Patent 3 736 147, 1973.
Kinsella, J. E., *CRC Crit. Rev. Food Sci. Nutr.* **7**, 219 (1976).
Lowe, V. A., Ferrier, L. K., Cheryan, M., 37th Annual Meeting of the Institute of Food Technologists, Philadelphia, PA, June 5–8, 1977, Paper No. 460.
Markley, K. S., "Soybeans and Soybean Products", Vol. 1, Interscience, New York, 1950.
Nash, A. M., Kwolek, K. F., Wolf, W. J., *Cereal Chem.* **48**, 360 (1971).
Nelson, A. I., Steinberg, M. P., Wei, L. S., *J. Food Sci.* **41**, 57 (1976).
Ohtsuru, M., Kanamoto, R., Kito, M., 5th International Congress of Food Science and Technology, Kyoto, Japan, Sept 17–23, 1978, Paper No. 5d-06.
Okubo, K., Waldrop, A. B., Iacobucci, G. A., Myers, D. V., *Cereal Chem.* **52**, 263 (1975).
Omosaiye, O., Cheryan, M., Matthews, M. E., *J. Food Sci.* **43**, 354 (1978).
Omosaiye, O., Cheryan, M., *Cereal Chem.* **56**, 58 (1979a).
Omosaiye, O., Cheryan, M., *J. Food Sci.* **44**, 1027 (1979b).
Pour-El, A., in "Proceedings of the World Soybean Research Conference", Hill, L. D., Ed., Interstate Printers and Publishers, Danville, IL, 1976.
Rand, R. P., in "Food Emulsions", Friberg, S., Ed., Marcel Dekker, New York, 1976, Chapter 6.
Saio, K., Koyama, E., Watanabe, T., *Agric. Biol. Chem.* **31**, 1195 (1967).
Saio, K., Watanabe, T., Kuji, M., *J. Food Sci.* **38**, 1139 (1973).
Sakakibara, M., Noguchi, H., *Agric. Biol. Chem.* **41**, 1575 (1977).
Shen, J. L., *J. Agric. Food. Chem.* **24**, 784 (1976).
Smith, A. K., Rackis, J. J., *J. Am. Chem. Soc.* **79**, 633 (1957).
Van Megen, W. H., *J. Agric. Food Chem.* **22**, 126 (1974).
Von Hippel, P., Schleich, T., in "Structure and Stability of Biological Macromolecules", Timasheff, S. N., Fasman, G. D., Eds., Marcel Dekker, New York, 1969.
Wheeler, E. L., Ferrel, R. E., *Cereal Chem.* **48**, 312 (1971).
Wolf, W. J., *J. Agric. Food Chem.* **18**, 969 (1970).

Received for review December 10, 1979. Accepted May 1, 1980.