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

Kay L. Franzen*[1] and John E. Kinsella

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

Department of Food Science, Cornell University, Ithaca, New York 14853.
[1]Present address: The Pillsbury Company, 311 2nd St. S.E., Minneapolis, Minnesota 55414.

market potential for protein ingredients, e.g. casein and sodium caseinate, dried milk, soy proteins, egg solids, hydrolyzed vegetable protein, and chicken meat, at approximately 3.1 billion pounds annually. Of this quantity 600 million pounds should be relatively nonfunctional ingredient protein while the remaining 2.5 billion pounds of protein should possess a high degree of functionality. The effective utilization of protein entails matching a wide variety of functional and nutritional characteristics to the complex needs of manufactured food products. The critical functional properties necessary in protein ingredients include solubility, heat coagulation, water and fat absorption, gelation, emulsion stabilization, whippability, and good organoleptic properties (Kinsella, 1976).

There is an urgent technical need for the development of methodology to manipulate plant proteins and endow them with desirable functional characteristics (Burrows et al., 1972; Krochta et al., 1975; Kinsella, 1976). Thus, hydrolysis with acid, alkali, and proteolytic enzymes is occasionally employed to improve functional properties (Tannenbaum et al., 1970; Cheftel et al., 1971; Arai et al., 1975). However, these methods have certain limitations, i.e., excessive hydrolysis and humin formation by acids, destruction of certain amino acids and formation of potentially toxic compounds by alkali (DeGroot and Slump, 1969; Provensal et al., 1975), and formation of bitter peptides by enzymes (Arai et al., 1975). Additional approaches for improving functional properties warrant study. Chemical derivatization, i.e., acylation of amino acid residues with compounds containing functional groups, e.g., succinic anhydride, has been suggested as one means of accomplishing this (Groninger, 1973; Grant, 1973; Melnychyn and Stapley, 1973; Chen et al., 1975).

Biochemists have frequently used chemical modification to determine the active sites of enzymes and antigens and conformation of proteins (e.g., see Nakagawa and Perlmann, 1972; Paik and Kim, 1972; Meighen and Schachman, 1970; Gounaris and Perlmann, 1967; Habeeb, 1967; Oppenheimer et al., 1967; Polyanovski, 1965; Hass, 1964; Riordan and Vallee, 1964; Habeeb et al., 1958; Fraenkel-Conrat et al., 1949). The procedures, mechanisms, and results of the chemical modification of proteins in these and other studies have been reviewed by Means and Feeney (1971).

Chemical modification has been applied to food proteins, e.g., fish protein concentrate, wheat flour proteins, casein, soy protein, single cell protein, and egg white protein, as a result of the need for proteins with improved functional properties for specific food uses (Groninger and Miller, 1975; Chen et al., 1975; Gandhi et al., 1968; Melnychyn and Stapley, 1973). Succinylated fish protein has a greater emulsifying capacity and a lower isoelectric point compared with unmodified fish protein extracted at pH 11.5 (Chen et al., 1975). According to Groninger and Miller (1975) succinylated and enzymatically hydrolyzed fish myofibrillar protein exhibited improved functionality in terms of dispersibility, aeration capacity, and foam stability. Groninger (1973) reported that succinylated fish myofibrillar protein possessed relatively good dispersion in the pH range 6.0–8.5, a high emulsification capacity, and bland odor and flavor. Exhaustive succinylation increased the solubility and viscosity of glutenin and gliadin (Grant, 1973). Unlike native casein, acetyl, succinyl, and maleyl casein derivatives were soluble at a pH of 4.4, and Creamer et al. (1971) successfully added them to acidic beverages and fruit purees. Melnychyn and Stapley (1973) noted that vegetable proteins, modified with mono- and dicarboxylic anhydrides, had decreased viscosities, lower isoelectric points, mild flavors, substantially no odor, and a pleasant taste. Moreover, unlike conventional vegetable protein isolates, they did not "feather", i.e., precipitate, when added to hot coffee or tea. Egg white modified with 3,3-dimethylglutaric anhydride had increased heat stability (Gandhi et al., 1968). N-Succinylated egg yolk proteins were useful in the production of emulsions, i.e. mayonnaise and salad dressing (Evans and Irons, 1970).

Soy protein is commonly incorporated into baked goods, processed meats, meat analogues, baby cereal, and pet food, and it is the most important source of meat extenders and meat analogues (Hammonds and Call, 1970; Horan, 1975). Much of the soy protein available lacks needed functional properties (Wolf, 1972). The purpose of this research was to investigate the effects of acetylation and succinylation on functional properties of soy protein.

MATERIALS AND METHODS

Acylation of Protein. Succinylation was performed by the procedure of Hoagland (1966). Soy isolate (2 g) (Promine D, Central Soya, Chicago, Ill.), consisting of 90–95% protein, was dispersed in phosphate buffer (75 mM, 250 ml), and 0.5-g increments of succinic anhydride (Eastman Organic Chemicals, Rochester, N.Y.) were added to a total of 2 g with stirring. During succinylation the pH was maintained between 7 and 8 with 3.5 M NaOH. After the pH stabilized the solution was dialyzed against distilled water (4 °C, 24 h) to remove impurities and excess reagents. A seamless cellulose dialyzer tubing with a pore diameter of 4.8 nm (Fisher Scientific Co., Pittsburgh, Pa.) was used. The chemically modified protein was recovered by lyophilization. No difficulties were encountered when this procedure was scaled-up to yield larger quantities of derivatized protein using the appropriate proportions of ingredients. Proteins were also acylated with 0.2- and 0.5-g quantities of succinic anhydride using this procedure.

The method of Hoagland (1966) adapted from the method of Fraenkel-Conrat et al. (1949) was employed to acetylate soy protein. Saturated aqueous sodium acetate solution (120 ml) was prepared at 25 °C by dissolving sodium acetate (55 g) in distilled water (120 ml). Soy isolate (2 g) was added to this solution and acetic anhydride (Eastman) was added in 0.2-ml increments to a total of 2.4 ml over a period of 1 h. The solution was dialyzed against distilled water at 4 °C for 24 h, and the acetylated protein was recovered by lyophilization.

Determination of Extent of Chemical Modification. The ninhydrin assay (Paik and Kim, 1972) adapted from the method of Moore and Stein (1954) was used to quantify the extent of chemical modification. Ninhydrin solution (1 ml) was added to a 1% aqueous protein solution (1 ml) and the mixture was heated at 100 °C (in a boiling water bath) for 5 min and cooled to 25 °C. Distilled water (5 ml) was added and the absorbance was determined at 580 nm against a distilled water/ninhydrin solution blank. The absorbance indicated the number of free amino groups available for reaction with ninhydrin reagent, and the difference in absorbancies between unmodified and acylated proteins reflected the extent of acylation.

The method of Ellman (1959) was used to estimate the extent of acylation of sulfhydryl groups. The ninhydrin solution was prepared by mixing citrate buffer (30 ml) (0.2 M, pH 5) and 4% ninhydrin in ethyl Cellosolve (2-ethoxyethanol) (30 ml) with a stannous chloride ($SnCl_2$) suspension containing 50 mg of $SnCl_2$ (1 ml).

Tests of Protein Functionality. *Solubility.* Dispersions (1%) of the protein were prepared and mixed for 1 min on a Vortex-Genie test tube mixer (Scientific Industries, Inc., Springfield, Mass.) at speed setting 5. The

samples were centrifuged for 10 min on a clinical centrifuge (Precision Vari-Hi-Speed Centricone, Precision Scientific Co., Chicago, Ill.). Aliquots (1 ml) of the supernatant were diluted with distilled water (3 ml), and this solution (3 ml) was added to the biuret reagent (3 ml). After 30 min the absorbances of these solutions at 540 nm were determined against a distilled water/biuret reagent blank. The absorbance values of the modified protein samples were compared to those of the corresponding native protein samples to quantify the differences in relative solubilities. The exact amounts of protein in the supernatant were calculated from a biuret standard curve of bovine serum albumin.

The biuret reagent (Paik and Kim, 1972) was prepared by first dissolving sodium tartrate (3 g) and copper sulfate ($CuSO_4$) (0.75 g) in distilled water (250 ml). A 10% aqueous NaOH solution (150 ml) was added with constant stirring, and the final volume was adjusted to 500 ml with water.

pH–Solubility Profile. Soy isolate (0.3 g) was dissolved in 0.1 M NaOH (30 ml), and 3.0-ml aliquots were pipetted into centrifuge tubes (10 ml size). The pH of the aliquots was adjusted with HCl (1 M) to cover the range 12.0 to approximately 1.5, and the samples were shaken on an Evapo-mix (Buchler Instruments, Fort Lee, N.J.) at 25 °C for 1 h. The pH was readjusted to the proper value where necessary. The samples were centrifuged at 27 000*g* for 20 min, and protein in the supernatant was quantified using the biuret reagent. The solubility profile was obtained by plotting protein solubility vs. pH where the solubility at pH 12 was 100%.

Emulsification. The method of Yasumatsu et al. (1972) was used for determining emulsifying activity and the heat stability of emulsions. Protein (0.7 g) was suspended in water (10 ml), and corn oil (Mazola, CPC International, Englewood Cliffs, N.J.) (10 ml) was added. This mixture was homogenized in an Eberbach semi-micro blender container at high speed (20 000 rpm) for 1 min. The emulsion was divided evenly into two 10-ml centrifuge tubes and centrifuged at 1300*g* for 5 min. The emulsifying activity (percent) was calculated as height of emulsified layer divided by height of total contents in the tube. The heat stability of these emulsions was measured by recentrifugation following heating at 80 °C for 30 min.

The method of Swift et al. (Swift et al., 1961; Swift and Sulzbacher, 1963) was used to determine the emulsifying capacities of native and succinylated proteins. Protein (50 mg) was added to water (5 ml) and dispersed at low speed (12 000 rpm) for 30 s in an Eberbach semi-micro blender container. To facilitate detection of the inversion point, Methyl Red O was added to the protein/water mixture. Corn oil was added to the protein dispersion from a 50-ml buret at the initial rate of 1 ml/s. The volume of oil at the inversion point was recorded, and emulsifying capacity was calculated as volume (milliliters) of oil emulsified per gram of protein in the sample.

To investigate the effect of pH on emulsifying capacity, each protein dispersion was adjusted to the desired pH value with HCl or NaOH. The pH was measured after dispersion but before emulsification.

Foam Expansion and Foam Stability. Protein solutions (0.3 g in 30 ml of distilled water) were prepared in 100-ml graduated cylinders. Stoppers were inserted, and they were placed horizontally in a mechanical shaking device described by Wang and Kinsella (1976). Following agitation for 1 min at 25 °C the cylinders were removed from the shaker, and a filter paper disk, having a diameter equal to that of the cylinder, was gently pushed onto the top of the foam layer to catch any foam remaining on the sides and to eliminate large air pockets. The foams resisted gentle compression by this disk at a point which was consistent among replicate samples. The height of the foam, i.e., foam expansion, was recorded as an index of the foaming power of the protein. The height of the foam recorded after 30 min of standing at 24 °C indicated foam stability.

To study the effects of pH on foam expansion and foam stability, each protein dispersion was adjusted to the desired pH with HCl or NaOH before agitation. Water was replaced with NaCl (1 M) or sucrose (50% w/v) solutions to determine the effect of these additives on foaming properties. Egg albumin (Nutritional Biochemicals Corp., Cleveland, Ohio) was used as a standard for comparison of foaming ability.

Viscosity and Heat Stability. The effects of several parameters influencing the viscosity of native and succinylated soy isolate, i.e., protein concentration, pH, temperature, and salts, were determined. All viscosity measurements were made using a Brookfield Viscometer Model LVT at 60 rpm fitted with a no. 4 spindle.

Amino Acid Analysis. Amino acid analyses were performed on native and succinylated soy isolate to determine whether succinylation altered the amino acid composition of this protein. Duplicate samples of native (17.1 mg) and succinylated (20.5 mg) soy isolate were used for amino acid analyses. Each sample was placed in a 10-ml glass ampule to which was added concentrated HCl (5 ml), distilled water (4 ml), and norleucine solution (1 ml). Each sample was then frozen in an acetone/dry ice mixture followed by evacuation of oxygen. The samples were sealed with an acetylene torch and hydrolyzed at 110 °C for 22 h. After acid hydrolysis each sample was filtered through a Millipore filter, and 2-ml portions were evaporated to dryness under NaOH in a vacuum desiccator. The residue was dissolved in pH 2.2 sodium citrate buffer (4 ml). An aliquot (1 ml) of the solution was analyzed on a Beckman Model 120C amino acid analyzer.

RESULTS

Extent of Chemical Modification. Treatment of soy protein isolate (2 g) with 0.2, 0.5, and 2 g quantities of succinic anhydride progressively acylated 50, 78, and 93% of the available amino groups, respectively. Although succinylation of all nucleophilic groups on amino acid residues is possible (Gounaris and Perlmann, 1967), the ϵ-amino group of lysine is most readily acylated because of its relatively low pK and its steric availability for reaction. A negligible amount of acylation of the sulfhydryl group occurred in these soy protein samples. Treatment of soy isolate with acetic anhydride acylated 92% of the amino groups.

Appearance. Succinylation substantially increased the specific volumes, i.e. volume/unit weight, of soy isolate and lightened its color from tan to chalk-white. Acetylation of soy isolate had the same effect on volume but produced no lightening of the color. No off-odors nor flavors were imparted by either the succinylation or acetylation process.

Hydration Characteristics. Succinylated soy isolate, at all three levels of acylation, hydrated and solubilized instantaneously when added to water in contrast to the unmodified protein which floated on the aqueous surface and hydrated slowly when stirred.

Succinylation using progressively increasing amounts of succinic anhydride enhanced the aqueous solubility of native soy isolate from 0.95 mg of protein/ml to 1.15, 1.65, and 1.70 mg of protein/ml, respectively (Figure 1). This represented increases in aqueous solubility of 20, 71, and

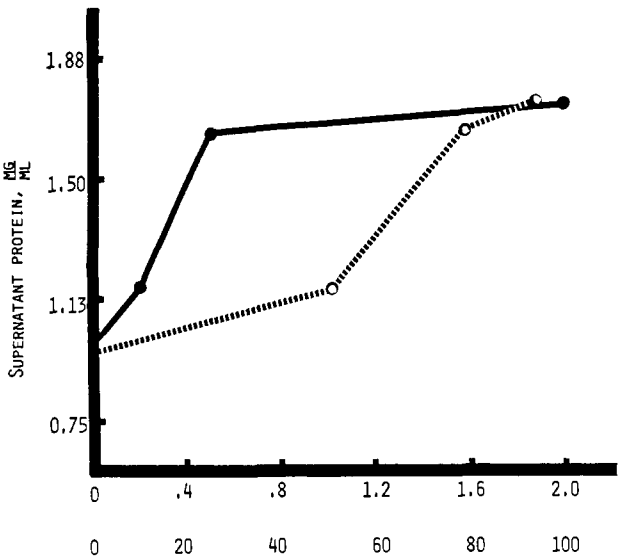

Figure 1. The effects of increasing degrees of succinylation on the solubility of soy protein at pH 7.0: (—) quantity of succinic anhydride, grams; (- - -) percent of amino groups succinylated.

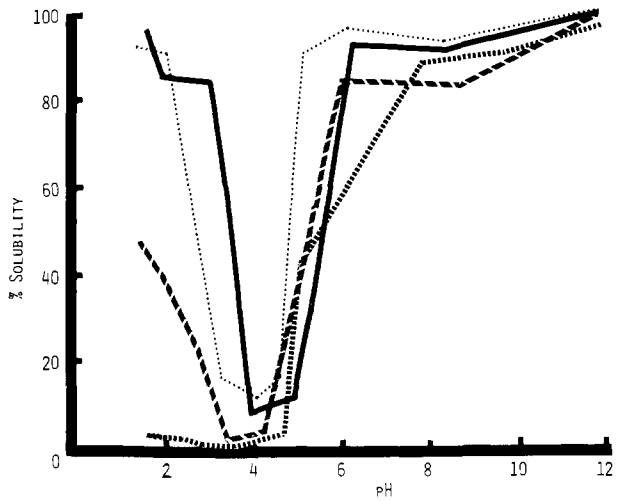

Figure 2. Solubility profile of soy isolate and succinylated soy isolate: (—) soy isolate and soy isolates succinylated with 0.2 (· · ·), 0.5 (- - -), and 2.0 (- · -) g of succinic anhydride per 2 g of protein, respectively.

80%, respectively. Noteworthy was the marked improvement in solubility at 50% succinylation. The aqueous solubility of acetylated soy protein isolate, with 92% of the available amino groups acylated, was greater (20%) than the unmodified protein. The solubilities of unmodified, acetylated, and succinylated soy protein were 0.95, 1.13, and 1.70 mg of protein per ml of water (pH 7.0) respectively.

The pH–solubility profile of soy isolate showed decreasing solubility with decreasing pH, minimum solubility at the isoelectric point (IEP), and resolubilization at pH values acidic to the IEP (Figure 2). Following exhaustive succinylation (over 90%), the solubility at alkaline pH values was high and decreased to a minimum at the IEP. Unlike the unmodified proteins, the acylated proteins did not resolubilize at pH values acidic to the IEP (Figure 2). As the extent of succinylation decreased, the solubility at pH values below the IEP increased and approached that of native soy isolate (Figure 2). Generally, succinylation shifted the IEP of soy isolate approximately 0.5 pH unit, i.e. from pH 4.5 to 4.0.

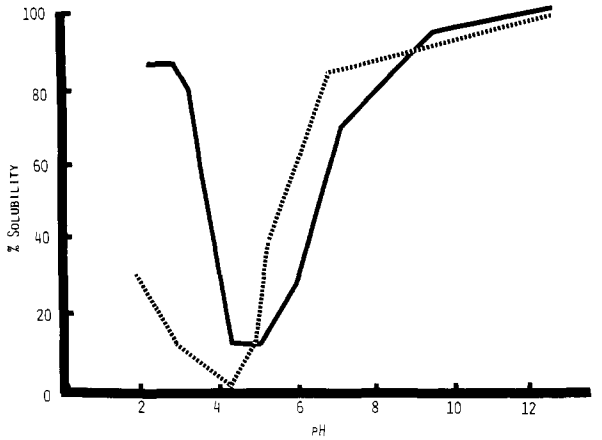

Figure 3. The pH-solubility profile of soy protein (—) and acetylated soy protein (- - -).

Table I. Relative Emulsifying and Foaming Capacities of Native, Succinylated, and Acetylated Soy Isolate

Protein soy isolate	Emul act., %	Emul stability, %	Foam expansion, ml	Foam stability, ml
Native	74.7	73.2	11.2	6.5
Succinylated	97.0	89.2	15.7	13.7
Acetylated	68.6	68.5	11.8	9.7
Egg albumin			30.0	29.0

Acetylation decreased the isoelectric point of the soy protein (Figure 3) and the solubility below pH 4.5. The solubility of the acetylated protein was enhanced between pH 5 and 6.5 compared to the unmodified protein.

Surfactant Characteristics. Exhaustive succinylation increased the emulsifying activity and emulsion stability of soy isolate from 74.7 and 73.2% to 97.0 and 89.2%, respectively (Table I). The emulsifying activity and emulsion stability of acetylated soy isolate were 68.6 and 68.5%, respectively.

When the emulsifying capacities of soy isolate and succinylated (using 1 g of succinic anhydride/2 g of protein) soy isolate were determined at pH 7.5, both proteins exhibited typical curves of decreasing emulsifying capacity with increasing protein concentration (Figure 4). Succinylation, however, markedly enhanced the emulsifying capacity of soy isolate at all three protein concentrations. The emulsifying capacities of 0.6, 1, and 2% aqueous solutions of soy isolate were 165.6, 128.0, and 87.3 ml of oil/g of protein, respectively, compared to values of 449.2, 287.1, and 192.0 ml of oil/g of protein for similar solutions of succinylated soy isolate. The soy isolate did not form stable emulsions during the addition of oil, and no detectable inversion point was observed. Microscopic examination of this emulsion revealed coalescing droplets of oil. This was in contrast to succinylated soy isolate which formed stable emulsions throughout the addition of oil and exhibited a sharp inversion point. Significantly, exhaustive succinylation was not required to achieve an enhancement in emulsifying capacity.

The pH–emulsifying capacity profiles of native and succinylated soy isolate followed the shape of their respective pH–solubility profiles (Figure 5). At pH values above the IEP, e.g. 5.5, 7.5, and 10, the emulsifying capacity of unmodified soy isolate was 12.3, 128.6, and 166.2 ml of oil/g of protein, respectively, compared with values of 107.8, 287.1, and 305.2 ml of oil/g of protein for succinylated soy isolate. However, at pH values below the IEP, e.g., 4 and 2, the emulsifying capacities of soy isolate

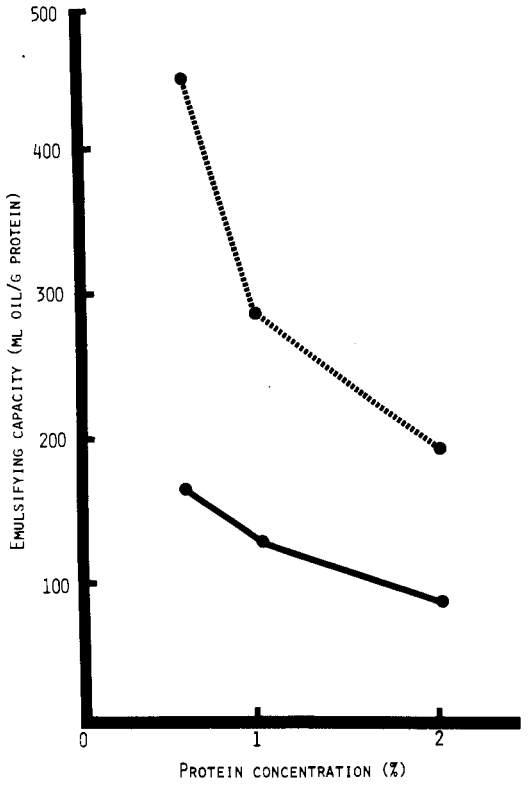

Figure 4. Comparison of the relative emulsifying capacities of soy protein isolate (—) and succinylated soy protein isolate (- - -) at different protein concentrations.

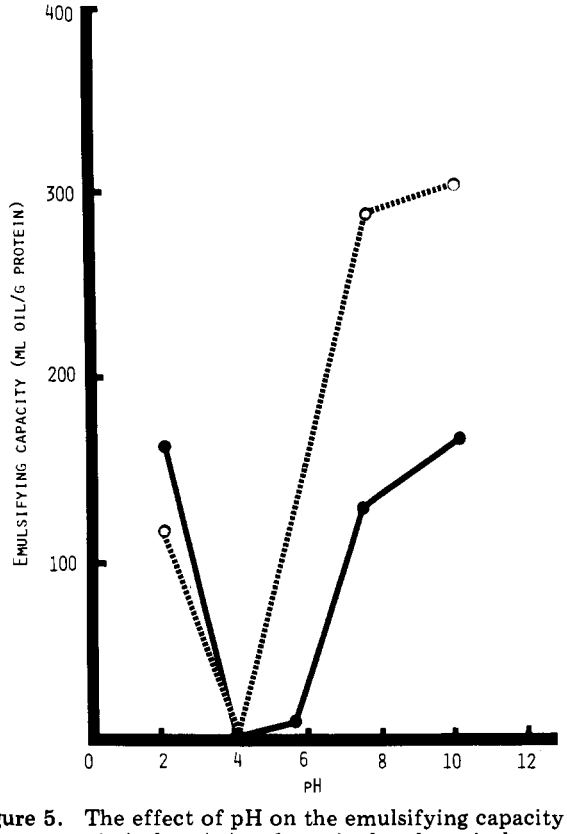

Figure 5. The effect of pH on the emulsifying capacity of soy protein isolate (—) and succinylated soy isolate (- - -).

were 6.6 and 162.0 ml of oil/g of protein, respectively, compared with values of 5.8 and 116.8 ml of oil/g of protein for succinylated soy isolate.

The foam expansion and foam stability of exhaustively

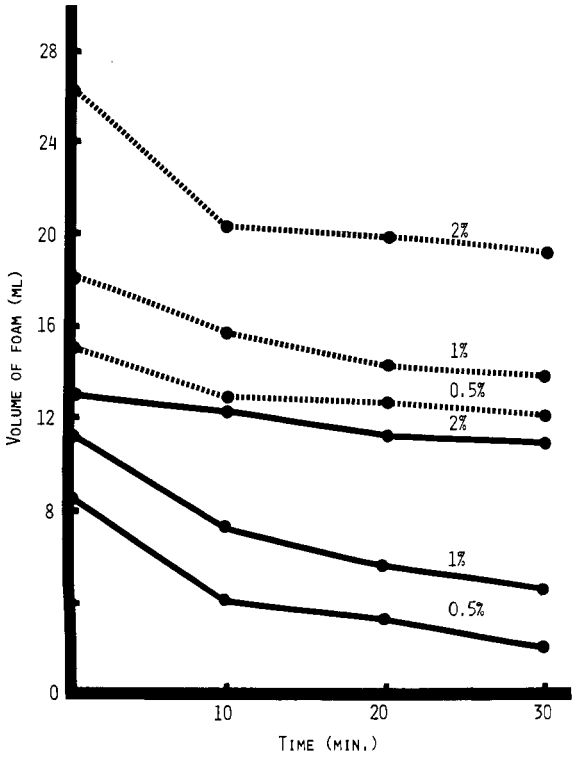

Figure 6. The effect of different protein concentrations of the initial foam volume and foam stability of soy protein isolate (—) and succinylated soy isolate (- - -). The protein concentration in solution initially is indicated in percent.

succinylated soy isolate were 15.7 and 13.7 ml compared with respective values of 11.2 and 6.5 ml for native soy isolate (Table I). Acetylated soy isolate had a foam expansion and foam stability of 11.8 and 9.7 ml while the egg albumin standard had respective values of 30.0 and 29.0 ml.

Foaming properties of unmodified and succinylated (50%) soy isolate diminished with decreasing protein concentration (Figure 6). At all three protein concentrations, however, the foaming ability of succinylated soy isolate was substantially greater than that of the soy protein. In fact, 0.5% solutions of succinylated soy isolate produced larger foam volumes than 2% solutions of unmodified soy isolate. Soy isolate, at concentrations of 0.5, 1, and 2%, produced foam expansions of 8.7, 11.3, and 13 ml and foam stabilities of 2, 4.7, and 11 ml, respectively. Corresponding concentrations of succinylated soy isolate yielded foam expansions of 15, 18.3, and 26.7 ml and foam stabilities of 12.3, 14, and 19.3 ml, respectively.

Sodium chloride (1 M) markedly enhanced the foaming abilities of both native and succinylated soy isolate whereas sucrose (50% w/v) slightly depressed their foaming (Figure 7).

The pH–foam expansion/foam stability profiles revealed that the succinylated soy isolate showed considerably greater foam expansions and foam stabilities than the unmodified protein at pH values above the IEP (Figure 8). Because of its lack of solubility at pH values acidic to the IEP, however, the foam volumes of succinylated soy isolate below pH 4 were severely depressed compared with those of unmodified soy isolate.

The emulsifying and foaming properties of the acetylated soy protein were not significantly changed from the unmodified protein.

Viscosity. The effects of pH, protein concentration, temperature, and salts, i.e. $CaCl_2$, on the viscosities of

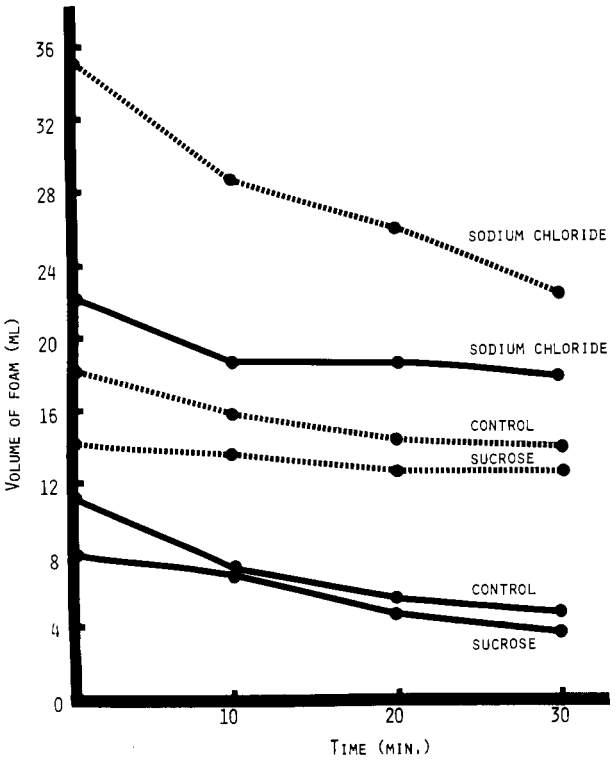

Figure 7. The effects of sodium chloride and sucrose on the foaming properties of unmodified (—) and succinylated soy protein (- - -).

Table II. Effects of $CaCl_2$ and KCl on the Viscosities of Aqueous Solutions of Native and Succinylated Soy Isolate

	Viscosity at 25 °C			
	pH 7		pH 10	
Protein	25 °C	After heating, 80 °C, 30 min	25 °C	After heating, 80 °C, 30 min
Soy isolate	0.663[a]	0.566	0.685	0.585
+ $CaCl_2$ (0.5%)	0.594	0.678	0.704	0.654
Succinylated soy isolate	0.713	0.650	0.610	0.622
+ $CaCl_2$ (0.2%)			0.531	0.560
+ $CaCl_2$ (0.5%)	0.672	0.744	0.516	0.557
+ KCl (0.2%)			0.632	0.650

[a] Brookfield viscosity, Model LVT no. 4 spindle.

native and exhaustively succinylated soy isolates were investigated (Table II). Neither succinylation, increases in protein concentration, nor heat significantly altered the viscosities of soy isolate at pH 7 according to the results of an analysis of variance determined at the 5% level of significance. No significant changes in viscosity were observed upon the addition of calcium cations to solutions of native and negatively charged succinylated soy isolate at both pH 7 and 10.

DISCUSSION

Protein acylation reactions presumably follow the carbonyl addition pathway (Means and Feeney, 1971). The rates of reaction depend upon the rate of nucleophilic attack, and acylation rates for homologous nucleophiles are inversely related to their pK values. High reactivity of a protein group is usually the result of low pK. The amino and hydroxyl groups of a protein are readily acylated compared with the other amino acid residues available for reaction. The tyrosine phenolic groups,

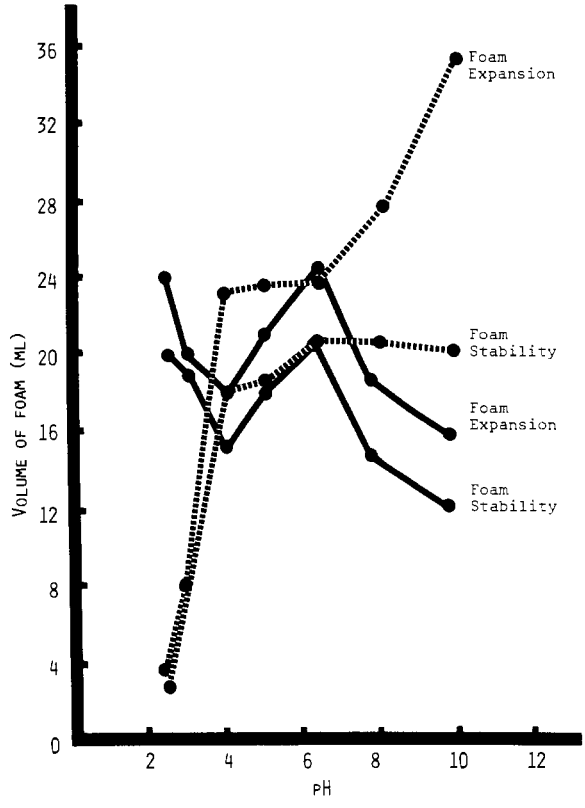

Figure 8. The effect of pH on foam expansion and foam stability of soy protein isolate (—) and succinylated soy protein isolate (- - -).

however, have a generally higher pK and are usually more protected from reaction than the amino groups. Serine and threonine hydroxyl groups are weak nucleophiles and are not easily acylated in aqueous solution. Most acylating agents react more readily with amino groups (Means and Feeney, 1971). The ϵ-amino group of lysine is most commonly acylated.

Succinylation of a protein converts the cationic amino groups to anionic residues and the increase in net negative charge produced by succinate anions alters the physicochemical character of the protein resulting in an enhanced aqueous solubility and subsequent changes in emulsifying and foaming capacity. Habeeb et al. (1958) explained the increase in aqueous solubility observed following succinylation and acetylation of soy protein. The structure of succinylated proteins results from their high net charge and the replacement of short range attractive forces in the native molecule with short range repulsive ones with subsequent unfolding of polypeptide chains. Thus, in soy isolate electrostatic attractions between neighboring ammonium and carboxyl groups enhance protein–protein interactions which lower solubility. Upon succinylation at neutral pH values, however, the ammonium cations of lysine are replaced by succinate anions. Electrostatic repulsions occur between the added carboxyl groups and the neighboring native carboxyl groups producing fewer protein–protein interactions and more protein–water interactions to enhance aqueous solubility. Since net negative charge is proportional to the extent of amino succinylation, the enhancement in aqueous solubility was positively related to the extent of derivatization. Significantly, the aqueous solubilities of succinylated soy isolate derivatized with 0.5- and 2-g quantities of succinic anhydride were similar, i.e., 1.65 and 1.70 mg/ml of protein. Hence, exhaustive succinylation was not required to produce a substantial increase in solubility.

Upon acetylation ammonium cations were replaced by neutral acetyl groups producing fewer electrostatic repulsions, and the aqueous solubility of acetylated soy isolate was intermediate in magnitude between that of soy and succinylated soy isolate. Habeeb et al. (1958) noted that the electrophoretic mobilities of acetylated bovine serum albumin and β-lactoglobulin were intermediate between those of the corresponding native and succinylated proteins.

The increase in aqueous solubility of proteins following succinylation has frequently been cited by other researchers. Melnychyn and Stapley (1973) observed that acylated, e.g., succinylated, soy protein had improved dispersibility characteristics and did not precipitate when added to hot beverages. Groninger (1973) and Groninger and Miller (1975) showed that succinylated fish myofibrillar protein had relatively rapid rehydration and relatively good dispersion characteristics in the pH range of 6.0–8.5. Oppenheimer et al. (1967) showed that succinylation rendered myosin soluble in water. Chen et al. (1975) partially solubilized fish protein concentrate by succinylation with succinic anhydride.

The exhaustive succinylation eliminated cationic ammonium groups from lysine and thus impaired solubilization of the protein at pH values acidic to the IEP. Apparently there were insufficient hydrophilic cationic groups to overcome the aggregating forces resulting from hydrophobic bonds between alkyl and aromatic groups of constituent amino acid residues. With lower amounts of succinic anhydride, i.e., 0.5 and 0.2 g, the ability of modified soy isolate to resolubilize at pH values below the IEP was restored. The insolubility of succinylated proteins at pH values below the IEP was observed with the succinylated forms of β-casein (Hoagland, 1966), myosin (Oppenheimer et al., 1967), bread flour proteins (Grant, 1973), and fish protein concentrate (Chen et al., 1975).

Succinylation shifted the IEP of soy isolate approximately 0.5 pH unit from pH 4.5 to 4. By appropriate chemical derivatization the IEP may be moved up or down so that it is out of the pH range where solubility is required. According to Creamer et al. (1971), however, the likelihood of enhancing the solubility at the IEP by chemical modification of the primary structure with hydrophilic groups is low since the most hydrophobic regions are buried in the molecular interior and least susceptible to chemical reaction. Nevertheless, the slight shift in the IEP upon succinylation may have implications in the successful development of food products such as high protein acidic beverages.

Succinylation increased the emulsifying activity, emulsion stability, and emulsifying capacity of soy isolate most likely because of its enhancement of solubility. This was evidenced by the substantial increases in the emulsifying capacity of succinylated soy isolate at pH values above the IEP and the depression in emulsifying capacity at pH values below the IEP. The positive correlation between solubility and the ability of a protein to emulsify has been documented by other researchers (e.g., Crenwelge et al., 1974; Yasumatsu et al., 1972; Pearson et al., 1965; Swift and Sulzbacher, 1963; Wang and Kinsella, 1976).

Groninger (1973) found a direct relationship between the emulsifying capacity of succinylated fish myofibrillar protein and the percentage of amino groups succinylated. Chen et al. (1975) showed that succinylated fish protein concentrate (FPC) had a greater emulsifying capacity and emulsion stability than alkaline-extracted FPC.

The effects of succinylation on the solubility and emulsifying properties of soy isolate are especially significant for its potential use in fabricated foods. For example, Pearson et al. (1965) concluded that soy sodium proteinate, with an aqueous solubility greater than native soy isolate, was a rather poor emulsifier in the usual pH range of meat (pH 5.6), had poor stability as an emulsifier, and probably did not serve any major function in emulsifying fat when added to sausage products. Succinylation may enhance the ability of soy isolate to emulsify fat in comminuted meat products.

Solubility is required for the production of stable protein foams (Eldridge et al., 1963), and succinylation substantially increased the foaming ability of soy isolate. Poor solubility below the IEP probably accounts for the depression in foam volumes of succinylated soy isolate at pH values of 4 and 2.

The effects of NaCl and sucrose on the foaming abilities of proteins have been observed by other researchers (Wang and Kinsella, 1976). Eldridge et al. (1963) reported increases in the foam expansion of solutions of soy sodium proteinate upon the addition of sucrose. The addition of sodium chloride to solutions of hydrolyzed, succinylated fish protein markedly increased the initial foam volume by 40% (Groninger and Miller, 1975). The addition of 50% sucrose, however, depressed the initial foam volume by 14%.

Groninger and Miller (1975) noted that succinylated fish myofibrillar protein, partially hydrolyzed by bromelin, had improved dispersibility, aeration capacity, and foam stability properties. This succinylated fish protein was successfully incorporated into products requiring the formation of stable foams, i.e., dessert toppings, souffles, and chilled and frozen desserts.

Amino acid analyses revealed little change in the amino acids of the derivatized soy isolate with the exception of lysine. Acylation may impair nutritional value of proteins. Exhaustive acylation lowers the PER of food proteins (Bjarnason and Carpenter, 1969; Groninger, 1973; Creamer et al., 1971). This effect can be minimized by reducing extent of acylation and by supplementing the diet with lysine. However, derivatized proteins which are intended primarily as functional ingredients should not constitute a significant source of nutritive protein in fabricated foods.

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Some Physicochemical and Nutritional Properties of Castor Bean (*Ricinus communis*) Protein

Pablo F. Polit[1] and Valdemiro C. Sgarbieri*

Defatted castor bean pomace (CBP) prepared by two different procedures was used for extraction and characterization of the proteins. Extractions were performed at pH 11 and at higher pH with 0.5% NaOH using a solid/liquid ratio of 1:5 (w/v) at 45 °C. The proteins of the two extractions at pH 11 and in 0.5% NaOH were precipitated at pH 5.8 and 4.5, respectively, and were isolated by filtration after 5 min of heating at 100 °C. These treatments eliminated the toxicity completely and apparently all of the allergens. Amino acid analysis of the isolates showed deficiencies in lysine and sulfur-containing amino acids. Feeding tests with weanling rats gave net protein utilization (NPU) ranging from 34 to 46%. Supplementation with 3% L-lysine plus 1.54% DL-methionine improved the NPU value from 37.5 to 49.2%.

The world production of castor bean seeds is 899 000 metric tons annually (F.A.O., 1974). After the extraction of castor oil the remaining material is the castor bean pomace (CBP), which contains 36% protein representing a source of 150 000 tons of protein per year.

The CBP contains a highly toxic albumin, ricin (Osborne et al., 1905), which can easily be inactivated by humid heat treatment (Jones, 1947). It also contains a powerful allergenic protein fraction which is more heat resistant (Coulson et al., 1960). Considerable effort has been made by many investigators to obtain a detoxified and deallergenized CBP for use in animal feedstuffs. Gardner et al. (1960) described the following treatments as promising: dry heating the CBP at 205 °C for 125 min or cooking under various conditions with alkali or acid, with or without added formaldehyde. Mottola et al. (1971, 1972a,b) described several procedures and proposed the following treatments: vapor cooking at 10 psi for 60 min; cooking at 80 °C for 45 min with ammonia; and cooking with lime at 120 °C for 15 min. Vilhjalmsdottir and Fisher (1971) described a hot water extraction to remove the growth-depressing factors of heat detoxified CBP.

Castor bean pomace was tested in animal feeding studies and was found to be an acceptable protein source for ruminants (Fuller et al., 1971; SANBRA, 1960; Weiss, 1971) and for chicks (Vilhjalmsdottir and Fisher, 1971), although its biological value is not very high. Other factors, such as the mildly toxic alkaloid ricinine and the residual oil, did not have adverse physiological effects on animals when administered in moderate doses (Fuller et al., 1971). Lowering the crude fiber content also increased the biological value (Vilhjalmsdottir and Fisher, 1971).

Isolation of the protein from CBP would permit the thermal detoxification of ricin as well as the elimination of most of the allergens because these compounds do not precipitate with the majority of the proteins. It also permits the elimination of fiber, thus improving the biological value of the proteins.

MATERIALS AND METHODS

Castor Beans. Dehulled castor bean seeds of the "Guarani" variety were used for all studies. They were obtained from the Oil Seed Collection of the Agronomic

Departamento de Planejamento Alimentar e Nutrição, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, 13.100-Campinas, Sao Paulo, Brazil.

[1]Present address: Instituto de Investigaciones Tecnológicas, Escuela Politécnica Nacional, Quito, Ecuador.