Effect of Sodium Sulfite, Sodium Bisulfite, Cysteine, and pH on Protein Solubility and Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis of Soybean Milk Base

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Protein modification is often used to improve the functional properties of proteins in many different food products. The purpose of this study was to investigate the effect of modification of soy proteins with sodium sulfite, sodium bisulfite, and cysteine on the protein solubility of a soy milk base, as determined by protein dispersibility index (PDI). An increase in the PDI was observed at 6−8 mmol/L concentration of each reagent; the most effective was sodium bisulfite, followed by sodium sulfite and cysteine. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) showed that chemical treatments resulted in an increase in the intensity of several bands with molecular weights of 16 000−20 000, 32 000−42 000, 60 000, and 90 000, which correspond to the polypeptides of the two major proteins of soybean, i.e., 11S (glycinin) and 7S (β-conglycinin) globulins. These data suggest that the chemical treatment has cleaved the disulfide bonds which link the polypeptides, thereby increasing the PDI. pH has a significant effect on PDI; the highest PDI was obtained at pH 9, and the intensity of several protein bands in SDS−PAGE increased with increase in pH from 6 to 9. Freezing soy milk base at −18 °C for 1−4 weeks resulted in a drastic decline in the PDI.

Keywords: *Soy milk; glycinin; β-conglycinin; SDS−PAGE; PDI*

INTRODUCTION

One of the most popular plant protein sources to serve as an ingredient in food formulation is soy proteins (Arrese et al., 1991). Among soy products, soybean milk shows considerable promise as it might be used as a suitable substitute for cow's milk (Hackler and Stillings, 1967; Smith and Circle, 1978), particularly in areas of the world where cow's milk is in short supply or for people who are allergic to cow's milk. Effective use of soy proteins in engineered foods is dependent on tailoring one or more of their characteristics to meet the complex needs of the product to be manufactured (Arce et al., 1991). One important physical characteristic of soy proteins is their water solubility, since this property significantly contributes to superior functional properties of soy product (Gupta et al., 1982).

Physical methods such as ultrafiltration (Lah and Cheryan, 1980), low-dose irradiation (Afify and Shousha, 1988), and use of chemicals such as sodium sulfite (Fukushima and Van Buren, 1970; Arce et al., 1991), sodium dodecyl sulfate (Arce et al., 1991), and cysteine (Aminlari et al., 1977; Fukushima and Van Buren, 1970) have been used to improve the protein solubility of soy products. In the present paper we describe the effect of different chemical and physical treatments on the protein dispersibility index (PDI) and sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) pattern of soy milk base proteins.

MATERIALS AND METHODS

Preparation of Soy Milk Base. Williams variety of soybeans from the 1994 crop were used to manufacture the soy milk base. For these experiments, soy milk base was prepared according to the method described by Aminlari et al. (1977), with some modifications. Briefly, whole cleaned soybeans were heated at 100 °C in a flow-through air dryer, dehulled by a FHT dehuller (Westinghouse), and blanched in a 0.25% sodium bicarbonate solution at 88 °C for 30 min. The cotyledons were then drained, allowed to cool to room temperature, ground with a meat grinder, and passed through a 3 mm opening screen. Enough tap water was added to make a slurry containing 12% solids. The slurry was homogenized in a Model 30 homogenizer (Virtis Co., Gardiner, NY) at 45 000 rpm for 1.5 min and passed through a 0.71 mm opening screen. The soy milk base thus produced was pasteurized at 82 °C for 20 min and cooled to room temperature; the pH was adjusted to 7.2 by adding 5 N HCl or 5 N NaOH, and the milk base was stored in plastic bottles for subsequent studies.

Chemical Modification of Soy Milk Base Proteins. A concentrated (1 mol/L) solution of sodium sulfite, sodium bisulfite, or cysteine in distilled water was made, and, while the final total solid of soy milk base was kept at 12%, aliquots of the concentrated solution of reagents were added to the 50 mL of soy milk base to give final concentrations of 2−10 mmol/L at 2 mmol/L increments. The solutions were mixed thoroughly at 25 °C by stirring for 20 min. The treated soy milk base was kept at 4 °C for 4 h (fresh samples) for PDI determination and SDS−PAGE analysis or stored at −18 °C for 1−4 weeks before being analyzed.

PDI Determination. The soluble and insoluble portions of fresh samples as well as those obtained by thawing of the frozen samples were separated by centrifugation as described in AOCS Official Method Ba 10-65 (AOCS, 1973) for determination of the PDI. Protein was determined by microKjeldahl method (N × 6.25). All analyses were run in duplicate.

pH Adjustment. The pH of 50 mL samples of soy milk base was adjusted to desired values by adding 5 N HCL or 5 N NaOH prior to storage at −18 °C for 1 or 4 weeks.

Electrophoresis. The fresh or frozen samples were subjected to SDS−PAGE to determine the effect of different treatments on the electrophoretic pattern of the proteins. The supernatants that were used for PDI determination were also subjected to SDS−PAGE. Slab SDS−PAGE was performed according to the discontinuous buffer system of Laemmli (1970) as described by Sato et al. (1986). Protein samples for elec-

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Table 1. Effect of Different Chemical and Physical Treatments on the PDI of Soy Milk Base[a]

	PDI (%)		
	storage conditions		
		stored at −18 °C	
treatment	fresh	1 week	4 weeks
no reagents	54.8−55.3 (55.05)	18.3−20.3 (19.3)	10.3−12.7 (11.5)
Na_2SO_3			
2 mmol/L	59.2−60.4 (59.8)	25.4−25.7 (25.5)	19.6−23.7 (21.6)
4 mmol/L	62.8−63.0 (62.9)	24.6−27.5 (26.1)	23.8−26.9 (25.3)
6 mmol/L	66.2−66.6 (66.4)	30.1−30.5 (30.5)	29−34.6 (31.8)
8 mmol/L	72.3−72.5 (72.4)	32.6−35.0 (33.8)	29.5−34.4 (32.0)
10 mmol/L	61.0−61.1 (61.05)	30.4−30.7 (30.6)	47.2−52.6 (50.0)
$NaHSO_3$			
2 mmol/L	63.2−63.5 (63.3)	26.4−27.6 (27.0)	30.6−32.3 (31.5)
4 mmol/L	69.5−69.8 (68.7)	33.2−32.5 (32.8)	30.3−33.8 (32.0)
6 mmol/L	70.1−70.7 (70.4)	37.1−38.8 (38.0)	30.1−35.0 (32.6)
8 mmol/L	67.4−68.1 (67.8)	38.0−40.2 (39.1)	31.9−31.9 (31.9)
10 mmol/L	66.0−66.4 (66.2)	30.8−31.9 (31.3)	28.6−33.8 (31.4)
cysteine			
2 mmol/L	50.5−51.0 (50.8)	21.5−22.3 (21.9)	20.8−23.7 (22.2)
4 mmol/L	66.4−66.6 (66.5)	22.1−23.4 (22.8)	28.8−33.7 (31.2)
6 mmol/L	67.4−67.7 (67.2)	16.3−16.9 (16.6)	22.9−27.2 (25.1)
8 mmol/L	70.9−71.1 (71.0)	15.6−16.3 (15.9)	16.6−19.9 (18.2)
10 mmol/L	60.7−61.1 (60.9)	12.7−13.0 (12.8)	16.5−18.4 (17.5)
pH			
6	nd[b]	19.9−19.9 (19.9)	17.0−17.6 (17.3)
7	nd	21.5−22.7 (22.1)	22.7−26.8 (24.8)
8	nd	24.9−25.5 (25.2)	26.3−30.4 (28.3)
9	nd	26.5−27.9 (27.2)	33.0−36.4 (32.7)

[a] Values are range of duplicates; values in parentheses represent average of duplicates. [b] Not determined.

trophoresis were prepared by diluting each sample in sample buffer to give final concentrations of 1 mg/mL protein, 0.01 mol/L Tris-HCl, pH 6.8, 0.4% SDS, 10% glycerol, and 0.004% bromophenol blue. The running gel, with dimensions of 140 × 140 × 1 mm, was made of 10% acrylamide in 1.2 mol/L Tris-HCl, pH 8.8, and 0.3% SDS. The stacking gel contained 3% acrylamide in 0.25 mol/L Tris-HCl, pH 6.8, and 0.2% SDS. Samples were heated in boiling water for 10 min, and 80 μL was applied to each slot. The electrode buffer comprised 0.025 mol/L Tris-HCl, 0.192 mol/L glycine, and 0.1% SDS, at pH 8.16. Electrophoresis was performed at constant 25 mA, and the gels were stained with 0.25% Coomassie Brilliant Blue in 50% acetic acid/25% methanol and destained with a 10% acetic acid/7.0% methanol solution. Molecular weight standard proteins used were myosin heavy chain (M_r = 205 000), β-galactosidase (M_r = 116 000), phosphorylase *b* (M_r = 97 400), bovine serum albumin (M_r = 66 000), ovalbumin (M_r = 46 000), carbonic anhydrase (M_r = 29 000), and chicken egg white lysozyme (M_r = 14 600).

RESULTS

The results of determination of the PDI of fresh and frozen and thawed soy milk base, treated with different

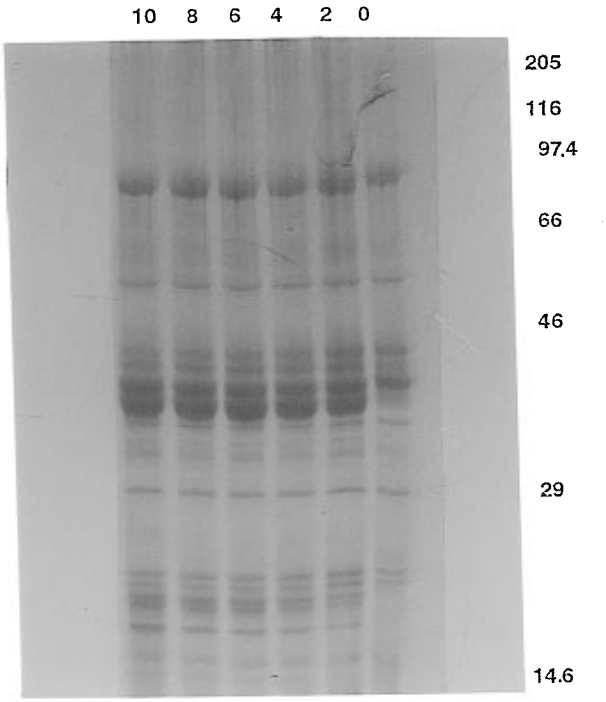

Figure 1. Effect of different concentrations of sodium sulfite on the SDS−PAGE pattern of fresh (unfrozen) soy milk base. Numbers on right are the molecular masses of standard proteins in kDa. Numbers on top are concentration of reagents used in mmol/L.

reagents, are shown in Table 1. In the case of fresh samples, all three reagents significantly increased the PDI and the increase was dependent on the concentration of each reagent. The most effective concentration was 6−8 mmol/L, after which a slight decrease in PDI was observed. In the case of untreated samples, a 65% or 80% reduction in PDI occurred when the product was stored at −18 °C for 1−4 weeks, respectively. The PDI of $NaHSO_3$- and Na_2SO_3-treated soy milk base, stored at −18 °C for 1 week, increased with increase in the concentration of these reagents up to 8 mmol/L, and a slight decrease was observed at 10 mmol/L (Table 1). For cysteine, the effect was less significant and the best result was obtained at 4 mmol/L, after which the PDI decreased. A similar result, but to a lesser degree, was obtained when samples were stored for 4 weeks.

The changes in protein solubility are reflected in the electrophoretic pattern of protein in treated soy milk base. Examples of SDS−PAGE data are shown in Figures 1−4. Figure 1 shows the results of Na_2SO_3 treatment of fresh (unfrozen) soy milk base. The results of $NaHSO_3$ and cysteine treatment were similar to those of Na_2SO_3; data for the effect of these reagents are not shown. Characteristic bands in the range of 16−20 and 30−42 kDa appear after treatment with these reagents, the intensity of which increases with increasing concentration of reagents, especially from 0 to 2 mmol/L. The intensity of the band of approximately 70 kDa decreases. Distinctive differences are noticed when one compares the results of Na_2SO_3, $NaHSO_3$, and cysteine treatment of fresh (unfrozen) milk base with frozen samples. When samples were stored at −18 °C for 1 week, an increase in the concentration of Na_2SO_3 caused increase in the appearance of several bands corresponding to proteins with molecular mass of 16−20 kDa and increase in the intensity of bands with molecular masses in the range of 30−42, 60, and 90 kDa (Figure 2). With this reagent a protein with molecular mass of <16 kDa

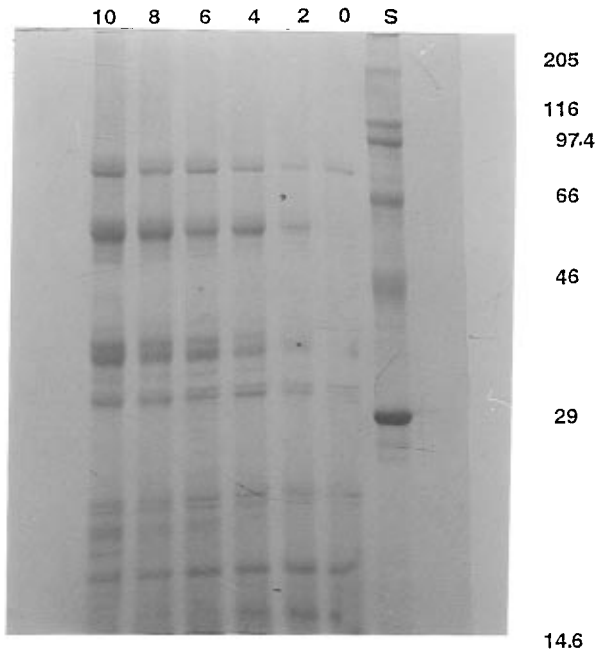

Figure 2. Effect of different concentrations of sodium sulfite on the SDS−PAGE pattern of soy milk base stored at −18 °C for 1 week. S, standard proteins with indicated molecular masses in kDa. Numbers on top are concentration of reagents used in mmol/L.

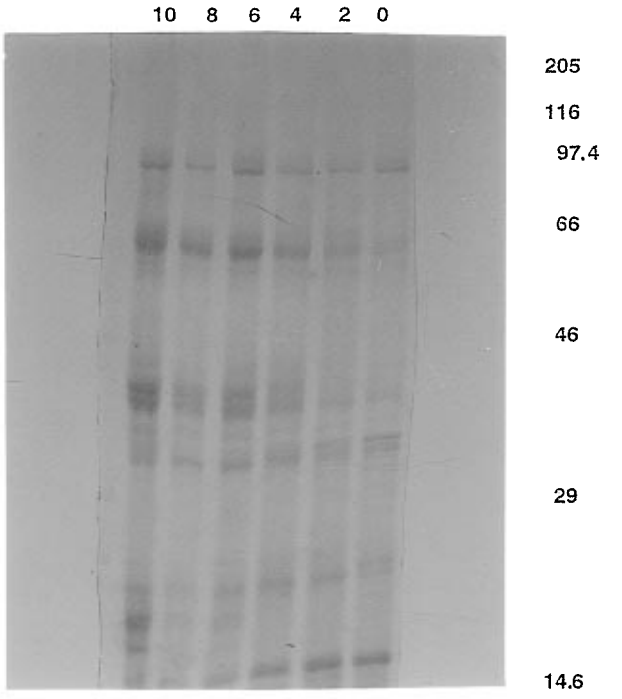

Figure 3. Effect of different concentrations of sodium sulfite on the SDS−PAGE pattern of soy milk base stored at −18 °C for 4 weeks. Numbers on right are the molecular masses of standard proteins in kDa. Numbers on top are concentration of reagents used in mmol/L.

appeared at 2−4 mmol/L concentrations and disappeared at higher concentrations. Similar results was observed for sodium bisulfite and not as much for cysteine (data not shown). When soy milk base was treated with these reagents and stored at −18 °C for 4 weeks, more profound effects occurred in the SDS−PAGE pattern of proteins (Figure 3). Most notably, increase in the intensity of 42, 60, and 90 kDa bands and decrease in the intensity of the band with molecular

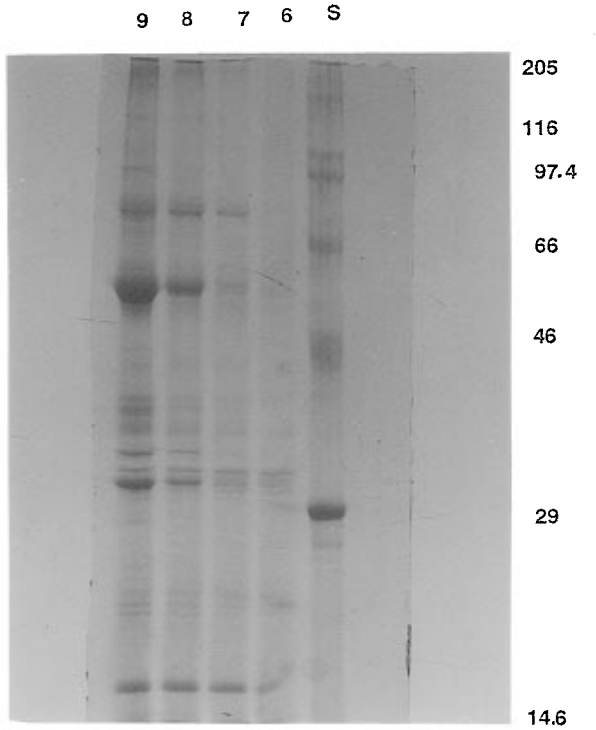

Figure 4. Effect of pH on the SDS−PAGE pattern of soy milk base, stored at −18 °C for 1 week. S, standard proteins with indicated molecular masses in kDa. Numbers on top indicate pH of the milk base.

mass of <16 kDa in Na_2SO_3-treated samples (Figure 3) are evident. Here again cysteine was less effective and the results of $NaHSO_3$ treatment were similar to those of Na_2SO_3; therefore, the SDS−PAGE data for cysteine and $NaHSO_3$ are not shown. A band of 25 kDa is present in fresh samples but does not exist in frozen product and its intensity is not affected by reagents.

pH has profound effects on the PDI and SDS−PAGE pattern of frozen soy milk base. Increase in pH significantly increased the PDI (Table 1) and resulted in an increase in the concentration of several proteins as evidenced by SDS−PAGE of the supernatants of samples after the soy milk base was frozen for 1−4 weeks. Figure 4 shows results for the samples stored for 1 week.

DISCUSSION

Soybeans have become an important protein source for the world populace. Given the world food shortage, soy proteins will be used even more frequently. However, before these proteins can be used effectively, food scientists and technologists must thoroughly understand their molecular and functional relationships (Peng et al., 1984). Protein solubility is a central functional property since a protein generally has to be in solution to exert its other desirable functional characteristics. Improvement in the solubility of proteins can be achieved by chemical and enzymatic modifications (Feeney, 1977; Feeney and Whitaker, 1986). In our studies of the effect of different factors on soy protein solubility in manufactured soy milk base, significant differences are observed as a function of type and concentration of chemicals, pH, and freezing conditions. These differences are manifested in PDI and SDS−PAGE patterns. Sodium bisulfite, sodium sulfite, and cysteine, in decreasing order, increase the solubility of soy proteins. Chemical treatments counterbalanced the adverse effects of freezing, and an improvement was seen in the

PDI of treated and frozen samples as compared with untreated ones. The effect of reagents is dose dependent; the solubility is maximum at 6−8 mmol/L and decreases thereafter. The positive effects of these chemicals on the protein solubility in different soy products have been reported by other investigators (Fukushima and Van Buren, 1970; Aminlari et al., 1977; Arce et al., 1991; Cole and Cousin, 1994). These effects are believed to involve reduction of disulfide bonds and decrease in the polymerization of proteins (Aminlari et al., 1977; Ohren, 1981; Friedman and Gumbmann, 1986). Soy proteins have been extensively studied (Smith and Circle, 1978; Kinsella, 1979; Hu and Esen, 1982; Lei et al., 1983; Mwandemele et al., 1984; Sato et al., 1986; Sathe et al., 1987; Visser and Thoma, 1987). The major proteins of soybean consist of glycinin (11S) and β-conglycinin (7S), each composed of several subunits (Brooks and Morr, 1985; Sato et al., 1986; Cole and Cousin, 1994). These two proteins account for >70% of the total protein (Smith and Circle, 1978). Glycinin has a molecular weight of 350 000 and is made of at least six nonidentical subunits. Each subunit contains an acidic polypeptide chain linked to a basic polypeptide by a single disulfide bond (Derbyshire et al., 1976). Molecular weights of the acidic polypeptides range between 37 000−42 000, and those of basic polypeptdes are in the range of 17 000−20 000 (Kinsella, 1979; Arrese et al., 1991). In the present study, the electrophoretic patterns showed that in the presence of increasing concentrations of reducing agents, the intensity of bands corresponding to 16 000−21 000 and 32 000−42,000 proteins increased. These data are consistent with the cleavage of disulfide bonds of 11S protein and the appearance of acidic and basic polypeptides in the supernatant of the centrifuged soy milk base. These proteins are responsible for the increase in the PDI of treated soy milk base. Increase in the concentration of reducing agents beyond certain points results in decline in PDI, disappearance of some protein bands (especially in the 13 000 molecular weight range), and concomitant increase in the high molecular weight bands. These changes might be attributed to an interplay of inter- and intramolecular disulfide bonds (Circle et al., 1964; Catsimpoolas and Meyer, 1970). According to this hypothesis, cleavage of intermolecular disulfide bonds by low concentrations of reducing agents causes depression of gelation, whereas high concentration of reducing agents result in cleavage of intramolecular disulfide bonds, which facilitates disruption of the native structure of globulin. Dissociation and unfolding of the subunits lead to exposure of reactive amino acid residues and, therefore, enhancement of gel formation and decrease in protein solubility. It is interesting to note that the chemical treatments are more effective in frozen samples than in fresh ones, as demonstrated in both PDI and SDS−PAGE patterns. This effect is probably related to the conformation change of soy proteins during freezing and increase in the accessibility of disulfide bonds. On the other hand, freezing seriously decreases the solubility of soy proteins. Many protein bands that are present in the fresh and untreated samples are either absent in the frozen samples or their intensity is significantly decreased. It is well-known that freezing alters the property of proteins (Fennema, 1994). During freezing the rate of chemical reactions is increased due to the concentration effect of freezing; that is, macromolecules are forced closer together, making interactions more probable with resultant insolubilization, aggregation, and precipitation of proteins.

β-Conglycinin also consists of at least four nonidentical subunits [α, α', β, and γ (Sathe et al., 1987)]. Depending on the pH, ionic strength, and presence of reducing agents, β-conglycinin can exist in multiple molecular forms, based on different compositions of subunits (Brooks and Morr, 1985; Cole and Cousin, 1994). At neutral pH and 0.5 ionic strength, the 7S globulin consists of three types, namely type I (B_1−B_6 β-conglycinin), type II (B_0 β-conglycinin), and type III (γ-conglycinin). At 0.1 ionic strength, type I is converted to a 9S dimer, type II is changed to an insoluble aggregate (13.4S), and type III does not change (Brooks and Morr, 1985). There are some disagreements regarding the molecular weight of subunits. Depending on the methods used for molecular weight determinations, values of molecular weights of α and α' subunits are reported to be in the range of 57 000−83 000, while those of β and γ subunits are 42 000−53 000 and 46 000, respectively (Brooks and Morr, 1985; Sathe et al., 1987; Arrese et al., 1991). The protein bands with molecular weight close to those of the subunits of β-conglycinin (especially in the range of 60 000−90 000) observed in the SDS−PAGE pattern increase in intensity as the concentration of reducing agents increases. These effects are the result of cleavage of the disulfide bonds in β-conglycinin, the effect of ionic strength, which is different in different treatments, or a combination of both factors. Furthermore, increased PDI and intensity of bands with molecular weights in the range of 32 000−42 000 and 60 000−90 000 with increase in pH indicate that at higher pH values different acidic polypeptides of glycinin and subunits of β-conglycinin dissociate and appear in the supernatant. These data suggest that these subunits are held together by ionic or hydrogen bonds as suggested by the effect of ionic strength on the molecular composition of different types of β-conglycinin (Brooks and Morr, 1985).

The results of this study show that the solubility of soy proteins can be improved by chemical treatments, increase in pH, and not freezing soy products. The safety of sulfite and bisulfite continues to be a matter of debate. On the basis of toxicological evaluations that have indicated an apparently low order of toxicity, the U.S. FDA has proposed to affirm "generally regarded as safe" status for sulfite and bisulfite as direct food additives for specific food applications (Wagon and Marleta, 1985). However, possible adverse effects of these treatments on the flavor contribution of the additives and nutritional value of soy products should be addressed before they are included in processing of these products for human consumption.

LITERATURE CITED

Afify, A. E. M. R.; Shousha, M. A. Effect of low-dose irradiation on soy protein solubility, trypsin inhibitor activity and protein pattern separated by polyacrylamide gel electrophoresis. *J. Agric. Food Chem.* **1988**, *36*, 810.

Aminlari, M.; Ferrier, L. K.; Nelson, A. I. Protein dispersibility of spray-dried whole soybean milk base: Effect of processing variables. *J. Food Sci.* **1977**, *42*, 985.

AOCS. *Official and Tentative Methods*, 3rd ed.; American Oil Chemists' Society: Champaign, IL, 1973.

Arce, C. B.; Pilosof, A. M. R.; Bartholomai, G. B. Sodium dodecyl sulfate and sulfite improve some functional properties of soy protein concentrates. *J. Food Sci.* **1991**, *56*, 113.

Arrese, E. L.; Sorgentin, D. A.; Wagner, J. R.; Anon, M. C. Electrophoretic, solubility and functional properties of commercial soy protein isolates. *J. Agric. Food Chem*. **1991**, *39*, 1029.

Brooks, J. R.; Morr, C. V. Current aspects of soy protein fractionation and nomenclature. *J. Am. Oil Chem. Soc.* **1985**, *62*, 1347.

Catsimpoolas, N.; Meyer, E. W. Gelation phenomena of soy globulin. 1-protein:protein interaction. *Cereal Chem*. **1970**, *47*, 559.

Circle, S. J.; Meyer, E. W.; Whitney, R. W. Rheology of soy protein dispersions: effect of heat and other factors on gelation. *Cereal Chem*. **1964**, *41*, 157.

Cole, K. D.; Cousin, S. L. Size exclusion chromatography of soybean proteins and isoflavones. *J. Agric. Food Chem*. **1994**, *42*, 2713.

Derbyshire, E.; Wright, D. G.; Boulter, D. Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry* **1976**, *15*, 3.

Feeney, R. E. Chemical changes in proteins. In *Evaluation of Proteins for Humans*; AVI Publishing: Westport, CT, 1977.

Feeney, R. E.; Whitaker, J. R. *Protein Tailoring for Food and Medical Uses*; Dekker: New York, 1986.

Fennema, O. R., Ed. Water and ice. In *Food Chemistry*, 2nd ed.; Dekker: New York, 1994.

Friedman, M.; Gumbmann, M. R. Nutritional improvement of soy flour through inactivation of trypsin inhibitors by sodium sulfite. *J. Food Sci.* **1986**, *51*, 1239.

Fukushima, D.; Van Buren, J. Effect of physical and chemical factors on the redispersibility of dried soy milk proteins. *Cereal Chem*. **1970**, *47*, 571.

Gupta, K.; Dhindsa, K. S.; Wagle, D. S. Electrophoretic and solubility characteristics of proteins in soybean varieties. *J. Food Sci. Technol.* **1982**, *19*, 264.

Hackler, L. R.; Stillings, B. R. Amino acid composition of heat-processed soy milk and its correlation with nutritive value. *Cereal Chem*. **1967**, *44*, 70.

Hu, B.; Esen, A. Hetreogenicity of soybean proteins: two-dimensional electrophoretic maps of three solubility fractions. *J. Agric. Food Chem*. **1982**, *30*, 21.

Kinsella, J. E. Functional properties of soy proteins. *J. Am. Oil Chem. Soc.* **1979**, *56*, 242.

Laemmli, U. K. Cleavage of structural proteins during the assembly of head of bacteriophage T4. *Nature* **1970**, *222*, 680.

Lah, C. L.; Cheryan., M. Protein solubility characteristics of an ultrfiltered full-fat soybean product. *J. Agric. Food Chem*. **1980**, *28*, 911.

Lei, M. G.; Tyrell, D.; Bassette, R.; Reeck, G. R. Two-dimensional electrophoretic analysis of soybean proteins. *J. Agric. Food Chem*. **1983**, *31*, 963.

Mwandemele, O. D.; McWhiter, K. S.; Chesterman, C. Improving the quality of soybean (*Glycine max* (L) Merril) for human consumption: Factors affecting the cookability of soybean seeds. *J. Food Sci. Technol.* **1984**, *21*, 286.

Ohren, J. A. Process and product characteristics for soya concentrates and isolates. *J. Am. Oil Chem. Soc.* **1981**, *58*, 333.

Peng, I. C.; Quass, D. W.; Dayton, W. R.; Allen, C. E. The physicochemical and functional properties of soybean 11S globulin—A review. *Cereal Chem*. **1984**, *61*, 480.

Sathe, S. K.; Lilley, G. G.; Mason, A. C.; Weaver, C. M. High-resolution sodium dodecyl sulfate poly acrylamide gel electrophoresis of soybean (*Glycine max* L) seed proteins. *Cereal Chem*. **1987**, *64*, 380.

Sato, K.; Yamagishi, T.; Yamauchi, F. Quantitative of soybean proteins by densitometry of gel electrophoresis. *Cereal Chem*. **1986**, *63*, 493.

Smith, A. K.; Circle, S. J. *Soybeans, Chemistry and Technology*; AVI Publishing: Westport, CT, 1978; Vol. 1.

Visser, A.; Thoma, A. Soy protein products—their processing, functionality and application aspects. *Food Rev. Int.* **1987**, *3*, 1.

Wagon, G. N.; Marleta, M. A. Undesirable or potentially undesirable constituents of foods. In *Food Chemistry*, 2nd ed.; Fennema, O. R., Ed.; Dekker: New York, 1994.

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