Effect of Salts on Soy Storage Proteins Defatted with Supercritical CO_2 and Alcohols

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ABSTRACT: The primary objective was to determine whether salts will stabilize soy storage proteins against the denaturing effects of alcohols or the heat and pressure used in supercritical CO_2 during the defatting process. Nitrogen solubility index (NSI) and differential scanning calorimetry (DSC) were used to monitor the denaturation of proteins. A variety of salt solutions used to hydrate full-fat soy grits increased the thermal stability of both 7S and 11S storage proteins. DSC was used to monitor their denaturation temperature. Neutral salt hydrations followed the lyotropic series for protein stabilization. Of the salts evaluated, the test results indicate that the reducing salt, sodium sulfite, and the neutral salt, sodium sulfate, when used to steep beans, yielded significantly higher NSI than did the water-steeped controls or other salt treatments after partial defatting with absolute isopropanol or ethanol and supercritical CO_2. However, these same salt treatments did not as effectively stabilize the proteins against the denaturing effects of ethanol more aqueous than 84% when these alcohols were used as the defatting medium.

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KEY WORDS: Defatting, differential scanning calorimetry, ethanol extraction of oil, heat denaturation, isopropanol extraction of oil, nitrogen solubility index, salt stabilization, soybeans, supercritical CO_2 extraction of oil.

For the development of new methodologies to process soy with desired functional properties, the researcher must control the protein denaturation process. It is generally recognized that a high level of solubility reflects a versatile protein with good potential for use in either food or industrial product systems. Denaturing conditions, such as exposure to heat, high pressure, high shear, or organic solvents, tend to lower protein solubility. The denaturation process for soy storage proteins in the heterogeneous cracked bean system is extremely complex, particularly at low to intermediate moisture. Fats, carbohydrates, and nonprotein constituents not only impact the moisture dynamics in the whole bean but also impact the expected protein-solvent and protein-protein interactions during denaturation. Salt stabilization of proteins in solution is an old technology that has already been well researched. However, limited information is available on salt stabilization to protect protein from denaturation during processing under limited-moisture conditions. At high salt concentration ($\mu > 0.5$), the ability of salts to stabilize proteins has been attributed to preferential hydration of the protein molecule as a result of the salt-induced alteration of the water structure in the vicinity of the protein (1). At high concentrations of neutral salts, proteins display decreased solubility, which may result in precipitation due to a salting-out phenomenon. Salting-out reduces solute–solvent contact and enhances solute–solute contact or entanglement by making water a poor solvent for the protein. Here, protein–protein interactions are favored over protein–solvent interactions owing to lack of water molecules, which leads to aggregation and precipitation of the protein molecules. Hence, the denaturation temperature of most proteins is generally higher in the presence of a high concentration of stabilizing salt. Conversely, at low salt concentration, salting-in may result from direct salt interactions with charged groups of the protein or salt–solvent interaction. Salting-in makes water a better solvent for the protein where viscosity-stabilizing effects relate to the reduction of entanglements from solute–solute contacts and subsequent enhancement of solute–solvent contacts. Increased solubility of protein increases its susceptibility to thermal denaturation. Consequently, in our current study to stabilize soy storage proteins, we will investigate neutral salts that will salt out these proteins as they exist in the bean *in situ*. Sessa and Nelsen (2) also demonstrated that reducing salts, particularly sodium sulfite, proved to effectively heat-stabilize the β-conglycinin (7S) and glycinin (11S) storage proteins in salt-steeped cracked beans. Sulfiting agents were used to inactivate soybean protease inhibitors (3–5) as well as to preserve the nitrogen solubility index (NSI) during defatting of salt-steeped beans with either *n*-hexane or supercritical CO_2 (SC-CO_2) (4). However, these authors (4) did not assess changes in NSI that may have occurred as a result of a lengthy steep treatment. Use of a reducing salt may cause a protein modification with elimination of undesirable toxic and/or antinutritional properties of some proteins, increased or decreased protein solubility, and protection of the protein against processing-induced modification, such as the Maillard reaction.

Our current objective is to determine whether neutral and/or reducing salts will affect the NSI and extractability of

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lipids from steeped beans when subjected to defatting with SC-CO_2, absolute isopropanol, and absolute or aqueous ethanol. NSI, which measures a fraction of protein that is soluble under a specific set of conditions, is commonly used in the protein industry to give a practical, though empirical, estimate of the extent of denaturation (6,7).

EXPERIMENTAL PROCEDURES

Materials. Whole soybeans, Williams 82, purchased from Kelly Seed Co. (Peoria, IL), were cracked into four to six parts by passage through cracking rolls and dehulled with a box aspirator. All salts used were Certified American Chemical Society grade.

Steep treatments. Cracked beans (50 g) were steeped at a bean-to-solvent ratio of 1:5 in both the absence (i.e., control) and presence of salt solutions that contained 1 or 2 M salt for 45 min at 65°C on a shaking (60 rpm) water bath. Salts investigated included sodium salts of acetate ($C_2H_3O_2^-$), chloride (Cl^-), metabisulfite ($S_2O_5^{2-}$), monobasic phosphate ($H_2PO_4^-$), sulfate (SO_4^{2-}), and sulfite (SO_3^{2-}), along with ammonium sulfate [$(NH_4)_2SO_4$], and calcium chloride ($CaCl_2$). Drained, cracked beans were dried in a hot-air oven for 16 h at 50°C. The steeped, cracked dried beans were then ground in a coffee mill and sieved to pass through a number 14 (0.0555 in.) sieve, followed by a number 35 (0.0197 in.) sieve. The ground beans retained on the number 35 sieve had the consistency of coarse sand. These served as our starting material.

Defatting methods. SC-CO_2 extraction was performed on either ground, unsteeped cracked beans or steeped cracked beans (5–10 g) according to the experimental procedure of King *et al.* (8). The samples were packed into 316SS tubing with dimensions 1.75 cm i.d. × 30.5 cm. Extraction proceeded for 1 h at 5000 psi and 50°C. Absolute isopropanol and ethanol as well as aqueous ethanols were also used to extract steeped beans. Beans (10 g), at a bean-to-solvent ratio of 1:5, were extracted for 2 h at 50°C on a Lab Line (Melrose Park, IL) Orbit Environ-Shaker, set at 200 rpm. After decanting the alcohol extracts, the bean residues were air-dried overnight and then oven-dried for 2 h at 50°C.

Thermal analyses. Thermal denaturation of soy samples was studied by differential scanning calorimetry (DSC) with a Rheometrics Gold+ (Rheometrics Scientific, Piscataway, NJ). Hermetic aluminum pans were packed with accurately weighed soy samples, which were weighed on a Cahn 29 automatic electrobalance; water was micropipetted onto the inner cover of the aluminum pan to adjust total moisture to about 50%; the weighed pan was then hermetically sealed and heated at 10.0°C/min from 30–160°C with an empty aluminum pan as a reference. Thermal denaturation of soy storage proteins gave endothermic events where temperature, designated T_d, was used to signify peak maximum. Calibration of the instrument was made with a sapphire crystal and checked with high-purity indium scanned as above.

Composition analyses. The coarsely ground unsteeped/steeped beans, either before or after defatting, were each finely ground prior to analyses for residual oil, Ac 3-44, moisture, Ac 2-41, and NSI determinations, Ac 4-41, according to AOCS methods (9).

Experimental design. The experimental design of this research is given in Table 1. The overall objective was not to effect a complete oil extraction with the defatting procedures used but to obtain consistent, reproducible sets of values. All samples were ground and sieved, and at least three replicates of each steep treatment and defatting procedure were performed. Because completeness of oil extraction was not our goal, we were able to evaluate whether individual salts or their concentration affected oil extractability.

Statistical analyses. Data were analyzed by using analysis of variance (ANOVA) in the General Linear Models procedure of the Statistical Analysis Systems software package (10).

RESULTS AND DISCUSSION

Salt effects on soy storage protein stability. Sessa (11) demonstrated that the DSC endothermic denaturation temperature of soy storage proteins (7S and 11S globulins) increased as moisture in cracked beans was decreased. Therefore, to keep data on our salt steeping of cracked beans comparable, all samples were wetted to 50% moisture and scanned at 10°C/min. In Figure 1, DSC thermograms of water and 1 M Na_2SO_4-steeped cracked beans are compared. Apparent are two endothermic events for each sample, with the lower T_d endotherm representing denaturation of 7S globulin and the higher T_d endotherm denaturation of 11S globulin. From the data in Table 2, the relative effectiveness of the salts used in our current study to stabilize both soy storage proteins in cracked beans, based on DSC endotherms, was: $Na_2SO_4 > Na_2SO_3 \geq Na_2S_2O_5 > NaH_2PO_4 > NaCl \geq NaC_2H_3O_2 > H_2O$. $(NH_4)_2SO_4$ at 2 M concentration (equivalent to 40% saturation) was also included to evaluate its ability to heat-stabilize soy 7S and 11S globulins by salting out these proteins. We used 2 M rather than 1 M concentration because, according to Wolf *et al.* (12), 40% saturation (i.e., 2 M) is the least concentration needed to precipitate 11S globulin. With 2 M $(NH_4)_2SO_4$, we obtained a T_d for 7S of 125.1°C, which was not significantly different (*P*

TABLE 1
Experimental Design

Salt steep	Molarity	Defatting procedure[a]
None	—	A, B, D, E
H_2O	—	A, B, C, D, E
Na_2SO_4	1	A, B, C, D, E
Na_2SO_4	2	A, B, C, D, E
Na_2SO_3	1	A, B, C, D, E
Na_2SO_3	2	A, B, C, D, E
$(NH_4)_2SO_4$	2	A, C, D
$Na_2S_2O_5$	1	A, B, C, D
NaH_2PO_4	1	A, C, D
$NaC_2H_3O_2$	1	A, C, D
NaCl	1	A, C, D
$CaCl_2$	1	A, B, C, D

[a]Designation of defatting procedure: A, none; B, supercritical CO_2; C, absolute isopropanol; D, absolute ethanol; E, aqueous ethanol.

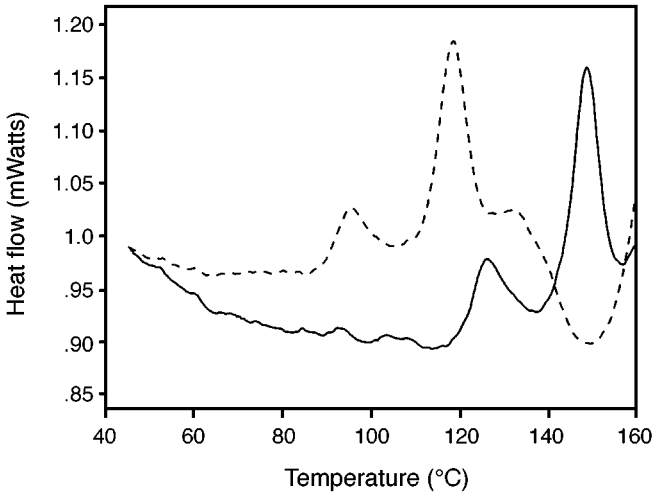

FIG. 1. Differential scanning calorimetric thermograms of SC-CO_2-extracted, ground soybeans that were steeped in either water only (- - -) or in 1 M Na_2SO_4 (——) for 45 min at 65°C. Each sample was wetted to 50% moisture and scanned at 10°C/min. SC-CO_2, supercritical CO_2.

< 0.01) from the 7S T_d with Na_2SO_4 and a T_d for 11S of 146.9°C, which was not significantly different from the 11S T_d with Na_2SO_4. In general, the salt-steep treatment had similar effects on both storage proteins. The relative effectiveness of the neutral salts in this series in stabilizing proteins against conformational change followed the classical Hofmeister series (13). Tanford (14) reported that salt concentrations of 1 M or higher will practically eliminate the electrostatic effects, i.e., direct binding of salts on proteins. The lyotropic stabilizing effects of salts at 1 M or higher have been attributed to the alteration of water in the vicinity of the protein (1). In general, salts that destabilize the folded conformation by weakening intramolecular contacts will decrease protein solubility (15). Hence, those salts that incur a salting-out effect possess the greatest stabilizing effect on the proteins. The reducing salts Na_2SO_3 and $Na_2S_2O_5$ also heat-stabilized 7S and 11S globulins. The sulfite anion is known to cleave disulfide groups to thiosulfonate derivatives (16), whereas the bisulfite anion generated by dissolution of $Na_2S_2O_5$ in water does not, unless the pH is adjusted to 8.0. The data in Table 2 show no significant differences in the T_d values of 7S globulin, which is expected because the 7S globulin is free of sulfhydryl and disulfide groups (17,18). However, we also observed no significant differences in the T_d values of 11S globulin, which is known to possess 20 disulfides (19). If a substantial number of disulfide groups were cleaved by Na_2SO_3 to generate thiosulfonate derivatives, the T_d of the Na_2SO_3-steeped beans should be lower than the $Na_2S_2O_5$-steeped beans. On the other hand, Kella *et al.* (20) reported that thiosulfonate derivatives of whey proteins, generated by action of Na_2SO_3, brought about structural changes that exposed nonpolar groups buried inside the protein interior. This finding would cause a heat-stabilizing rather than heat-destabilizing effect. Because we did not quantitate the conversion of disulfide groups in 11S globulin, we attribute the heat-stabilizing effects of both Na_2SO_3 and $Na_2S_2O_5$ to the salt/water competition with protein. In the previous study of Sessa and Nelsen (2), the T_d for 7S and 11S globulins were lower than in our current investigation. The lower T_d reflects a higher moisture content in their cracked beans as well as a lower scan rate of 5.0°C/min when analyzed by DSC. The total moisture content of the ground, steeped, cracked bean samples affects the thermal denaturation of the protein logarithmically rather than linearly (11) which would cause the subtle differences observed in T_d.

TABLE 2
Effect of Salt[a] on the DSC Denaturation Temperature (T_d)[b] of β-Conglycinin (7S) and Glycinin (11S) in Cracked Soybeans

Salt	7S, T_d (°C)[c]	11S, T_d (°C)[c]
Na_2SO_4	124.6 a	145.4 a
Na_2SO_3	121.7 ab	139.4 b
$Na_2S_2O_5$	118.9 bc	138.1 b
NaH_2PO_4	114.6 cd	132.4 c
NaCl	111.9 d	131.3 c,d
$NaC_2H_3O_2$	105.5 e	126.9 d
H_2O	95.0 f	115.5 e

[a]Salt concentrations were all 1 M.
[b]Differential scanning calorimetry (DSC) conditions: sample moisture 50%; heat rate 10°C/min.
[c]Column values followed by the same letter are not significantly different (P < 0.01).

Effect of salt steeping on oil extractability. From data in Table 3, we observe that in ANOVA results, the H_2O-steeped soybeans had a higher ($P > 0.01$) percentage of residual lipid than did salt-steeped soybeans. Residual lipid contents were similar for either water- or salt-steeped beans defatted by procedures C and D (i.e., absolute alcohols) and were higher (P < 0.01) than residual lipid contents of soybeans defatted by procedure B (i.e., SC-CO_2). The individual salts used for the steep treatments showed no significant differences ($P < 0.01$) when compared with data from all salts. Therefore, high concentrations of salts, in general, effected better extractability of lipid from the cracked bean. The triacylglycerols (TAG) of soybeans are stored in discrete organelles, called oil bodies, which are surrounded by a monolayer of phospholipids and alkaline proteins, termed oleosins (21). These oil bodies are stable and, because they maintain a hydrophilic surface, hydrophobic solvents, such as hexane, benzene, and chloroform, cannot extract the matrix TAG from these organelles. Relatively hydrophilic organic solvents, such as diethyl ether and acetone, can penetrate the hydrophilic surface and dissolve the matrix TAG. According to Tzen *et al.* (22), some salts, such as $CaCl_2$ and $MgCl_2$, can cause the aggregation, but not

TABLE 3
Effect of Water vs. Salt Steeping on Extractability of Lipids from Cracked Soybeans

Defatting procedure[a]	Water-steeped[b]	Salt-steeped[b]
B	13.5	10.9
C	15.1	12.0
D	15.2	12.1

[a]See Table 1 for procedure definitions.
[b]Residual lipids from all steeped, defatted cracked soybeans.

the coalescence of oil bodies, while NaCl does neither. Because coalescence is essential for extraction of TAG from the oil bodies, the salt-steep treatments in our current investigation are not likely to cause disruption of the oil body. Therefore, the better oil extractability that we observed with all salt steep treatments, when subjected to either absolute alcohol or SC-CO_2 extraction, most likely resulted from an enhanced porosity of the cracked bean matrix that resulted from steeping. Hence, the more porous salt-steeped sample, when ground, allowed better passage of the extraction medium.

Effect of salt-steep treatment on NSI of lipid-extracted beans. Wu and Bates (23) optimized the conditions necessary to improve protein-lipid film formation from soy milk. These authors found that optimizing the conditions for subsequent production of soy milk and films required steeping at 65°C for 1 h because the water uptake of dry whole beans was about 100% with this condition. Using these conditions, these authors demonstrated a slight loss of soluble solids in the soak water. In a previous study, Sessa (11) achieved maximal water uptake in cracked beans when soaked in water at a total water/bean ratio of 5:1, and heated at 65°C for 45 min when agitated on a shaking water bath. As shown in Tables 4 and 5, the steep treatments with either water alone or with all salts except for Na_2SO_3, $(NH_4)_2SO_4$, and Na_2SO_4, caused significant decreases in NSI when compared with an NSI of 65.8% from full-fat unsteeped beans. The data in Table 4 demonstrate that the water-steep treatment caused a significant ($P < 0.01$) decrease in NSI-db (NSI, dry basis). In ANOVA, no ($P > 0.10$) difference was found between SC-CO_2 extractions and water-steeped beans or in the water-steep × SC-CO_2 interaction. The apparent drop in NSI is attributed to leaching of protein into the steep liquor. As shown in Table 5, Na_2SO_3, $(NH_4)_2SO_4$, and Na_2SO_4 yielded significantly higher ($P < 0.05$) NSI than did the H_2O-steeped beans. All other salt treatments showed significantly lower NSI. Least-square means comparisons within defatting procedures C and D showed differences in NSI for water- or salt-steeped cracked beans. The isopropanol-defatting procedure C showed less difference in NSI than did the ethanol-defatting procedure. While Na_2SO_3, $(NH_4)_2SO_4$, and Na_2SO_4 showed minimal effects on NSI, they possessed the highest heat-stabilizing effects, ranking in the order of $(NH_4)_2SO_4 > Na_2SO_4 > Na_2SO_3$, as previously discussed. Apparently, these three salts are best at salting out the protein within the cracked bean matrix, whereas all other salts and H_2O alone tend to have a more solubilizing effect.

The data in Table 6 show the salting-out effects of Na_2SO_3 and Na_2SO_4 at 1 and 2 M concentrations. Cracked beans steeped with 2 M salts gave significantly higher NSI compared to 1 M salts when defatted with either SC-CO_2 or alcohols. In ANOVA results, the least-square means of NSI-db within defatting procedures B, C, and D were 54.1, 55.1, and 57.9%, respectively, where no significant differences ($P > 0.05$) were observed. Therefore, the defatting procedure did not make a significant difference in NSI. NSI-db from the Na_2SO_3 steep was greater ($P < 0.01$) than NSI-db from the Na_2SO_4 steep. The 2 M salt concentration produced greater ($P < 0.01$) NSI-db than did 1 M. The salt × molarity interaction was not significant ($P > 0.05$).

The data in Table 7 show the effects of defatting procedures B, C, and D on the NSI of salt- and water-steeped cracked beans. In ANOVA results, the least-square means of NSI-db within defatting procedures B, C, and D were 34.6, 37.9, and 38.6%, respectively, where no significant differences ($P > 0.05$) were observed. The overall results from Ta-

TABLE 4
Effect of Water Steeping and Supercritical CO_2 Extraction on the NSI-db[a] of Cracked Soybeans

Sample description	NSI-db (%)
Unsteeped/full-fat	65.8
Unsteeped/defatted	64.5
Steeped/full-fat	43.2
Steeped/defatted	40.4

[a]NSI-db, nitrogen solubility index, dry basis.

TABLE 5
Effect of Defatting Procedures C and D[a] on the NSI-db of Salt- and Water-Steeped Cracked Soybeans

Salt	Molarity	NSI-db[b] (%)	C[c]	D[c]
Na_2SO_3	2	70.6 a	a	a
$(NH_4)_2SO_4$	2	61.7 b	b	b
Na_2SO_4	2	56.8 b	b	a,b
H_2O	—	41.9 c	c	c
NaCl	1	36.1 c,d	c	c,d
$NaC_2H_3O_2$	1	34.7 d	c	d,e
NaH_2PO_4	1	18.0 e	d	e,f
$Na_2S_2O_5$	1	11.2 e,f	d	f
$CaCl_2$	1	7.2 f	d	f

[a]Procedures are described in Table 1.
[b]Column values followed by the same letter are not significantly different ($P < 0.05$).
[c]Least-square means comparisons with defatting procedures C and D. For abbreviation see Table 4.

TABLE 6
Effect of Salt-Steep Concentration on the NSI-db of Steeped Cracked Soybeans Defatted by Procedures B, C, and D[a]

Salt	Molarity	NSI-db (%)
Na_2SO_3	1	59.7
Na_2SO_3	2	69.3
Na_2SO_4	1	36.9
Na_2SO_4	2	54.4

[a]See Table 1 for procedure definition. For abbreviation see Table 4.

TABLE 7
Effect of Defatting Procedures B, C, and D[a] on the NSI-db of Salt- and Water-Steeped Cracked Soybeans

Salt	Molarity	NSI-db[b] (%)
Na_2SO_3	2	69.4 a
Na_2SO_4	2	56.2 b
H_2O	—	41.5 c
$Na_2S_2O_5$	1	10.1 d
$CaCl_2$	1	6.8 d

[a]See Table 1 for procedure definition. For abbreviation see Table 4.
[b]Column values followed by the same letter are not significantly different ($P < 0.05$).

bles 4–7 demonstrate that the significantly lower NSI observed with all salts except Na_2SO_3, Na_2SO_4, and $(NH_4)_2SO_4$ result from loss of protein during the steep treatment and do not result from the defatting procedure. These three salts salt out the protein as it exists in the cracked, ground bean matrix.

Effect of salt-steeping on NSI of aqueous ethanol-defatted cracked beans. Our data demonstrated that defatting with absolute isopropanol or ethanol caused no decrease in NSI of the steeped, ground/cracked soybeans. Alcohol-water mixtures as solvent systems possess both hydrophobic and hydrophilic characteristics. Alcohols destabilize proteins by weakening hydrophobic interactions between nonpolar residues, as well as by perturbing the characteristic water structure around the protein molecule (24,25). Effects of aqueous ethanol extraction on the NSI to defat unsteeped cracked beans at 50°C for 2 h are shown in Figure 2. Based on these results, calculation of NSI is:

$$\text{NSI} = 11.23 + \frac{59.26}{1 + [(\%\text{EtOH})/83.69] - 35.837} \quad [1]$$

with a correlation coefficient of $R^2 = 0.99$.

Under the conditions used in this investigation, 83.69% aqueous ethanol represents the midpoint where we observe a sharp decrease in NSI, which results from protein denaturation. Fukushima (26) devised a model system to interpret the aqueous alcohol denaturation of soybean proteins. To evaluate the effects that aqueous ethanol extraction of salt- and water-steeped cracked beans have on their respective NSI we subjected both salt- and water-steeped cracked beans to a series of ethanol-water mixtures. The salts used in this evaluation were Na_2SO_3 and Na_2SO_4, each at 2 M. From the results of this investigation, shown in Figure 3, regression lines for NSI were: NSI = −98.76 + 1.5295(% EtOH) with $R^2 = 0.99$ (6 data points) for water-steeped cracked beans; NSI = −126.49 + 2.0571(% EtOH) with $R^2 = 0.99$ (6 data points) for

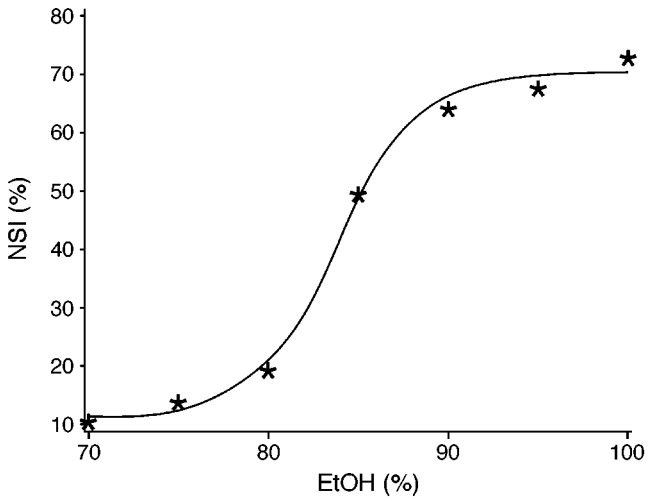

FIG. 2. Effect of aqueous ethanol (EtOH) extraction on the nitrogen solubility index (NSI) of defatted cracked soybeans.

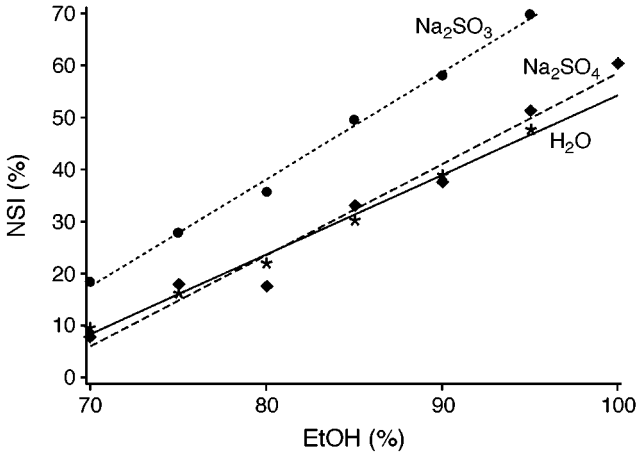

FIG. 3. Effect of EtOH extraction on the NSI of 2 M salt- vs. water-steeped, cracked beans. See Figure 2 for abbreviations.

Na_2SO_3 steeped cracked beans; and NSI = −116.55 + 1.7503(% EtOH) with $R^2 = 0.97$ (7 data points) for Na_2SO_4-steeped cracked beans. These data show that Na_2SO_4 steep treatment provides no significant difference in NSI values when compared with water-steeped cracked beans, whereas the Na_2SO_3-steeped beans exhibited a significantly higher NSI than did the other two treatments (comparisons are based on the following slopes and intercepts, calculated from the data in Figure 3, where H_2O vs. SO_3^{2-}, slope < 0.01, intercept < 0.01; H_2O vs. SO_4^{2-}, slope 0.11, intercept 0.12; SO_3^{2-} vs. SO_4^{2-}, slope 0.05, intercept 0.25). These findings clearly demonstrate that significantly higher NSI is achieved with a 2 M Na_2SO_3 steep treatment prior to defatting with aqueous alcohols at 70–95%. A higher NSI does not necessarily imply that less denaturation has occurred. NSI, which measures the certain fraction of protein that is soluble under the specific conditions of a particular method, is only a practical, empirical estimate of the extent of denaturation (6,7). Protein solubility results from the balance of the attraction of protein molecules for each other and the solvent molecules for the solute (27). The reducing salt Na_2SO_3 will cleave disulfide bonds with introduction of a negatively charged sulfonate group: PS–SP + SO_3^{2-} ⇒ PS–SO_3^- + PS^- where P = protein (20). This reaction should result in an apparent increase in the surface charge of the protein, with a consequent increase in protein solubility.

In conclusion, this investigation established that salts with the capability of salting out proteins will stabilize soy 7S and 11S globulins against heat denaturation. Salts, at 2 M concentration, can salt out proteins within the cracked bean matrix. We assume that high ionic strength is necessary to rupture the membranes of the protein bodies to achieve this result. We found that SC-CO_2 and absolute alcohols did not denature proteins during defatting of the cracked beans under the conditions used. Salt-steep treatments of cracked beans significantly enhanced the extractability of lipids. This may have resulted from some loss of solubles from the cracked beans during the

steep treatment, which resulted in a higher porosity in the bean matrix. With a more porous matrix, solvent or SC-CO_2 penetration into the matrix should result in more effective removal of oil. Salt-steeping did not protect the protein against the denaturing effects of aqueous ethanol. However, SO_3^{2-}-steeped cracked beans did retain significantly higher NSI values when defatted with 70–95% aqueous ethanol. This effect was achieved by a salt interaction with the protein's disulfide bonds and did not result from a salting-out phenomenon.

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