Effect of Simulated Freeze Damage on Soybean Seed Composition and Functionality

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Two soybean varieties (Beeson and Williams) were harvested at various maturation levels by applying paraquat directly to the growing plant. The application of this herbicide to arrest growth was used to simulate freeze damage. The free fatty content and the photometric index declined from 0.61% to 0.19% and 301.56 to 201.90, respectively, as maturation progressed for the Beeson variety. Similar trends were found with the Williams variety. However, the amount of trypsin inhibitor differed between the two varieties depending upon maturation level.

The soybean crop is used primarily for oil crushing in the United States, whereas the use of soybeans for direct human consumption is important in Asian countries. University of Illinois researchers, Nelson *et al.* (1), have developed many foods from whole soybeans, but a problem arose when frost- or freeze-damaged beans were used in soybean products. The use of damaged beans significantly reduced the flavor and color acceptance of food products prepared from this raw material. Urbanski *et al.* (2) suggested a need for stricter quality standards for soybeans used in food.

Unpredictable weather can result in serious quality problems during the harvest season of soybeans. The term "weather damage" refers to frost, freeze, or field damage, although it may also refer in other instances to damage caused by hail or drought. In the northern states especially, frost or freeze damage may occur due to low temperatures early in the harvest season. Freeze damage occurs when the green soybean plant is killed by temperatures of 25°F or less. When this happens, no further maturation takes place and the plant dies and the beans dry to a dark green color. Frost damage occurs at 28–29°F and the leaves are killed. However, this effect is much less severe than freeze damage and the seeds usually continue to mature and lose their green color except for a slight tinge of green in some seedcoats.

Field damage can occur when the soybean crop is approaching maturity and is subjected to intermittent damp weather preventing normal maturing and harvest. Moisture uptake by the pods and beans encourages mold growth. Urbanski (3) suggested that field-damaged soybeans should not be used for direct human consumption. Weather- or field-damaged soybeans occur frequently and there is not much known about the effect of the damage on utilization.

On September 20, 1984, the temperature dropped to 20–25°F in the southeast part of Nebraska. According to Duane Reese (Reese, D., personal communication, University of Nebraska, Lincoln, NE, 1984), the leaves and pods were green when the freeze killed the plants. After the freeze occurred, rain and wet conditions prevailed for two weeks. Mold developed where the seed was attached to the pod.

Since weather-damaged soybeans continue to be a problem, it is important to investigate various properties of damaged beans. The purpose of this study was to examine various degrees of simulated freeze damage on the quality of soybeans used in the food processing industry. Two soybean varieties, Williams and Beeson, grown and harvested on the South Farms at the University of Illinois were used. Soybean seed evaluation included chemical analyses for oil and protein contents, free fatty acids, color of crude oil, trypsin inhibitor and lipoxygenase activities. Microbial assays of raw soybeans as well as solubility and emulsification of soy protein concentrates obtained from these soybeans were studied.

MATERIALS AND METHODS

Simulation of freeze-damaged soybeans. Two soybean varieties were grown on the South Farms at the University of Illinois. Paraquat was sprayed (38 ml/gallon water) on the plants at various percent seed solids to simulate freeze damage. The plants turned brown and lost moisture after spraying, thus allowing mechanical harvest within one to two weeks. The harvested beans were dried in a through-flow air drier at ambient temperature until the solids content was 88% or more.

Solids content. The solids content of the harvested soybeans was determined by the A.O.A.C. (4) procedure. One hundred beans were stripped from the pods and weighed. A sample of the beans was ground in an analytical micromill (Tekmar Company) placed in a tared aluminum dish and weighed. The samples were dried in a vacuum oven for 24 hr at 60°C.

Oil. The oil content was determined by extracting the oil from ground soybeans with petroleum ether according to A.O.C.S. Official Method Ac 3-44 (5).

Free fatty acid. The free fatty acids (FFA) in soybean oil were determined according to the A.O.C.S. Official Method Ac 5-41 (5).

Photometric index of crude oil. The Photometric index of soybean oil was determined by measuring the absorbance at 440 and 550 nanometers, A.O.C.S. Official Method Td 2a-64 (5).

Microbial counts. The aerobic and yeast and mold counts of whole soybeans were determined according to the *Standard Methods for the Examination of Dairy Products* edited by Marth (6).

Trypsin inhibitor. Trypsin inhibitor content was determined by the A.O.C.S. Official Method Ba 12-75 (5) with a modification by Hamerstrand *et al.* (7).

Lipoxygenase. The method for the lipoxygenase assay developed by Surrey (8) and modified by Lao (9) was based on conjugated diene formation determined at 234 millimicrons.

Preparation of soybean protein concentrate. Soybean concentrate was prepared from defatted, dehulled soybean meal. The meal was mixed with distilled water

(60°C) in a ratio of 1:8. The pH was lowered to 4.5 with 2N HCl and the mixture was stirred for 30 min in a water bath at 50°C. The stirred mixture was centrifuged, the precipitate was resuspended in the solution and the entire procedure was repeated five times. The final precipitate was adjusted to pH 6.75 and freeze-dried.

Protein dispersibility index. The protein dispersibility index (PDI) was determined according to the A.O.C.S. Official Method Ba 10-65 (5) with a pH modification by Volkert and Klein (10). In addition, the PDI was determined at various sodium chloride concentrations (0, 1, 2, and 3 percent), and the pH of the dispersion was held constant at 5.75 to simulate meat emulsions.

Emulsifying activity and emulsion stability. The emulsifying activity and emulsion stability were determined by the method developed by Yasumatsu *et al.* (11) with a similar modification by Volkert and Klein (10). Sodium chloride concentrations of 0, 1, 2 and 3 percent of the final emulsion weight (200 grams) were evaluated at pH 5.75 to simulate meat emulsion systems. The protein content of the soybean concentrates was approximately 70% on a dry basis. The oil was added at 50% of the final emulsion weight because this percentage is the highest fat content likely to be encountered in processed meats.

RESULTS AND DISCUSSION

Simulated freeze damage: preparation of simulated freeze-damaged soybeans. Paraquat was sprayed on soybean plots of Williams and Beeson varieties periodically as soybean seed solids were increasing to simulate freeze damage of the soybean plant and seeds. Within a week after spraying, the soybean leaves wilted and turned brown which facilitated combine harvesting. The harvested soybeans were arbitrarily defined as stages 1, 2 and 3 with increasing solids content (Table 1). The control samples did not receive the paraquat spray and were harvested as "normal" soybeans.

This method of simulating freeze-damaged soybeans follows that of Yao *et al.* (12). In his study, soybean seed development was arrested in the field by spraying paraquat on the plant at three different solids contents and then harvesting the beans at 36.95%, 38.65% and 86.21% solids.

Instead of chemical desiccation to halt maturation, Melvin *et al.* (13) picked soybeans from the field and froze them at 0°F to obtain freeze-damaged soybeans. Urbanski *et al.* (14) harvested green soybean pods by hand and held them at −5.5°C for six hr to impart freeze damage to the beans. However, these freezing methods are difficult to optimize and very labor intensive if a large sample is desired.

Therefore, paraquat application was chosen to simulate freeze damage of soybeans because a large sample size was required. With this treatment, combine harvesting was possible.

Free fatty acid. The free fatty acid (FFA) contents of the Williams and Beeson varieties stage 1 were much higher initially than their respective stages 2, 3 and control (Fig. 1,2). All samples increased in FFA content after twelve months of storage. It is interesting to note that the Williams stage 2 and Beeson stages 2 and 3 were much lower than their respective control samples after twelve mo of storage.

The results are not in complete agreement with other researchers. Both Yao *et al.* (12) and Urbanski *et al.* (14) found that the FFA of crude oil from immature or freeze-damaged samples was higher initially and increased at a faster rate during storage than their respective control samples. MacMillan and Melvin (15) found that as the soybeans matured, FFA content decreased. Milner *et al.* (16) found that free fatty acids increased with increasing frost damage. In the present study, only the most immature soybeans were high in FFA values at initial storage time. Perhaps some of the present samples were more mature than those of other researchers. In addition, the plants were not exposed to freezing temperatures but were exposed to severe chemical treatment. It seems reasonable to believe that perhaps the paraquat treatment might have affected the FFA content.

Oil that is high in FFA is undesirable from both a qualitative and an economic standpoint. The neutral oil yield is reduced if the oil is high in FFA because the impurities must be removed or the end product will have off-flavors. In addition, the longer the beans are stored, the greater the increase in FFA due to hydrolytic enzymes in the beans.

Photometric index. The Williams and Beeson simulated freeze-damaged soybean oils decreased in Photometric indices from stage 1 to the control sample (Table 2). The higher values for stage 1 are attributed to chlorophyll which is undesirable because the processor must remove the green color, which adds to the refining cost. After twelve mo of storage, the green color did not dissipate in the oils. These results agree with those of Yao *et al.* (12), Urbanski *et al.* (14), Milner *et al.* (16) and MacMillan and Melvin (15).

Microbial counts. The Williams and Beeson varieties from this study were analyzed for both aerobic plate counts and yeast and mold counts (Table 3). The Beeson stage 3 soybeans were much lower in aerobic plate counts than the control, stage 1 or stage 2. The high counts for stage 1 of both varieties may be attributed to some visible foreign material and numerous splits in the samples. The Beeson soybeans declined in yeast and mold counts as the bean matured except for stage 3 and control which were similar. Although not shown, all counts declined after twelve mo of storage.

The results are in agreement with Milner *et al.* (16) and Tervet (17) who found that the percentage of seeds

TABLE 1

Solids Content of Williams and Beeson Soybeans at the Time of Harvest

	Williams—1981		Beeson—1982	
Stage	Date of spray	g Solids/ 100 seeds at harvest	Date of spray	g Solids/ 100 seeds at harvest
1	Sept. 8	9.68	Sept. 7	10.95
2	Sept. 11	11.13	Sept. 20	17.26
3	Sept. 22	14.52	Sept. 28	18.28
Control	No spray	17.53	No spray	17.46

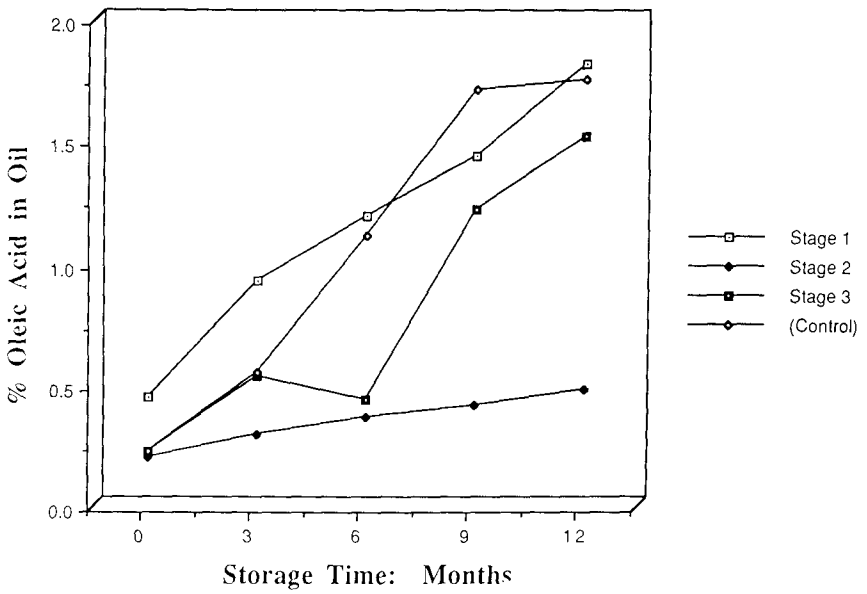

FIG. 1. Effects of storage time on FFA content of Williams simulated freeze-damaged and control samples.

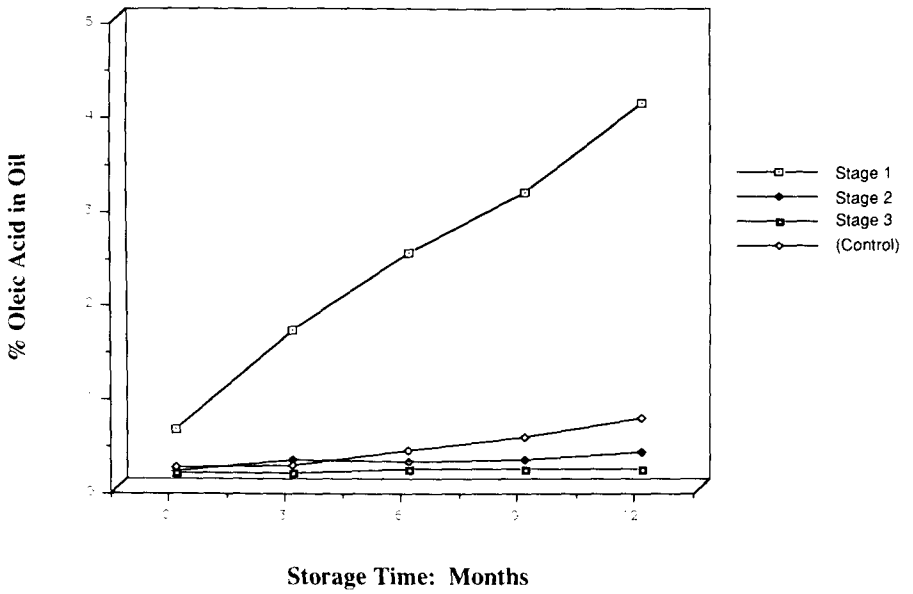

FIG. 2. Effects of storage time on FFA content of Beeson simulated freeze-damaged and control samples.

infected with fungi and bacteria increased in proportion to the frost injury. High microorganism counts may lower the quality of the soybean. This results in economic losses to the seller. The processor will also experience economic losses if storage conditions are such that microbial growth is enhanced.

It is speculated that stage 3 Beeson, which was harvested at physiological maturity (maximum dry-matter content), might be consistent with lower aerobic plate counts and FFA content.

Trypsin-inhibitor and lipoxygenase activity. The trypsin inhibitor values of the Williams soybeans decreased as the beans matured, but appeared to undergo little change after twelve mo of storage (Table 4). The Beeson soybeans were similar in trypsin-inhibitor values initially. However, after storing these beans for twelve mo, stages 1 and 2 increased slightly while stage 3 and the control remained the same. Thus, the trend of the two varieties was somewhat different.

The literature contains conflicting reports concerning trypsin-inhibitor activity during maturation. The results with the Beeson variety were consistent with

TABLE 2

Effects of Storage Time (months) on the Oil Color of Williams and Beeson Simulated Freeze-Damaged and Control Samples[1,2]

	Williams Photometric index		Beeson Photometric index	
Sample	0 mo	12 mo	0 mo	12 mo
Stage 1	365.1 ± 6.9^a	358.6 ± 2.3^a	301.6 ± 0.9^a	295.0 ± 3.0^a
Stage 2	221.5 ± 0.5^b	222.6 ± 0.5^b	205.6 ± 0.6^b	209.3 ± 0.4^b
Stage 3	211.0 ± 0.7^c	217.3 ± 1.3^c	202.0 ± 0.1^c	202.6 ± 0.1^c
Control	209.5 ± 0.4^c	208.0 ± 0.4^d	201.9 ± 0.4^c	204.1 ± 0.1^c

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

TABLE 3

Microbial Counts of Williams and Beeson Simulated Freeze-Damaged and Control Samples

	Williams		Beeson	
Sample	Aerobic plate count per gram	Yeast & mold count per gram	Aerobic plate count per gram	Yeast & mold count per gram
Stage 1	8.8×10^5	1.0×10^4	1.5×10^4	4.7×10^3
Stage 2	1.1×10^4	4.0×10^3	1.3×10^4	4.5×10^2 E
Stage 3	8.4×10^3	4.5×10^2 E	6.5×10^2 E	< 100 E
Control	3.0×10^3 E	< 100 E	1.1×10^4	< 100 E

E = estimated.

TABLE 4

Effects of Storage Time (months) on the Trypsin Inhibitor Activity of Williams and Beeson Simulated Freeze-Damaged and Control Samples[1,2]

	Williams Trypsin inhibitor, mg per gram defatted soy solids		Beeson Trypsin inhibitor, mg per gram defatted soy solids	
Sample	0 mo	12 mo	0 mo	12 mo
Stage 1	30.3 ± 0.6^a	30.3 ± 0.6^a	26.2 ± 1.4^a	29.7 ± 0.2^a
Stage 2	28.8 ± 1.1^b	28.6 ± 0.7^b	26.4 ± 1.8^a	29.9 ± 0.2^a
Stage 3	26.2 ± 0.2^c	28.5 ± 0.3^b	27.2 ± 0.6^a	26.1 ± 0.6^b
Control	26.1 ± 0.2^c	24.7 ± 0.4^c	25.7 ± 1.1^a	25.0 ± 0.1^c

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
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Urbanski *et al.* (14) before and after storage. However, Yao *et al.* (12) found no significant difference between the immature and control samples before or after storage. Collins and Sanders (18) found that the Dare (field-type) variety soybeans increased almost linearly in trypsin-inhibitor activity as the bean matured.

Off-flavor production is a problem in raw soybeans and is attributed to lipoxygenase activity. Lipoxygenase is very specific and will catalyze the *cis-cis*-1, 4-pentadiene group contained in the fatty acids arachidonic, linoleic, and linolenic to hydroperoxides in the presence of molecular oxygen according to Eskin *et al.* (19). The lipoxygenase activity of both Williams and Beeson soybeans increased as the beans matured at initial storage time, but differences in Williams stages 2 to 3 and Beeson stage 3 to the control were not significant (Table 5). After twelve mo of storage, the lipoxygenase activity of the Beeson soybeans decreased significantly for all stages; the Williams soybeans appeared to decrease slightly in lipoxygenase activity, yet increased significantly for stage 3 and the control.

The results of the Beeson variety were consistent with Yao *et al.* (12) who found that the lipoxygenase-1 (L-1) activity increased from 10.50 LAU/mg soy solids to 21.39 LAU/mg soy solids as the beans matured. Rackis *et al.* (20) found that the lipoxygenase activity increased from 18.9 (24 days after flowering) to 32.4 (mature) microliters O_2/min/mg dry matter. Urbanski *et al.* (14) found that the Williams and Clark 63 freeze-damaged soybeans had much lower lipoxygenase ac-

TABLE 5

Effects of Storage Time (months) on the Lipoxygenase Activity of Williams and Beeson Simulated Freeze-Damaged and Control Samples[1,2]

	Williams Lipoxygenase activity units per milligram defatted soy solids		Beeson Lipoxygenase activity units per milligram defatted soy solids	
Sample	0 mo	12 mo	0 mo	12 mo
Stage 1	10.0 ± 1.4^c	8.9 ± 0.1^c	13.8 ± 0.8^c	9.2 ± 1.8^c
Stage 2	14.2 ± 1.7^b	11.4 ± 0.7^b	27.6 ± 0.5^b	17.5 ± 1.2^b
Stage 3	15.7 ± 0.4^b	23.9 ± 0.9^a	32.6 ± 0.8^a	21.9 ± 1.3^a
Control	20.6 ± 0.2^a	24.4 ± 0.2^a	32.3 ± 1.8^a	18.8 ± 1.7^b

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.

[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

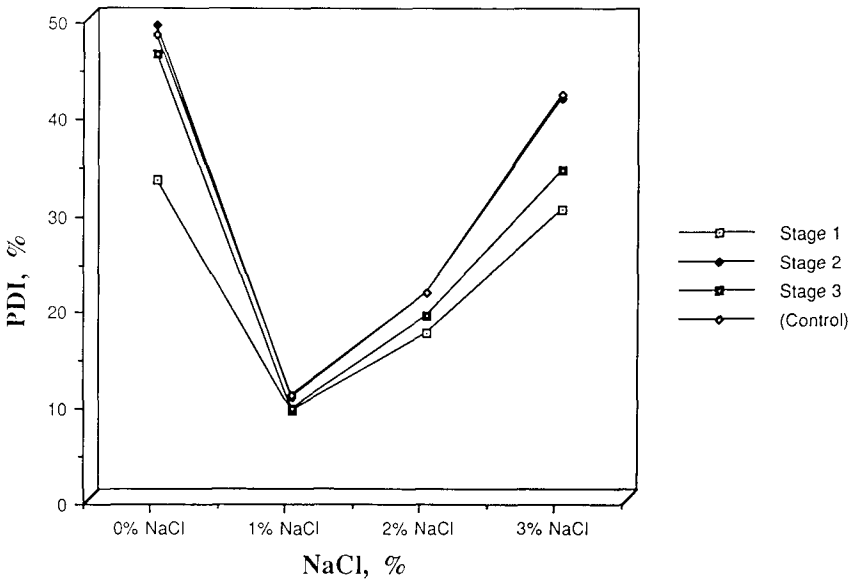

FIG. 3. Effects of sodium chloride on the protein dispersibility index (PDI) at pH 5.75 of Williams simulated freeze-damaged and control soybean concentrates.

tivities than the control soybeans of the same variety which is consistent with the present results.

Protein dispersibility index. The protein dispersibility index (PDI) of protein concentrates obtained from both the Williams and Beeson varieties showed a large response to changes in sodium chloride concentrations (Fig. 3,4). The PDI of the concentrates was lowest at near 1% NaCl regardless of bean maturity. Stage 1 concentrate from both varieties was generally lower in PDI at a given salt concentration.

At the isoelectric point for soy flour suspensions, McWatters and Holmes (21) found that the nitrogen solubility index (NSI) of suspensions with water were lower than suspensions with sodium chloride (0.1 M). At the pH levels outside of the isoelectric point, the NSI was higher than suspensions without sodium chloride.

In the McWatters and Holmes' study, the NSI values extrapolated at pH 5.75, which is the pH used in this study, were much lower for suspensions with sodium chloride (0.1 M). In this study, 1% sodium chloride resulted in the lowest PDI regardless of maturation; the PDI increased as the sodium chloride concentration increased from 1% to 3%.

Although the ratio of 7s to 11s protein fractions was not determined in this study, Yao (22) found that the ratio increased with increasing maturation. Thus, stage 1 in the present study may have more 11s protein than the control sample; therefore, there may be decreased solubility for stage 1 for both Williams and Beeson varieties. However, Yao (22) found no differences in solubility of soybean isolates prepared from various maturation stages.

Emulsifying activity and emulsion stability. The emulsifying activity (EA) and emulsion stability (ES) of soybean protein concentrates were carried out under conditions to simulate meat emulsions. The EA test is similar to the ES test except that heat is applied to the latter. Some differences did exist between soybean protein concentrates prepared from simulated freeze-

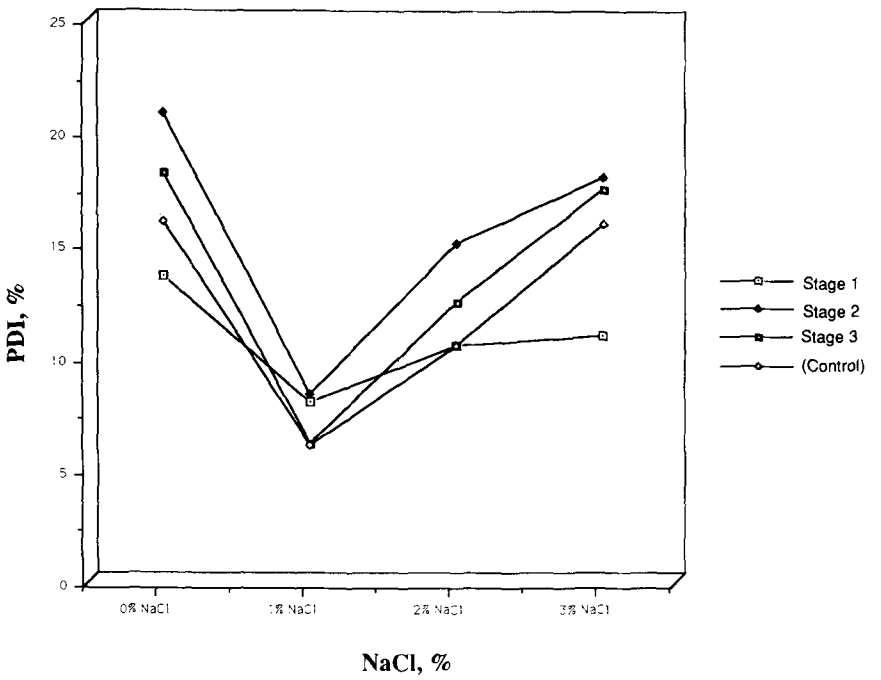

FIG. 4. Effects of sodium chloride on the protein dispersibility index (PDI) at pH 5.75 of Beeson simulated freeze-damaged and control soybean concentrates.

TABLE 6

Effects of Sodium Chloride on the Emulsifying Activity and Emulsion Stability at pH 5.75 of Williams Simulated Freeze-Damaged and Control Soybean Concentrates[1,2]

	Emulsifying activity (%)				Emulsion stability (%)			
Sample	0% NaCl	1% NaCl	2% NaCl	3% NaCl	0% NaCl	1% NaCl	2% NaCl	3% NaCl
Stage 1	87.5 ± 0.6^{a}	84.2 ± 0.7^{a}	83.8 ± 0.4^{a}	84.1 ± 0.3^{a}	96.7 ± 1.6^{a}	93.2 ± 1.4^{a}	$91.9 \pm 0.9^{a,b}$	87.4 ± 0.7^{a}
Stage 2	88.5 ± 0.6^{a}	84.8 ± 0.4^{a}	83.2 ± 0.8^{a}	82.5 ± 0.8^{b}	93.4 ± 0.4^{b}	95.4 ± 1.2^{a}	93.9 ± 1.1^{a}	88.6 ± 0.8^{a}
Stage 3	87.2 ± 0.8^{a}	80.0 ± 0.4^{b}	80.8 ± 0.7^{b}	81.1 ± 0.4^{b}	97.1 ± 1.2^{a}	94.3 ± 2.1^{a}	90.6 ± 0.7^{b}	83.1 ± 0.9^{b}
Control	83.7 ± 0.5^{b}	80.9 ± 0.6^{b}	80.3 ± 0.5^{b}	78.6 ± 0.4^{c}	$96.2 \pm 0.1^{a,b}$	94.4 ± 1.3^{a}	89.6 ± 0.7^{b}	81.6 ± 1.7^{b}

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

TABLE 7

Effects of Sodium Chloride on the Emulsifying Activity and Emulsion Stability at pH 5.75 of Beeson Simulated Freeze-Damaged and Control Soybean Concentrates[1,2]

	Emulsifying activity (%)				Emulsion stability (%)			
Sample	0% NaCl	1% NaCl	2% NaCl	3% NaCl	0% NaCl	1% NaCl	2% NaCl	3% NaCl
Stage 1	76.9 ± 0.4^{a}	76.9 ± 1.1^{a}	76.1 ± 0.8^{a}	$76.3 \pm 0.4^{a,b}$	88.8 ± 0.4^{b}	86.0 ± 0.8^{a}	82.7 ± 0.4^{a}	77.4 ± 1.5^{b}
Stage 2	$76.2 \pm 0.2^{a,b}$	79.1 ± 1.3^{a}	76.4 ± 0.2^{a}	77.1 ± 0.7^{a}	$91.4 \pm 1.7^{a,b}$	87.9 ± 0.9^{a}	84.1 ± 0.4^{a}	81.0 ± 0.2^{a}
Stage 3	$76.1 \pm 0.1^{a,b}$	76.0 ± 1.0^{a}	75.9 ± 2.1^{a}	76.7 ± 0.4^{a}	92.6 ± 0.2^{a}	85.9 ± 2.1^{a}	82.2 ± 0.3^{a}	$80.0 \pm 1.8^{a,b}$
Control	74.9 ± 0.9^{b}	75.4 ± 0.2^{a}	75.8 ± 1.1^{a}	75.1 ± 0.2^{b}	$91.2 \pm 1.4^{a,b}$	85.3 ± 0.7^{a}	83.0 ± 1.2^{a}	$79.2 \pm 0.5^{a,b}$

[1]Means with a common underline in the same horizontal row do not differ significantly at the 5% level.
[2]Means in the same vertical column bearing different superscripts differ significantly at the 5% level.

damaged and control samples. The Williams control sample was significantly lower in EA than the Williams stage 1 sample at each salt concentration (Table 6). This trend was not as pronounced in the Beeson variety (Table 7). The salt effect was minimal on the EA of the Beeson concentrates. However, the Williams 0% NaCl emulsions were significantly higher in EA than the 1, 2 or 3% sodium chloride emulsions, regardless of maturation stage.

The ES appeared to decrease in both varieties as the sodium chloride concentrations increased (Tables 6,7). The ES of emulsions prepared from Williams soybean protein concentrates decreased slightly as maturation increased. However, no such trend was found in the Beeson variety.

Although the ratio of 7s/11s was not determined in the experiment, the ratio may be important in the functionality of soy proteins. Saio *et al.* (23) found tofu-gel prepared from a soybean variety with lower 7s/11s (0.616) was firmer than that from a variety with higher 7s/11s (0.983). Yao *et al.* (12) found that as the soybean matured, the 7s increased from 0.254 to 0.388 grams of 7s per gram of isolated soy protein, but the 11s decreased from 0.746 to 0.612 grams of 11s per gram of isolated soy protein. Thus, the ratio 7s/11s increased from 0.340 to 0.634 as the beans matured. Most likely, the present samples differed in the ratio of 7s/11s. This difference may have effected a significant influence on the quantification of the functional properties tested.

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