**Conclusions.** An automated procedure, based on an improved method for analyzing trypsin inhibitor activity, has been developed. The method has advantages in speed and in reproducibility, eliminating much of the tedium and resulting potential for error of manual methods. It is applicable to a large variety of foods and soy products.

The results of this work demonstrate that processing of soy flour and soy isolates produces products with very low TI activity, especially when compared to the TI activity of other commonly consumed foods.

**Registry No.** BAPNA, 911-76-2; trypsin inhibitor, 9035-81-8.

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# Varietal and Environmental Differences in Soybean Glycinin and β-Conglycinin Content

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The amounts of glycinin and β-conglycinin were measured in 12 soybean varieties by using rocket immunoelectrophoresis. These two proteins constitute 55–75% of the soluble protein in the soybean seed. When 10 varieties (Corsoy, Hodgson, Kitamusume, Tokachi-nagaha, Toyosuzu, Vinton, Wasekogane, Weber, and Yuuzuru) were grown in a uniform environment in 1980 and 1981, the glycinin content (as seed protein) was 46.9–54.4% and 46.7–57.2%, respectively. The average glycinin content was 51.0%. β-Conglycinin content for the 2 years examined averaged 18.5%, with a range of 16.8–20.1% and 16.5–20.9%, respectively. Vinton and Weber soybeans from several growing seasons and different environments had glycinin contents with a range of 11.8% and 14.5%, respectively. β-Conglycinin content of these soybeans varied by 5.0%. There seemed to be no relationship between glycinin and β-conglycinin content in these soybeans. Environmental influences seem to have a much greater impact on glycinin concentration in soybeans than on β-conglycinin content. Genetics also has an influence on the expression of these two proteins but to a lesser extent than environment.

Glycinin and β-conglycinin are the two major protein fractions in the soybean seed. Their physical, chemical, and functional properties are an area of considerable research interest. These two proteins are reported to produce different properties in a number of food products (Saio et al., 1973, 1974). The heat stabilities of the two proteins are quite different (Saio et al., 1975; German et al., 1982; Damodaran and Kinsella, 1982). The reactivity of the two proteins to metal ions, and in particular $Ca^{2+}$, is different (Saio et al., 1973; Briggs and Wolf, 1957). Damodaran and Kinsella (1981) have reported a difference in binding constants with the two proteins for a number

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Table I. Distribution of 11S and 7S Fractions

| variety source | method[a] | % of total seed protein 11S | 7S | reference |
|---|---|---|---|---|
| Clark-1958 | U | 36 | 40 | Wolf et al. (1961) |
| Hakuhou-1959 | U | 26 | 52 | Wolf et al. (1961) |
| Clark-1957 and Hawkeye-1957 | U | 31 | 37 | Wolf et al. (1962) |
| unknown | I | 40 | 28 | Fukushima (1980) |
| unknown | U | 42 | 34 | Fukushima (1980) |
| unknown | unknown | 52 | 35 | Kinsella (1979) |
| Hakuho-1968 | U | 36 | 46 | Saio et al. (1969) |
| Akasaya-1968 | U | 38 | 46 | Saio et al. (1969) |
| Aobata-1968 | U | 34 | 50 | Saio et al. (1969) |
| Norin-2-1968 | U | 37 | 48 | Saio et al. (1969) |
| Shirotsuruno'o-1968 | U | 30 | 53 | Saio et al. (1969) |
| Shofuku-1968 | U | 38 | 44 | Saio et al. (1969) |

[a] Method utilized for determination of the concentration of glycinin and β-conglycinin: U = ultracentifugal; I = immunological.

of soy off-flavor compounds. Saio et al. (1969) have reported different gel strengths for the two proteins. The phosphorus and phytic acid fractions of soybeans seem to be associated with the β-conglycinin fraction (Prattley and Stanley, 1982). All these reports suggest that glycinin and β-conglycinin play very different roles in the functionality of soy protein foods. One would anticipate that changes in the amounts of these two proteins in soybean seeds would yield different food properties.

There are several reports in the literature evaluating the content of 11S and 7S fractions from ultracentrifugal data. The literature values show considerable variation in the concentration of these two protein fractions (Table I). Wolf et al. (1962) have suggested that these variations are due to environmental and genetic differences. Ultracentrifugal data report only percentage areas for specific sedimentation values, namely, 11S and 7S. These sedimentation values are commonly used to refer to the glycinin and β-conglycinin fractions, respectively. The 7S fraction, however, contains β-conglycinin plus the lipoxygenases, hemagglutinins, and β-amylase. A 15S fraction is also usually observed in ultracentrifugal data but is not found with gel filtration or gel electrophoresis (Murphy, 1982; Nielsen, 1981). The composition of the 15S fraction has been suggested to be the result of aggregations of glycinin (Wolf and Cowan, 1975).

A recent report by Skurray et al. (1980) presented data that they claim were ratios of β-conglycinin to glycinin for 15 varieties of soybeans used in tofu production. The method that Skurray et al. (1980) reported using to evaluate the protein amounts in their soybean varieties could not have given data for glycinin and β-conglycinin on their gels. The method (Larson, 1967) utilizes a pH 4.5 buffer supernatant of a soybean extract for the electrophoresis sample. One would expect all the glycinin and β-conglycinin to precipitate at this pH. The conclusion by Skurray et al. (1980) that the ratio of glycinin to β-conglycinin has little effect on tofus produced appears to have no support. Thus, the effect of glycinin and β-conglycinin on tofu texture has only barely begun to be investigated (Saio et al., 1969).

Recently, Hughes and Murphy (1983) investigated the content of glycinin in 10 varieties of soybeans grown in a uniform environment. The study evaluated the effect of genetics on the expression of the glycinin genes. A variation of 7% was observed in glycinin content. This suggested to us that the genetics of the soybean had a significant effect on glycinin content.

In this study, we examined the glycinin and β-conglycinin concentration from the 10 varieties used earlier (Hughes and Murphy, 1983). The protein contents from the 1980 and the 1981 crop years were evaluated by rocket immunoelectrophoresis in this current study. This method is more specific for the two proteins than other methods previously reported. We were able to segregate differences due to genetics and to differences between the summers of 1980 and 1981.

## MATERIALS AND METHODS

**Plant Materials.** Ten varieties of soybeans, five of Japanese lineage (Kitamusume, Tokachi-nagaha, Toyosuzu, Wase-Kogane, and Yuuzuru) and five of American lineage (Coles, Corsoy, Hodgson, Vinton, and Weber), were grown in Ames, IA, during the summers of 1980 and 1981 in a single experimental unit by the Agronomy Department. Other soybean varieties used were from unspecified lots produced by the Agronomy Department: Amsoy, 1981 crop; Prize, 1982 crop; Vinton (sample 1), 1982 crop; Weber, 1981 and 1982 crops [Weber (sample 2) and Weber (sample 1)]. Vinton, 1981 crop [Vinton (sample 2)], was provided by Midwest Soya International, Inc., Clear Lake, IA. The soybeans and their flours were stored at −20 °C prior to extraction. All soybean varieties were analyzed for protein by a micro-Kjeldahl technique (AOAC, 1970, Method 38.012). The samples were defatted by hexane extraction (AACC, 1969). Protein in solution was measured by using the biuret method (AOAC, 1970, Method 2.066).

**Glycinin and β-Conglycinin Purification.** Glycinin was purified for antibody production and rocket immunoelectrophoresis standards as previously reported (Hughes and Murphy, 1983). Glycinin for antibody (IgG) production was further purified by $NaDodSO_4$–polyacrylamide gradient gel electrophoresis (Hughes and Murphy, 1983). All the glycinin bands were cut from Coomassie blue stained gels and electrophoresed from the $NaDodSO_4$–gel matrix into dialysis bags (Stephens, 1975). β-Conglycinin was initially purified by the method reported by Thanh and Shibasaki (1976). β-Conglycinin for antibody production was further purified by gel electrophoresis as glycinin reported above (Stephens, 1975). The purity of both proteins was estimated according to Fenner et al. (1975). Cross-reactivity of IgG antiglycinin and IgG anti-β-conglycinin was also used to estimate purity of β-conglycinin and glycinin, respectively. IgG antiglycinin reacted only with intact glycinin. No reaction occurred with any isolated glycinin subunits. Moreira et al. (1981) also have reported this observation for their IgG antiglycinin. IgG anti-β-conglycinin reacted with α, α′, and β subunits as well as the intact trimer in Ouchterlony plates.

**Antibody Production.** Young adult rabbits and goats were used to prepare antisera to glycinin and β-conglycinin, respectively. Animals were injected at 2-week intervals with the respective antigen in Freund's complete adjuvant until a suitable titer was obtained. Animals were bled, and the serum was processed by ammonium sulfate precipitation to concentrate the antibodies (Mayer and Walker, 1980). Concentrated serum was frozen at −20 °C until use.

**Rocket Immunoelectrophoresis.** A 1% agarose gel (9.5 × 9.5 × 0.15 cm) containing 1–3 mL of concentrated IgG solution was prepared as described previously (Axelsen et al., 1973). The agarose was buffered with 24 mM Tricine, 81 mM Tris, 0.34 mM calcium lactate, and 3 mM sodium azide (pH 8.6). The proteins of defatted flours from the different soybean varieties were extracted with phosphate buffer (2.6 mM $KH_2PO_4$, 32.5 mM $K_2HPO_4$, 0.4

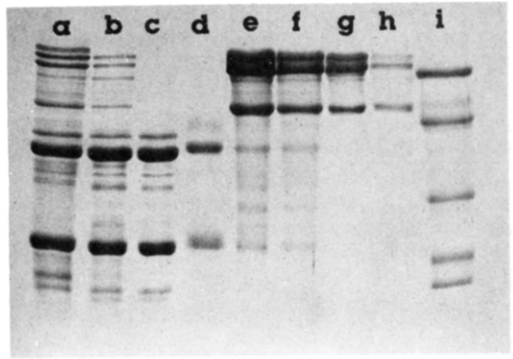


**Figure 1.** Glycinin and β-conglycinin purification on a 10–15% $NaDodSO_4$–polyacrylamide gradient gel. Lanes contain the following: (a) whole soy extract; (b) isoelectrically precipitated glycinin; (c) column-purified glycinin, rocket electrophoresis standard; (d) immunologically pure glycinin used for antisera production; (e) isoelectrically precipitated β-conglycinin after Sepharose 6B-CL chromatography; (f) (e) after second chromatography with Sepharose 6B-CL; (g) (f) after 2 times through concanavalin A–Sepharose and 2 more times through Sepharose 6B-CL, rocket electrophoresis standard; (h) immunologically pure β-conglycinin used for antisera production; (i) molecular weight marker proteins—albumin, bovine (66K), albumin, egg (45K), trypsinogen (24K), β-lactogobulin (18K), and lysozyme (14.3K) (Sigma).

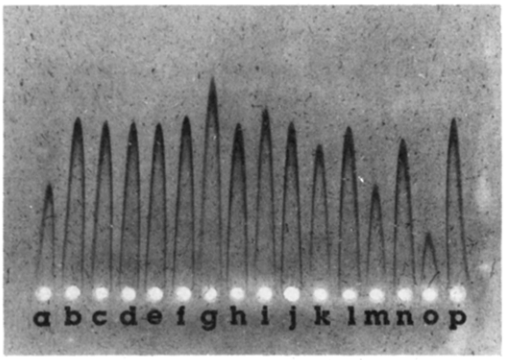


**Figure 2.** Rocket immunoelectrophoresis of glycinin from different soybean varieties. Wells a, g, i, k, m, and o are glycinin standards containing 1.0, 2.5, 2.0, 1.5, 1.0, and 0.5 μg of protein, respectively. The remaining wells are (b) Yuuzuru, (c) Weber, (d) Wase-kogane, (e) Vinton, (f) Toyusuzu, (h) Tokachi-nagaha, (j) Kitamusume, (l) Hodgson, (n) Corsoy, and (p) Coles.

M NaCl, pH 7.6). Ten microliters of freshly extracted sample and standard solutions were applied to 3.0-mm wells cut in the agarose gel. The concentration of standards was 0.05–0.25 μg/μL. Electrophoresis was run at 80 V for 15 h by using the Tris–Tricine buffer just described. The gel was presssed, dried, and stained with Coomassie Brilliant Blue R-250 and destained, and the heights of the rockets were measured.

**Statistical Analysis.** The data were evaluated by using a general linear model of the SAS Institute, Inc., statistical package (SAS Institute, Inc., Cary, NC). Duncan's multiple-range test in the SAS package was used to compare the means.

## RESULTS AND DISCUSSION

Rocket immunoelectrophoresis is a quantitative method for the determination of a single protein (antigen) in a mixture of proteins with no prior purification (Axelsen et al., 1973). This method requires highly purified protein to produce an antibody in the host animal and purified protein to use as standards. Figure 1 is a photograph of an $NaDodSO_4$–gradient polyacrylamide gel containing the different fractions from soybeans. Impurities in the rocket electrophoresis standards averaged 13% for glycinin and 10% for β-conglycinin (Figure 1C,G).

Figure 2 is a photograph of a typical rocket immunoelectrophoresis gel. The rocket-shaped precipitate has a height and area proportional to the amount of antigen (Mayer and Walker, 1980; Axelsen et al., 1973). Standard curves for glycinin and β-conglycinin from Vinton soybeans were prepared, relating peak height to protein concentration. The standard curves had correlation coefficients averaging 0.9975 for glycinin and 0.9971 for β-conglycinin. Glycinin purified from Weber soybeans gave the same peak heights as an equal concentration of Vinton glycinin. The rocket patterns of the standards and the samples were the same, indicating that the proteins were immunologically identical.

Glycinin and β-conglycinin contents were determined in four different extractions of the 10 soybean varieties grown in a homogeneous environment for 1980 and 1981 (Table II). These data indicate that glycinin and β-conglycinin constitute between 55 and 75% of the protein in the soybean seed. These data are quite different in terms of the distribution between glycinin and β-conglycinin and the 11S and 7S fractions from data obtained earlier with use of ultracentrifugal analysis (Table I). The reports by Fukushima (1980) and Kinsella (1979) using immunological and unknown techniques, respectively, are similar to data reported here. The difference in methods may be entirely responsible for the contrasts observed between the literature values and our data. Because ultracentrifugation techniques would not distinguish between the various proteins associated with the 7S fraction, variations in the amount of β-conglycinin in the 7S fraction would not be reflected in these data. The ultracentrifuge technique does not differentiate the aggregates due to glycinin from other high molecular weight material in the 15S fraction. Therefore, glycinin could be underestimated with this ultracentrifuge technique.

**Table II. Storage Protein Composition of Soybean Varieties Grown in a Single Experimental Unit**

| variety | % of total protein: glycinin 1980[a] | glycinin 1981 | β-conglycinin 1980[a] | β-conglycinin 1981[b] | glycinin/β-conglycinin |
|---|---|---|---|---|---|
| Coles | 51.7$^{bc}$ | 52.4$^{b}$ | 19.0$^{ab}$ | 20.5 | 2.73$^{bc}$ |
| Corsoy | 46.9$^{e}$* | 51.5$^{b}$* | 19.2$^{ab}$ | 20.9 | 2.46$^{d}$ |
| Hodgson | 50.1$^{cd}$ | 51.7$^{b}$ | 20.0$^{a}$ | 19.4 | 2.59$^{cd}$ |
| Kitamusume | 48.9$^{de}$ | 47.5$^{c}$ | 18.8$^{ab}$ | 17.3 | 2.68$^{bcd}$ |
| Tokachi-nagaha | 48.8$^{de}$ | 46.7$^{c}$ | 19.4$^{ab}$ | 17.4 | 2.60$^{cd}$ |
| Toyosuzu | 48.3$^{de}$* | 51.1$^{b}$* | 20.1$^{a}$ | 18.1 | 2.61$^{cd}$ |
| Vinton | 54.4$^{a}$* | 57.2$^{a}$* | 17.7$^{ab}$ | 17.5 | 3.17$^{a}$ |
| Wase-kogane | 52.9$^{ab}$ | 53.4$^{b}$ | 19.6$^{a}$ | 19.2 | 2.74$^{bc}$ |
| Weber | 51.9$^{bc}$ | 52.6$^{b}$ | 16.8$^{b}$ | 16.5 | 3.14$^{a}$ |
| Yuuzuru | 49.7$^{cd}$* | 52.1$^{b}$* | 18.6$^{ab}$ | 17.3 | 2.84$^{b}$ |
| average | 50.3* | 51.6* | 18.9 | 18.2 | 2.84 |
| range, % | 7.5 | 10.5 | 3.3 | 4.4 | |
| mean square error | 2.32 | 2.26 | 2.57 | 7.15 | 0.008 |

[a] Columns not sharing common superscripts are significantly different at $\alpha = 0.05$. In the rows, values with an asterisk are significantly different at $\alpha = 0.05$. Sample size: $n = 4$. [b] $n = 5$.

Glycinin content in whole soybeans has been reported to vary from 7.5% for soybeans grown in a uniform environment (Hughes and Murphy, 1983) to 10% for soybeans grown at different locations (Wolf et al., 1961). In this study, the data indicate a variation of 7.5% for the 10 varieties grown in 1980 and 10.5% for those grown in 1981. The combined yearly averages for the 10 varieties was significantly greater in 1981 than in 1980. This suggests to us that the environmental conditions are having a significant effect on the expression of the glycinin genes

in the mature seed. Although the glycinin concentration yearly average was significantly different, only Corsoy, Toyosuzu, Vinton, and Yuuzuru were significantly greater in 1981 than in 1980 when compared on an individual basis.

Our estimates for glycinin concentration are higher in this study than our previously reported results (Hughes and Murphy, 1983). We feel that our first study underestimated glycinin concentrations in the varieties examined. The amount of dye bound by the gels can change depending on slight variations in dye concentration and slight variations in staining and destaining times. The dye-destaining method of Fenner et al. (1975) assumes one knows exactly where the peptide bands of interest are in order to excise them from the gel (Hughes, 1981). We may have not cut out all bands belonging to glycinin. The 19000 molecular weight peptide band was not cut out of these gels because of the danger of quantitating (as glycinin) other similar molecular weight peptides. This contributed to the underestimation of glycinin concentration (Hughes and Murphy, 1983).

The data for glycinin and β-conglycinin content reported in this paper are comparable with those reported recently by Medeiros (1982) using an enzyme-linked immunosorbent assay (ELISA) and the same extraction buffer as this report. He reports glycinin contents of 23.3–58.2% and β-conglycinin contents of 14.4–36.3%. CX635-1-1-1, a very high protein cultivar used extensively by Nielsen and coworkers at Purdue University, contained 15.7% β-conglycinin and 45.6% glycinin. Commerical cultivars had glycinin contents of 38.1–50.8% and β-conglycinin contents of 18.6–29.0%. Therefore, it is reasonable to assume that in high-protein varieties of soybeans, 50% of the protein is glycinin.

There was no difference in glycinin concentration between Japanese and American varieties as groups. This is in agreement with results reported by Hughes and Murphy (1983) but in contrast to work reported much earlier (Saio et al., 1969; Wolf et al., 1961).

There was a significant difference among varieties in glycinin concentration within both years. In both years, Vinton contained the highest concentration of glycinin. The ranking of the varieties changed little between two growing seasons except for Corsoy, which had a difference of almost 5% in glycinin concentration between the 2 years.

β-Conglycinin concentration of soybeans in the varieties and years ranged from 16.8 to 20.9%. These value are much lower than reported in the literature for the 7S fraction (Table I). However, because most of these studies report the amount of 7S fraction rather than the β-conglycinin concentration, our results are not surprising. There was more variance in the means of β-conglycinin concentrations for each variety than observed with the glycinin content measurements. Therefore, in 1981, no significant difference was observed between varieties nor between the 1980 and 1981 variety average. The sample size was increased to 5 in the 1981 group, but this did not decrease the variance in the data to any signficant extent. Significant differences were observed in the 1980 group in β-conglycinin concentration. Wase-Kogane, Toyosuzu, and Hodgson contained the highest β-glycinin content. Weber soybeans contained the lowest and were significantly lower than the former three varieties in β-conglycinin content. The other varieties were not significantly different from the four varieties just mentioned in β-conglycinin concentration. It is not known why the variances associated with the β-conglycinin data are so much larger than the variance of the glycinin data. There appeared to be no relationship between changes in glycinin concentration and the changes in β-conglycinin concentration between crop year groups, although this effect could be obscured by the larger variance in the β-conglycinin data.

**Table III. Storage Protein Composition of Soybean Varieties Grown in Several Environmental Conditions**

| variety[a] | year | glycinin[b] | β-conglycinin | glycinin/β-conglycinin |
|---|---|---|---|---|
| Amsoy | 1981 | 44.4$^{def}$ | 16.0$^{cdef}$ | 2.77 |
| Prize | 1982 | 42.6$^{f}$ | 19.7$^{a}$ | 2.16 |
| Vinton (sample 1) | 1982 | 45.6$^{de}$ | 19.0$^{ab}$ | 2.41 |
| Vinton (sample 2) | 1981 | 46.6$^{d}$ | 15.2$^{ef}$ | 3.10 |
| Vinton (sample 3) | 1980 | 54.6$^{b}$ | 17.4$^{bcd}$ | 3.15 |
| Vinton (sample 4) | 1981 | 57.4$^{a}$ | 16.7$^{cde}$ | 3.44 |
| Weber (sample 1) | 1982 | 38.2$^{g}$ | 17.9$^{bc}$ | 2.13 |
| Weber (sample 2) | 1981 | 43.7$^{ef}$ | 14.7$^{f}$ | 2.98 |
| Weber (sample 3) | 1980 | 52.1$^{c}$ | 16.6$^{cdef}$ | 3.14 |
| Weber (sample 4) | 1981 | 52.7$^{bc}$ | 15.6$^{def}$ | 3.39 |
| range | | 19.2 | 5.0 | 1.31 |
| mean square error | | 1.73 | 1.03 | |

[a] Vinton (sample 3) and Vinton (sample 4) are the 1980 and 1981 beans, respectively, grown in a single experimental unit of the 10 varieties in Table II. Weber (sample 3) and Weber (sample 4) are the 1980 and 1981 beans from the same study, respectively. Sample size: $n = 4$. [b] Values in column not sharing common superscripts are significantly different at $\alpha = 0.05$.

The ratio of glycinin to β-conglycinin was evaluated. Because glycinin and β-conglycinin were not estimated from the same extracts (due to technical parameters), only the combination of the 1980 and 1981 ratios could be statistically evaluated. There was a significant increase in the ratio in 1981 soybeans as compared with the 1980 crop. The statistical ranking of the varieties was quite different than rankings for either of the two proteins individually. The Vinton ratio (the variety with the highest glycinin content) and Weber ratio (the variety with the lowest β-conglycinin content) were the highest values observed and not statistically different from each other. The Yuuzuru ratio was the same as the group average ratio. All other variety ratios were below the group average value. The significance of this ratio to soy food systems is yet to be determined.

In addition to examining the glycinin and β-conglycinin content of soybean varieties grown in a uniform environment, we also had the opportunity to examine these same proteins in two varieties (Weber and Vinton) grown in several different environments. These two varieties were chosen because of their difference in protein concentration and seed size. Vinton variety soybeans grown in 1980 were reported to be popular with tofu producers in the United States, but the 1981 Vinton crop was not (Wilson, 1983). These producers preferred the Prize variety to replace Vinton. Therefore, these varieties were chosen to estimate what differences in glycinin and β-conglycinin, if any, could be observed. Amsoy was included in the study for its intermediate protein content.

The data from these experiments are presented in Table III. It is obvious immediately that environment was affecting glycinin and β-conglycinin content to a greater extent than variety. In this data set, there was almost a 20% range of values for glycinin content and 5% for β-conglycinin. Within Vinton and Weber, there was an 11.8 and a 14.5% range of values for glycinin content, repectively. Although, β-conglycinin contents vary to a smaller extent than those of glycinin, the mean square error is small enough in this sampling to observe significant dif-

ferences between varieties. There seemed to be no relationship betwen glycinin and β-conglycinin concentration in these randomly grown soybean varieties as observed with the varieties grown in a uniform environment (Table II). Interestingly, even with no discernible relationship between glycinin and β-conglycinin contents, the ratios of glycinin to β-conglycinin increased in almost an identical manner for both Weber and Vinton as glycinin content increased.

Wang et al. (1983) have reported that there is probably little relationship between glycinin and β-conglycinin content and tofu curd formation and strength. Their hardness (kg) data for tofus produced from the same varieties of soybeans reported in Table II indicate extremely soft tofus and probably do not represent a typical commercial product. Johnson and Wilson (1984) have reported a number of physical sensory parameters utilizing an Instron Universal Testing Machine for tofus produced from Prize, Vinton 1, and Weber 1 soybeans reported in this paper. In preliminary comparisons, we find high correlation coefficients (>0.9) between glycinin content and hardness, brittleness, elasticity, and gumminess of tofu. β-Conglycinin content did not correlate with any texture parameters examined. These results will be published elsewhere. These data suggest to us that the contents of these two proteins in soybeans do play a significant role in soy food structure as suggested by Saio et al. (1969).

## CONCLUSION

The results of this work indicate that genetics has an impact on glycinin and β-conglycinin content in soybeans. This is in agreement with data reported recently for glycinin (Hughes and Murphy, 1983). Environmental conditions seem to affect glycinin concentration to a much greater extent than genetics. A range of almost 20% was observed in glycinin concentrations in soybeans grown in different locations. β-Conglycinin also varied in concentration due to genetics and environment but to a lesser extent than glycinin. Preliminary experiments suggest that glycinin is significantly correlated with textures of tofus made for Western preferences. The effects the concentration of these two proteins has on other soy foods are yet to be determined.

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