

The Influence of Genotypic Variation in Protein Composition on Emulsifying Properties of Soy Proteins

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ABSTRACT: This study describes the relationship between the emulsifying properties of soybean proteins and their composition, i.e., glycinin (11S) and β -conglycinin (7S). Twelve investigated soybean genotypes showed significant differences in storage protein composition. The β -conglycinin concentration positively correlated with extractable soluble protein content, which was positively correlated with protein extractability. These data suggest that the level of β -conglycinin has a positive influence on protein extractability. The emulsion activity index (EAI) was strongly and positively correlated with the 11S:7S ratio and strongly and negatively correlated with the concentration of β -conglycinin. The emulsion stability index (ESI) showed a moderate positive correlation with the monomeric form of glycinin and a strong positive correlation with the ratio of the monomeric to dimeric form of glycinin. No association was evident between ESI and EAI. Also, no relationship was found between ESI or EAI and extractability. Based on these data, it appears that the 11S:7S ratio strongly reflects the ability of soybean proteins to form emulsions, whereas the ratio of the two different forms of glycinin may be crucial factors for the stability of soybean protein emulsions. Thus, understanding the relationship between protein composition and functionality could be useful for further improvement of functional behavior of soy proteins in food systems.

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KEY WORDS: β -Conglycinin, emulsifying properties, extractability, glycinin, soybean genotypes, storage protein.

Soy proteins have been used in a variety of food applications for many years. Some of the reasons for their use include their relatively low cost and availability compared with other competing food ingredients. The primary reason for the use of soy proteins is their wide range of functional properties that help to stabilize food systems. In the last few years, the interest in soy proteins has increased. One of the key factors is the 1999 U.S. Food and Drug Administration ruling that approves the use of a label claim that soybeans can lower cholesterol and reduce the risk of heart disease. Furthermore, anticarcinogenic and other therapeutic activities of soy proteins have been reported recently (1).

Emulsifying properties of soy proteins have been extensively studied (2–4). These properties depend on factors such

as pH, ionic strength, protein concentration, structure, surface hydrophobicity, chemical, physical or enzymatic modifications (3,5). Many approaches have been carried out on protein isolate (5), glycinin (11S), and β -conglycinin (7S) soy protein fractions (4–6), or their subunits (7). Purification as well as processing affects protein composition of the obtained protein or protein product, which reflects not only on functional properties (4,6) but also on the content of bioactive components of soy proteins (8,9). Furthermore, protein composition varies among genotypes and is influenced by environment (10). However, for many years food scientists considered all soybeans the same with regard to their functional properties. Recently, Riblett *et al.* (6) and Khatib *et al.* (4) examined several soybean genotypes and reported that each genotype and their respective protein fractions contributed to different functional properties. They noticed that differences in amino acid profiles might alter functionality. Cai and Chang (11) examined 13 soybean genotypes and found that soybean variety had highly significant effects on the 7S and 11S protein contents and the 11S:7S protein ratio of soybean seed, soymilk, and tofu. Furthermore, they showed that variety influences soymilk and tofu yield as well as quality parameters. Also, variation in subunit composition within fractions may affect functionality such as gelling characteristics of soybean glycinin (12). The variation in concentration of bioactive components of soy proteins was observed in a large number of cultivars (9). Such findings present a unique opportunity for soybean breeders and food scientists to develop soybeans with specific protein composition for specific food applications.

Further evaluation of soy protein is needed with regard to genotype and its effects on soy protein functionality, especially in light of increasing interest in beneficial health effects of bioactive soy proteins. It has been reported that there are no components in soybeans that antagonize its efficacy and there might even be compounds that enhance it (9). Although commercially prepared soy proteins used in today's food applications are mainly denatured and modified, native soybean proteins may be used more widely in the future owing to the influence of large-scale processing of soy on the content of bioactive compounds.

The aim of this research was to contribute to understanding the influence of soybean genotypes on functionality of native soybean proteins. To avoid changes of physicochemical properties of proteins provoked by isolation and subsequent purification, the analyses were performed on protein extracts. In this

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study, glycinin and β -conglycinin from selected soybean cultivars were determined directly from whole soybean protein extract, and their relationship to protein extractability and emulsifying properties was investigated. Understanding the relationship between protein composition and functionality could be useful to predict functional behavior of the protein products. Information of this type might help growers in the proper selection of genotypes for certain types of processing and specific food applications.

MATERIALS AND METHODS

Materials. Twelve soybean genotypes grown in 2001 in field conditions were evaluated. Six genotypes (Nena, ZPS-015, Lana, L91-31022, L94-1171, SG1-1) were selected by the Maize Research Institute Zemun Polje (Belgrade, Serbia and Montenegro) and the others (Krajina, Novosadjanka, Vojvodjanka, Proteinka, Balkan, and Ravnica) by the Institute of Field and Vegetable Crops (Novi Sad, Serbia and Montenegro). Proteinka and Novosadjanka are high seed-protein cultivars, and the genotype Lana lacks the Kunitz type of trypsin inhibitor. Reagents and chemicals used in this work were of analytical grade and were obtained from standard commercial sources.

Protein extractability. To determine protein extractability, protein was extracted for 1 h at room temperature from defatted meal in a 1:20 ratio with 0.03 M Tris-HCl buffer, pH 8, which contained 0.01M β -mercaptoethanol. The mixture was centrifuged at $17,000 \times g$ for 15 min at room temperature. The protein content in the supernatant was determined by the procedure of Lowry *et al.* (13) at 750 nm. The total protein content in the sample was determined by the Kjeldahl method using the conversion factor of 6.25. The protein extractability was expressed as the percentage (w/w) of the extractable soluble protein compared with the total protein content in the sample.

Emulsifying properties. Emulsifying properties were measured according to a method modified from Wu and others (14). Pure sunflower oil (15 mL) and 45 mL of a 0.1% protein solution, prepared using protein extracted from defatted meal as described for protein extractability determination, were homogenized in a mechanical homogenizer at the highest settings for 1 min. Fifty microliter portions of the emulsions were pipetted from the bottom of the container at 0 and 10 min after homogenization. Each portion was diluted with 10 mL of 0.1% (wt/vol) SDS solution. Absorbances of these diluted emulsions were measured at 500 nm. The absorbances measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the emulsifying activity index (EAI) and the emulsifying stability index (ESI):

$$EAI(m^2/g) = 2T \left(\frac{A_0 \times F}{C \times \phi \times 10.000} \right) \quad [1]$$

where $T = 2.303$; A_0 = absorbance measured immediately after emulsion formation; dilution factor = 200; C = weight of protein/unit volume (g/mL) of aqueous phase before emulsion formation; ϕ = oil volume fraction of the emulsion; and

$$ESI(\text{min}) = A_0 \times \frac{\Delta t}{\Delta A} \quad [2]$$

where $\Delta t = 10$ min and $\Delta A = A_0 - A_{10}$.

SDS-PAGE. Dissociating electrophoresis was carried out according to the procedures of Fling and Gregerson (15) in 1.5-mm thick gels with 12.5% (wt/vol) separating gels and 5% (wt/vol) stacking gels. The protein extract was diluted to 2 mg/mL with sample buffer [0.055 M Tris-HCl, pH 6.8, 2% (wt/vol) SDS, 7% (vol/vol) glycerin, 5% (vol/vol) 2-mercaptoethanol, 0.0025% (wt/vol) bromophenol blue]. After boiling for 2 min, 25 μ L of the cooled solution was loaded onto each well. The gels were run in a buffer solution [0.05 M tris(hydroxymethyl)aminomethane, 0.19 M glycine, 0.1% (wt/vol) SDS, pH 8.5] for 3 h to completion. Gels were fixed, stained with 0.23% (wt/vol) Coomassie brilliant blue R250 (dissolved in 3.9% (wt/vol) TCA, 6% (vol/vol) acetic acid, and 17% (vol/vol) methanol) for 1.5 h and destained with 18% (vol/vol) ethanol and 8% (vol/vol) acetic acid. The destained gels were scanned and then were analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA). The determination of glycinin and β -conglycinin was made, and their concentrations and ratio were calculated from the sum of the total area of their subunits (11). To investigate varietal effect, electrophoresis of the storage proteins in 12 soybean varieties was performed in duplicate. Namely, two aliquots of the same sample were analyzed at the same time. Two gels were run simultaneously in the same electrophoretic cell.

M.W. of the proteins were estimated by means of the LMW Pharmacia kit (phosphorylase B, 94.0; bovine albumin, 67.0; ovalbumin, 43.0; carbonic anhydrase, 30.0; trypsin inhibitor, 20.1; and α -lactalbumin, 14.4).

PAGE. PAGE was performed according to the method of Davis (16). The separating gels were 7% (wt/vol), pH 8.9 and stacking gels were 5% (wt/vol), pH 6.7. A 25 μ L sample of the extract (2 mg protein/mL) diluted with sample buffer [0.03 M Tris-HCl buffer with 0.01 M 2-mercaptoethanol, pH 8, 10% (vol/vol) glycerol, 0.0025% (wt/vol) bromophenol blue] was loaded per well. The gels were run in a buffer solution [0.05 M tris(hydroxymethyl)aminomethane, 0.19 M glycine, 0.1% (wt/vol) SDS, pH 8.3] for 2.30 h to completion and then were fixed, stained, destained, and analyzed in the same way as in SDS-PAGE.

Statistical analysis. Experiments were performed in triplicate. The data were analyzed using Statistica software version 5.0 (StatSoft Co., Tulsa, OK). The significance of differences between means was determined by *t*-test procedure for independent samples at $P < 0.05$. The EAI and ESI were measured on two different days, producing each day two different emulsions of the same sample, and taking two aliquots of each emulsion. The results are given as the mean values. Regression analyses were also carried out.

RESULTS AND DISCUSSION

Electrophoretic analysis. PAGE and SDS-PAGE separated total soybean proteins into multiple components. Glycinin and

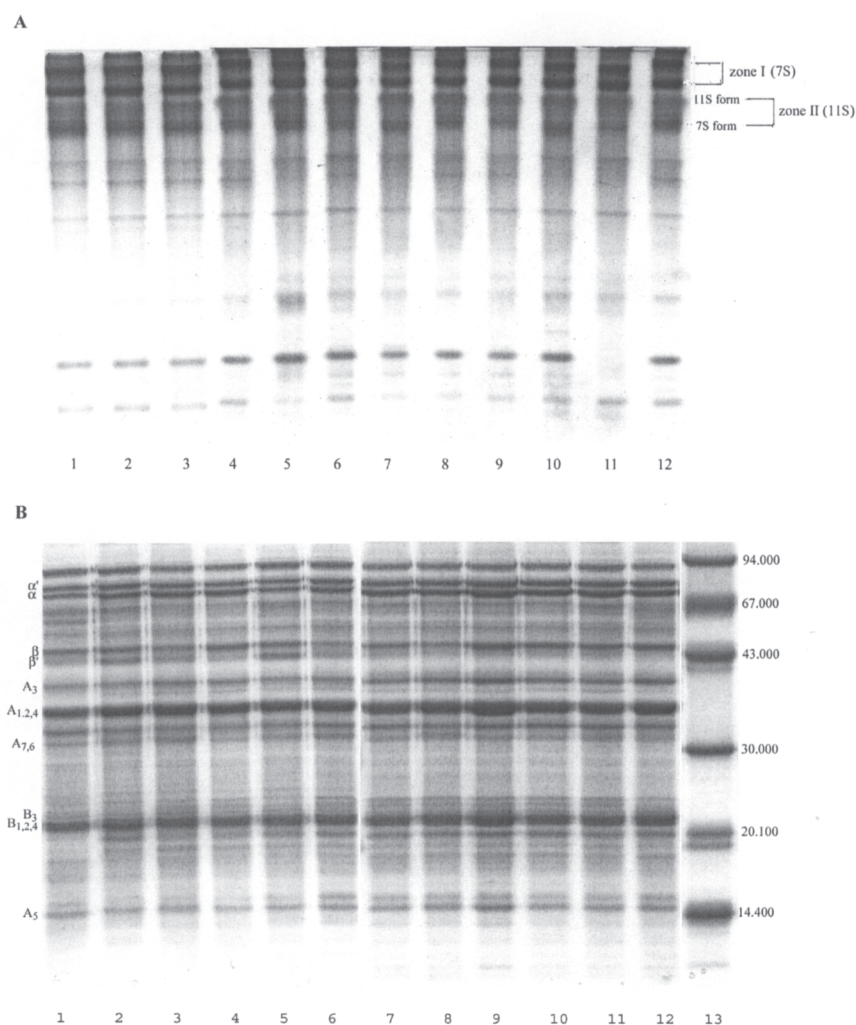


FIG. 1. Electrophoretic pattern of the 12 soybean genotypes. (A) Native PAGE: Lanes 1–12 represent the PAGE patterns of the genotypes Proteinka, Balkan, Ravnica, Vojvodjanka, Krajina, SG1-1, L94-1171, L91-31022, Nena, ZPS-015, Lana, and Novosadjanka, respectively. (B) SDS-PAGE: Lanes 1–12 are polypeptides in soybeans of the genotypes Lana, ZPS-015, Nena, L91-31022, L94-1171, SG1-1, Ravnica, Balkan, Proteinka, Novosadjanka, Vojvodjanka, and Krajina, respectively. Lane 13 represents protein molecular mass markers (molecular masses are shown on the right). A_3 , $A_{1,2,4}$, A_5 , $A_{7,6}$, B_3 and $B_{1,2,4}$ are polypeptides of glycinin (A, acidic; B, basic), and α' , α , β , and β' are subunits of β -conglycinin.

β -conglycinin are the major storage proteins in soybeans. PAGE separated these proteins into several bands (Fig. 1A). Two bands (zone I) were identified as β -conglycinin, and two (zone II) as glycinin (17,18). The band with lower electrophoretic mobility in zone II represents the 11S form of glycinin, whereas the other represents the dissociating form of 11S, i.e., the 7S form of glycinin. The 7S and 11S forms of glycinin are considered as monomeric and dimeric forms of the glycinin molecule, respectively. The electrophoretic mobility of the monomeric form of glycinin is not the same in all electrophoretic patterns. This reflects heterogeneity of glycinin molecular species.

SDS-PAGE confirms these results. SDS-PAGE separated β -conglycinin and glycinin into subunits and polypeptides (Fig. 1B). The protein concentration of subunits is shown in Table 1.

As one can see, the investigated soybean varieties had different storage protein composition as well as polypeptide composition ($P < 0.05$).

The concentration of β -conglycinin (7S) and glycinin (11S) of 12 soybean varieties ranged from 17.9 to 24.6% and 32.9 to 39.9% of total extractable proteins, respectively; these values are similar to 17.2 to 23.5% β -conglycinin and 36.3 to 51.3% glycinin of varieties reported by Cai and Chang (11). Glycinin and β -conglycinin constituted 52 to 64% of total extractable protein, which is slightly lower than ranges of values that Murphy and Resurreccion (10), using rocket immunoelectrophoresis, found for 10 commercial soybean varieties (55 to 75% of protein) as well as values for 45 wild soybean genotypes (57 to 72% of soluble protein) reported by Kwanyuen *et al.* (19). No significant correlation was found between total protein content

TABLE 1
Protein Composition of the Investigated Soybean Genotypes^a

| Genotype | Seed protein (% dry wt) | 7S subunits | | 11S subunits | | Total | | Forms of glycinin ^b | | | |
|--------------|------------------------------|--------------------------|----------------------|------------------------------|----------------------------|------------------------------|------------------------|--------------------------------|-------------------------|------------------------------|---------------------|
| | | α' + α | β + β' | Acidic (% ESP ^c) | Basic | 7S | 11S | 11S:7S (ratio) | M (% ESP ^c) | D | M/D (ratio) |
| Lana | 45.88 ^a | 9.31 ^a | 8.63 ^{ab} | 22.72 ^{ab} | 14.54 ^{ab} | 17.94 ^{ab} | 37.27 ^a | 2.08 ^a | 16.62 ^a | 12.67 ^{ab} | 1.31 ^a |
| ZPS-015 | 48.86 ^{b,c,d,e} | 10.8 ^b | 9.81 ^c | 21.28 ^c | 13.81 ^{cd} | 20.61 ^{c,d,e} | 35.09 ^b | 1.7 ^{b,c,d,e,f} | 17.46 ^b | 11.98 ^{a,c,d} | 1.46 ^b |
| Nena | 47.16 ^{b,f} | 11.02 ^{b,c,d} | 8.89 ^a | 24.15 ^d | 13.38 ^{a,c,e,f,g} | 19.91 ^{c,d,e,f,g,h} | 37.53 ^{a,c} | 1.88 ^{g,h,i} | 14.85 ^c | 13.45 ^{ef} | 1.10 ^c |
| L91-31022 | 49.45 ^{c,h} | 12.74 ^{ef} | 11.88 ^d | 22.70 ^{ab} | 16.60 ^h | 24.62 ⁱ | 39.30 ^d | 1.60 ^{j,k,l} | 18.56 ^{b,d,e} | 11.81 ^c | 1.57 ^d |
| L94-1171 | 46.07 ^f | 10.7 ^{b,c,g} | 11.12 ^d | 21.92 ^{ef} | 15.38 ^{ei} | 21.82 ^{c,d,e,i} | 37.30 ^{a,c} | 1.71 ^{b,j,m,j} | 19.41 ^d | 13.80 ^{eg} | 1.41 ^{b,e} |
| Sgt-1 | 47.73 ^{d,i} | 11.02 ^c | 7.53 ^e | 20.03 ^{a,c,e,g} | 13.85 ^{cg} | 18.55 ^{a,f,j,k} | 33.88 ^{b,e,f} | 1.83 ^{g,m,j} | 17.70 ^{ab} | 12.24 ^{a,c,e,g,h,i} | 1.45 ^{b,e} |
| Ravnica | 47.76 ^{a,b,d,e,f} | 12.21 ^{eh} | 6.28 ^f | 22.42 ^{gh} | 13.51 ^{cd} | 18.49 ^{a,b,c,h} | 35.92 ^e | 1.94 ^{a,b,g,m} | 21.11 ^{ef} | 10.93 ^{a,c,i} | 1.93 ^f |
| Balkan | 48.83 ^{a,b,d,e,f,h} | 13.04 ^{d,e,h,i} | 6.7 ^f | 23.81 ^{di} | 13.51 ^{a,c,g} | 19.74 ^{b,g,l,m} | 37.32 ^a | 1.89 ^{c,h} | 19.45 ^{de} | 12.94 ^{b,d,h} | 1.50 ^{b,d} |
| Proteinka | 53.28 ^{g,k} | 15.25 ^{f,j} | 6.58 ^f | 23.35 ^{b,f,h,i} | 16.51 ^{b,d,h,i,j} | 21.83 ^{b,i,n,o} | 39.87 ^g | 1.83 ^{d,i} | 22.85 ^f | 12.81 ^{a,c,f,h,j} | 1.76 ^g |
| Novosadjanka | 50.40 ^{h,i,j,k} | 14.21 ^j | 9.43 ^{b,c} | 21.5 ^{b,c,d,i,g} | 14.83 ^{b,d,i,j} | 23.64 ⁱ | 36.34 ^{e,h} | 1.54 ^{e,k} | 19.55 ^d | 13.48 ^{g,i} | 1.45 ^b |
| Vojvodjanka | 47.80 ^{e,j} | 11.49 ^{gh} | 8.88 ^a | 20.47 ^c | 12.39 ^f | 20.37 ^{d,j,l,n} | 32.86 ^f | 1.61 ^l | 19.45 ^d | 14.11 ^{b,e,g} | 1.38 ^e |
| Krajina | 54.49 ^g | 12.77 ^{ef} | 7.2 ^{a,e,f} | 22.91 ^{b,h} | 14.18 ^{g,i} | 19.97 ^{e,k,m,o} | 37.09 ^{c,h} | 1.86 ^{b,c,d,m} | 17.57 ^b | 9.82 ⁱ | 1.79 ^{f,g} |

^aMeans in the same column with different superscript roman letters are significantly different ($P < 0.05$).

^bM, monomeric form of glycinin; D, dimeric form of glycinin.

^cESP, extractable soluble protein.

and the concentration of major storage proteins, 7S and 11S, respectively (Table 2). This is in accordance with the findings of Kwanyuen *et al.* (19).

The ratio of 11S:7S proteins varied from 1.54 to 2.08 among the soybean varieties (Table 1). Lana and Ravnica had the highest 11S:7S protein ratios (≥ 1.94), whereas L91-31022 and Novosadjanka had the lowest (≤ 1.60).

These results suggest that soybean variety had a highly ($P < 0.05$) significant effect on the 7S and 11S protein concentration as well as on the 11S:7S protein ratio. The 11S:7S ratio of soybean varieties reported in the literature varies widely. To investigate the relationship with tofu quality, Cai and Chang (11) studied 12 cultivars and found that the 11S:7S protein ratio ranged from 1.64 to 2.51. Studying protein composition and nutritional quality, Kwanyuen *et al.* (19) reported an 11S:7S protein ratio of 1.7 to 4.9 among 45 wild soybean genotypes. On the other hand, Murphy and Resurreccion (10) reported that the 11S:7S protein ratio ranged from 2.1 to 3.4 among 12 soybean varieties, and they suggested that the differences in glycinin and β -conglycinin content were due to genetic and environmental differences.

Protein extractability. Protein extractability was significantly different among investigated genotypes (Table 3). The highest extractability was found in L91-31022 (90.3%) and the lowest in Balkan (77.7%). The average extractability of all genotypes was about 85%. A moderate positive correlation exists between protein extractability and extractable soluble protein content (Table 2). Also, a moderate positive correlation was found between extractable soluble protein content and β -conglycinin concentration. The increase in β -conglycinin concentration results in an increase of extractable soluble protein content. On the other hand, the increase of extractable soluble protein content leads to an increase of protein extractability. These facts indicate that genotypes with a higher level of β -conglycinin would have higher extractability than others. This is probably due to differences in protein structure of glycinin and β -conglycinin. Glycinin is a hexameric protein with compact quaternary structure, whereas β -conglycinin is a trimeric protein. β -Conglycinin is glycoprotein with about 5% carbohydrate moieties (20). It is known that the carbohydrate moieties

TABLE 2
Correlation Coefficients Between Investigated Factors in 12 Soybean Genotypes

| Factors | Protein | ESP | Extractability | ESI | EAI |
|----------------|-------------------|-------------------|-------------------|-------------------|--------------------|
| Protein | | 0.58 ^a | | 0.66 ^a | |
| ESP | | | 0.64 ^a | 0.16 | -0.47 |
| 7S | 0.33 | 0.61 ^a | 0.38 | 0.001 | -0.78 ^b |
| 11S | 0.35 | | 0.02 | 0.18 | 0.01 |
| 11S:7S | | | -0.36 | 0.14 | 0.86 ^b |
| M/D | 0.60 ^a | | | 0.87 ^b | -0.24 |
| Monomer (M) | | | -0.21 | 0.63 ^a | -0.22 |
| Dimer (D) | | | 0.21 | -0.55 | -0.02 |
| Extractability | | 0.64 ^a | | -0.47 | -0.26 |
| ESI | 0.66 ^a | | -0.47 | | -0.05 |

^aThese numbers correspond to correlations that are significant at $P < 0.05$.

^bThese numbers correspond to correlations that are significant at $P < 0.01$.

TABLE 3
Functional Properties of the Investigated Soybean Genotypes^a

| Genotype | Seed | | Functional properties | | |
|--------------|-----------------------------|---|------------------------------|------------------------|----------------------------|
| | Protein (% dry wt) | Extractable soluble protein (mg/mL) | Extractability (%) | ESI (min) | EAI (m ² /g) |
| Lana | 45.88 ^a | 17.75 ^{a,e} | 87.02 ^{a,b,e,g} | 13.22 ^a | 124.75 ^{a,d} |
| ZPS-015 | 48.86 ^{b-e} | 19.79 ^{b,d,f,g} | 88.63 ^{a-e} | 14.59 ^{b,c} | 113.23 ^{a,d} |
| Nena | 47.16 ^{b,f} | 17.98 ^{a,i} | 86.30 ^{a-e,g} | 14.34 ^b | 121.42 ^{a,d} |
| L91-31022 | 49.45 ^{c,h} | 20.43 ^{b,j,g} | 90.27 ^{a,c,e,g} | 14.37 ^{b,f} | 93.65 ^b |
| L94-1171 | 46.07 ^f | 17.56 ^a | 84.22 ^{b,d,h,i} | 14.74 ^{b,c} | 105.55 ^a |
| SG1-1 | 47.73 ^{d,i} | 18.81 ^{d,i,k,l} | 88.73 ^{c,j} | 15.02 ^c | 108.24 ^{a,d} |
| Krajina | 54.49 ^g | 19.21 ^{e,f,j,k,g} | 79.32 ^{a,c,d,f,g,h} | 18.23 ^d | 109.00 ^{c,d} |
| Vojvodjanka | 47.80 ^{e,j} | 17.63 ^{a,e} | 83.43 ^{d,i} | 14.82 ^{b,c,e} | 104.92 ^{b,e} |
| Novosadjanka | 50.40 ^{h-k} | 19.78 ^{g,l,f} | 88.13 ^{e,i,j,k} | 16.13 ^e | 100.91 ^c |
| Proteinka | 53.28 ^{g,k} | 20.43 ^{g,f} | 85.51 ^{a-k} | 17.88 ^d | 112.21 ^{c,d} |
| Balkan | 48.8 ^{a,b,d,e,f,h} | 16.13 ^c | 77.75 ^{f,k} | 15.63 ^{c,e,f} | 115.15 ^{d,e} |
| Ravnica | 47.76 ^{a,b,d,e,f} | 17.42 ^{a,f} | 81.73 ^{i,k} | 18.50 ^d | 113.74 ^{a,d,e} |

^aESI, emulsion stability index; EAI, emulsion activity index. Means in the same column with different superscript roman letters are significantly different ($P < 0.05$).

contribute to solubility (7). Furthermore, β -conglycinin has lower surface hydrophobicity than glycinin (6). However, no correlation was found either for β -conglycinin concentration or for 11S:7S ratio with protein extractability (Table 2), probably because extractability is expressed on the basis of total protein content. Our results suggest that some other proteins contribute also to enhancement of protein extractability.

Emulsifying properties. Significant differences in ESI and EAI were found among investigated genotypes (Table 3). The highest EAI and the lowest ESI were found in Lana, a cultivar lacking the Kunitz type of trypsin inhibitor. The ESI was highest in Ravnica whereas the lowest mean value for EAI was in L91-31022. No correlation was found between ESI and EAI. Also, no significant correlation was found between ESI or EAI and extractability.

A strong positive correlation ($P < 0.01$) was found between the 11S:7S ratio and EAI. Also, a strong negative correlation ($P < 0.01$) was found between EAI and concentration of β -conglycinin (Table 2). Essentially no correlation was found between 11S concentration and EAI, most likely due to the lesser extent of variation in glycinin concentration of the investigated genotypes. These results indicate that genotypes with the highest 11S:7S ratio and the lowest β -conglycinin concentration have the highest EAI. Based on these data, it appears that the 11S:7S ratio strongly reflects the ability of soybean proteins to form emulsions.

Different emulsifying ability could be explained by differences between the glycinin and β -conglycinin protein structure. Glycinin has more protein surface hydrophobicity or exposed hydrophobic groups than β -conglycinin (6), which may lead to more adsorbed oil/protein on the interface. The results of Khatib *et al.* (4) showed that there were about 15% more hydrophobic amino acid residues in the glycinin fraction than β -conglycinin. Additionally, glycinin partially dissociates from the 11S form into the 7S form, which, under the applied experimental conditions, can be detected on a PAGE gel. It has been

shown that dissociation improves the ability of the protein to diffuse to the interface. The 7S form of glycinin has a less structured conformation and contributes to easier anchorage into the interfacial layer (21).

A moderate positive correlation exists between ESI and total protein content (Table 2). This suggests that although the protein levels were the same among samples, seed total protein composition and structure had significant influences on emulsion stability. But no association was evident between ESI and the 11S:7S ratio or glycinin and β -conglycinin concentration. It is known that (i) the 7S form of glycinin has a lower M.W. than 11S and diffuses more quickly to the interface; (ii) dissociation leads to higher surface hydrophobicity by increasing the accessibility of the buried hydrophobic B polypeptides; and (iii) the 7S form has higher flexibility, which improves the ability of glycinin to adsorb at the interface. In looking for factors that influence emulsion stability, different forms of glycinin were evaluated (Table 1). Within the 12 investigated genotypes, the ESI showed no significant correlation with the dimeric form of glycinin, but it had a significant, moderately positive correlation ($r = 0.63$) with the monomeric form of glycinin and a strongly positive correlation ($r = 0.87$, $P < 0.01$) with the ratio of monomeric/dimeric form of glycinin (M/D) (Table 2). It has been shown that deamidation, reduction, and dissociation of glycinin resulted in enhanced emulsifying properties (3,21). Explaining emulsifying properties of food proteins, Kato and Nakai (22) and Nakai (23) showed that surface hydrophobicity and solubility were the major factors determining emulsifying activity, whereas the molecular flexibility of the proteins was important for emulsion stability. The results obtained in this work suggest that, when soybean protein extracts are tested for emulsifying properties, the concentration of the monomeric form of glycinin is an important parameter for enhancing emulsion stability. Furthermore, these results indicate that the ratio of two different forms of glycinin (M/D) might be a crucial factor for the stability of soybean protein emulsions.

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